NovaTec Immundiagnostica GmbH

Product List – CE Marked

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ISO 13485:2016

EC – Directive 98 / 79 EC
For In-Vitro-Diagnostics

2020-02-1

NovaTec Immundiagnostica GmbH

NovaLisa® Virology

Prod. No.	Name
ADVA0010	Adenovirus IgA
ADVG0010	Adenovirus IgG
ADVM0010	Adenovirus IgM
CHIG0590	Chikungunya Virus IgG capture
CHIM0590	Chikungunya Virus IgM µ-capture
CMVG0110	Cytomegalovirus (CMV) IgG
ACMV7110	Avidity Cytomegalovirus (CMV) IgG
CMVM0110	Cytomegalovirus (CMV) IgM
DENG0120	Dengue Virus IgG
DENM0120	Dengue Virus IgM
DVM0640	Dengue Virus IgM µ-capture
NS1D4020	Dengue Virus NS1 Antigen
EBVA0150	Epstein-Barr Virus (VCA) IgA
EBVG0150	Epstein-Barr Virus (VCA) IgG
AEBV7150	Avidity Epstein-Barr Virus (VCA) IgG
EBVM0150	Epstein-Barr Virus (VCA) IgM
EBVG0580	Epstein-Barr Virus (EBNA) IgG
HANG0670	Hantavirus IgG
HANM0670	Hantavirus IgM
HEVG0780	Hepatitis E Virus (HEV) IgG
HEVM0780	Hepatitis E Virus (HEV) IgM
HSVG0250	Herpes simplex Virus 1+2 (HSV) IgG
HSVM0250	Herpes simplex Virus 1+2 (HSV) IgM
HSV1G0500	Herpes simples Virus 1 (HSV 1) IgG
HSV1M0500	Herpes simplex Virus 1 (HSV 1) IgM
HSV2G0540	Herpes simplex Virus 2 (HSV 2) IgG
HSV2M0540	Herpes simplex Virus 2 (HSV 2) IgM
INFA0290	Influenza Virus A IgA
INFG0290	Influenza Virus A IgG
INFM0290	Influenza Virus A IgM
INFA0300	Influenza Virus B IgA
INFG0300	Influenza Virus B IgG
INFM0300	Influenza Virus B IgM
MEAG0330	Measles Virus IgG
AMEA7330	Avidity Measles Virus IgG
MEAM0330	Measles Virus IgM
MUMG0340	Mumps Virus IgG
MUMM0340	Mumps Virus IgM
PAIA0360	Parainfluenza Virus 1,2,3 IgA
PAIG0360	Parainfluenza Virus 1,2,3 IgG
PARG0370	Parvovirus B 19 IgG
PARM0370	Parvovirus B 19 IgM
RSVA0380	Respiratory syncytial Virus IgA
RSVG0380	Respiratory syncytial Virus IgG
RSVM0380	Respiratory syncytial Virus IgM
RUBG0400	Rubella Virus IgG

ARUB7400	Avidity Rubella Virus IgG
RUBM0400	Rubella Virus IgM µ-capture
TICG0440	TBE / FSME IgG
TICM0440	TBE / FSME IgM
PTICG044	TBE / FSME IgG plus
VZVA0490	Varicella-Zoster Virus (VZV) IgA
VZVG0490	Varicella-Zoster Virus (VZV) IgG
VZVM0490	Varicella-Zoster Virus (VZV) IgM
ZVG0790	Zika Virus IgG capture
ZVM0790	Zika Virus IgM µ-capture

NovaLisa ® Bacteriology

Prod. No.	Name
BAR0900	Bartonella
BOPA0030	Bordetella pertussis IgA
BOPG0030	Bordetella pertussis IgG
BOPM0030	Bordetella pertussis IgM
BPTA0610	Bordetella pertussis toxin (PT) IgA
BPTG0610	Bordetella pertussis toxin (PT) IgG
BORG0040	Borrelia burgdorferi IgG
BORM0040	Borrelia burgdorferi IgM
BRUG0050	Brucella IgG
BRUM0050	Brucella IgM
CHLA0070	Chlamydia trachomatis IgA
CHLG0070	Chlamydia trachomatis IgG
CHLM0070	Chlamydia trachomatis IgM
CHLA0510	Chlamydia pneumoniae IgA
CHLG0510	Chlamydia pneumoniae IgG
CHLM0510	Chlamydia pneumoniae IgM
CORG0090	Corynebacterium diphtheriae toxin IgG
CORG5009	Corynebacterium diphtheriae toxin 5S IgG
PCORG009	Corynebycterium diphtheriae toxin 5S IgG plus
COX1G0600	Coxiella burnetii (Q-Fever) Phase 1 IgG
COX2G0600	Coxiella burnetii (Q-Fever) Phase 2 IgG
COX2M0600	Coxiella burnetii (Q-Fever) Phase 2 IgM
HELA0220	Helicobacter pylori IgA
HELG0220	Helicobacter pylori IgG
PHELA022	Helicobacter pylori IgA plus
PHELG022	Helicobacter pylori IgG plus
LEGG0650	Legionella Pneumophila IgG
LEGM0650	Legionella Pneumophila IgM
LEPG0660	Leptospira IgG
LEPM0660	Leptospira IgM

MYCA0350	Mycoplasma pneumoniae IgA
MYCG0350	Mycoplasma pneumoniae IgG
MYCM0350	Mycoplasma pneumoniae IgM
TETG0430	Clostridium tetani toxin IgG
TETG5043	Clostridium tetani toxin 5S IgG
PTETG043	Clostridium tetani toxin 5S IgG plus

NovaLisa ® Parasites

Prod. No.	Name
CHAG0560	Chagas (Trypanosoma cruzi) IgG
TRYP0570	Chagas
ENTG0140	Entamoeba histolytica IgG
LEIG0310	Leishmania infantum IgG
MAL0620	Malaria
TOXA0460	Toxoplasma gondii IgA
TOXG0460	Toxoplasma gondii IgG
ATOX7460	Avidity Toxoplasma gondii IgG
TOXM0460	Toxoplasma gondii IgM µ-capture

NovaLisa ® Worms

Prod. No.	Name
ASCG0020	Ascaris lumbricoides IgG
ECHG0130	Echinococcus IgG
FIL0760	Filariasis
SCHG0410	Schistosoma mansoni IgG
SCHM0410	Schistosoma mansoni IgM
STRO0690	Strongyloides
TAEG0420	Taenia solium IgG
TOCG0450	Toxocara canis IgG
TRIG0480	Trichinella spiralis IgG

NovaLisa ® Fungi

Prod. No.	Name
ASPG0680	Aspergillus fumigatus IgG
ASPM0680	Aspergillus fumigatus IgM
CANA0060	Candida albicans IgA
CANG0060	Candida albicans IgG
CANM0060	Candida albicans IgM

NovaLisa ® Hormones

THYROID HORMONES
(ELISAs for the determination of thyroid hormones and antibodies)

Prod. No.	Name
ATG1010	Anti-TG
ATPO1020	Anti-TPO
FT41050	Free T4
TSH1030	TSH

Hormones

STEROID HORMONES
(ELISAs for the determination of steroid hormones in plasma and serum)

Prod. No.	Name
DNOV001	Cortisol
DNOV002	Testosterone
DNOV003	17 beta-Estradiol
DNOV004	17-OH Progesterone
DNOV005	DHEA-S
DNOV006	Progesterone
DNOV008	Androstenedione
DNOV009	Free Testosterone
DNOV011	Total Estriol
DNOV012	Aldosterone

STEROID HORMONES IN URINE
(ELISAs for the determination of steroid hormones in urine)

Prod. No.	Name
DNOV010	Urinary Cortisol

STEROID HORMONES IN SALIVA
(ELISAs for the determination of steroid hormones in saliva)

Prod. No.	Name
DSNOV20	Cortisol Saliva
DSNOV21	Testosterone Saliva
DSNOV24	DHEA-S Saliva
DSNOV27	Androstenedione Saliva

PROTEIN HORMONES
(ELISAs for the determination of proteins in plasma and serum)

Prod. No.	Name
DNOV030	LH
DNOV031	FSH
DNOV032	Prolactin
DNOV033	AFP
DNOV034	beta HCG

THYROID HORMONES
(ELISAs for the determination of thyroid hormones and antibodies)

Prod. No.	Name
DNOV051	Free T3
DNOV053	Total T3
DNOV054	Total T4
DNOV057	Thyroglobulin

DIABETES MONITORING
(ELISAs for the determination of specific analytes in plasma and serum)

Prod. No.	Name
DNOV111	Insulin
DNOV112	C-Peptide

CIRCULATING IMMUNO COMPLEXES
(ELISAs for the determination of specific analytes in plasma and serum)

Prod. No.	Name
DNOV093	CIC-C1q
DNOV094	CIC-C3d
DNOV096	CH-50

TUMOR MARKERS
(ELISAs for the determination of specific analytes in plasma and serum)

Prod. No.	Name
DNOV 060	CEA
DNOV061	CA 125
DNOV062	CA 15-3
DNOV063	CA 19-9

MISCELLANEOUS
(ELISAs for the determination of specific analytes in plasma and serum)

Prod. No.	Name
DNOV100	Ferritin
DNOV101	HGH
DNOV102	IgE

NovaLisa ® Autoimmune

Autoimmune
(ELISAs for the determination of specific autoimmune antibodies)

Prod. No.	Name
ATG1010	Anti-TG
ATPO1020	Anti-TPO

Rheumatology
(ELISAs for the determination of specific analytes in plasma and serum)

Prod. No.	Name
RFM3010	Rheumatoid Factor IgM

NovaLisa ® Recombinant Antigens

Prod. No.	Name
BORG0040	Borrelia burgdorferi IgG
BORM0040	Borrelia burgdorferi IgM
CHAG0560	Chagas (Trypanosoma cruzi) IgG
TRYP0570	Chagas
HANG0670	Hantavirus IgG
HANM0670	Hantavirus IgM
HELA0220	Helicobacter pylori IgA
PHELA022	Helicobacter pylori IgA plus
HEVG0780	Hepatitis E Virus (HEV) IgG
HEVM0780	Hepatitis E Virus (HEV) IgM
HSV1G0500	Herpes simples Virus 1 (HSV 1) IgG
HSV1M0500	Herpes simplex Virus 1 (HSV 1) IgM
HSV2G0540	Herpes simplex Virus 2 (HSV 2) IgG
HSV2M0540	Herpes simplex Virus 2 (HSV 2) IgM
MAL0620	Malaria
STRO0690	Strongyloides
ZVG0790	Zika Virus IgG capture
ZVM0790	Zika Virus IgM µ-capture

NovaLisa ® Quantitative Assays (WHO standardized)

Prod. No.	Name
BPTA0610	Bordetella pertussis toxin (PT) IgA
BPTG0610	Bordetella pertussis toxin (PT) IgG
CORG0090	Corynebacterium diphtheriae toxin IgG
CORG5009	Corynebacterium diphtheriae toxin 5S IgG
PCORG009	Corynebycterium diphtheriae toxin 5S IgG plus
RFM3010	Rheumatoid Factor IgM
RUBG0400	Rubella Virus IgG
TETG0430	Clostridium tetani toxin IgG
TETG5043	Clostridium tetani toxin 5S IgG
PTETG043	Clostridium tetani toxin 5S IgG plus
TOXG0460	Toxoplasma gondii IgG
ATOX7460	Avidity Toxoplasma gondii IgG
TSH1030	TSH

NovaLisa ® Quantitative Assays

Prod. No.	Name
ATG1010	Anti-TG
ATPO1020	Anti-TPO
BPTA0610	Bordetella pertussis toxin (PT) IgA
BPTG0610	Bordetella pertussis toxin (PT) IgG
CORG0090	Corynebacterium diphtheriae toxin IgG
CORG5009	Corynebacterium diphtheriae toxin 5S IgG
PCORG009	Corynebacterium diphtheriae toxin 5S IgG plus
FT41050	Free T4
HELA0220	Helicobacter pylori IgA
HELG0220	Helicobacter pylori IgG
PHELA022	Helicobacter pylori IgA plus
PHELG022	Helicobacter pylori IgG plus
RFM3010	Rheumatoid Factor IgM
RUBG0400	Rubella Virus IgG
ARUB7400	Avidity Rubella Virus IgG
TETG0430	Clostridium tetani toxin IgG
TETG5043	Clostridium tetani 5S toxin IgG
PTETG043	Clostridium tetani toxin 5S IgG plus
TICG0440	TBE / FSME IgG
PTICG044	TBE / FSME IgG plus
TOXG0460	Toxoplasma gondii IgG
ATOX7460	Avidity Toxoplasma gondii IgG
TSH1030	TSH

Antigen Assays

Prod. No.	Name
NS1D4020	Dengue Virus NS1 Antigen

NovaLisa ® IgM µ-capture Assays

Prod. No.	Name
CHIM0590	Chikungunya Virus IgM µ-capture
DVM0640	Dengue Virus IgM µ-capture
RUBM0400	Rubella Virus IgM µ-capture
TOXM0460	Toxoplasma gondii IgM µ-capture
ZVM0790	Zika Virus IgM µ-capture

NovaLisa ® Antibody Assays

Prod. No.	Name
ASCG0020	Ascaris lumbricoides IgG
CHAG0560	Chagas (Trypanosoma cruzi) IgG
TRYP0570	Chagas
ENTG0140	Entamoeba histolytica IgG
LEIG0310	Leishmania infantum IgG
MAL0620	Malaria
STRO0690	Strongyloides
TAEG0420	Taenia solium IgG
TOCG0450	Toxocara canis IgG
TRIG0480	Trichinella spiralis IgG

NovaLisa ® Avidity Assays

Prod. No.	Name
ACMV7110	Avidity Cytomegalovirus (CMV) IgG
AEBV7150	Avidity Epstein-Barr Virus (VCA) IgG
AMEA7330	Avidity Measles Virus IgG
ARUB7400	Avidity Rubella Virus IgG
ATOX7460	Avidity Toxoplasma gondii IgG

NovaLisa ® Liquor Diagnostic

Prod. No.	Name
BORG0040	Borrelia burgdorferi IgG
BORM0040	Borrelia burgdorferi IgM

NovaTec Immundiagnostica GmbH

NovaLisa®

Bordetella pertussis IgM

ELISA

CE

Only for in-vitro diagnostic use

Product Number: BOPM0030 (96 Determinations)

ENGLISH

1. INTRODUCTION

Bordetella pertussis is a respiratory pathogen that causes pertussis, commonly known as whooping cough, a localized infection of the ciliated epithelium of the bronchial tree. Pertussis is characterized by a prolonged paroxysmal cough often accompanied by an inspiratory whoop.

The disease affects mainly children, but adults have also been increasingly reported to be affected. The pathogen produces toxins which cause local damage to the cilia of epithelial cells, which leads to prolonged illness and pertussis. Disease presentation varies with age and history of previous exposure or vaccination. Severe disease is infrequent in healthy, vaccinated persons. Infants, particularly those who have not received the primary vaccination series against pertussis, are at risk for complications and mortality.

In addition to B. pertussis, three other Bordetella species can cause disease in humans: B. parapertussis, B. holmesii, and B. bronchiseptica. B. parapertussis causes a pertussis-like illness that is generally milder than pertussis because the bacteria do not produce pertussis toxin. Co-infection of B. pertussis and B. parapertussis is not unusual.

B. pertussis is of worldwide prevalence. Globally, 20-40 million cases of pertussis occur each year, 90 % of which are in developing countries, and there are up to 400,000 fatalities each year, mostly in young infants.

Transmission of B. pertussis occurs primarily via close direct contact with an infected person or inhalation of airborne droplets. Symptoms develop following inhalation of the airborne pathogen. The organism is highly contagious, with up to 90 % of household contacts developing the disease. Infected persons are most contagious in the catarrhal and the paroxysmal stages.

The incubation period is usually seven to 10 days, with a range of 4-21 days.

Typical pertussis symptoms occur in three different stages: catarrhal, paroxysmal, and convalescent.

The catarrhal stage lasts for about 1-2 weeks, and is characterized by non-specific symptoms such as rhinorrhea, sneezing, low-grade fever and cough. The second stage is the paroxysmal stage, lasting for about 4-6 weeks, and is characterized by various pathognomonic symptoms of pertussis such as episodes of paroxysmal cough with a characteristic whooping sound. The final stage is the convalescent stage. During this stage, the respiratory symptoms gradually decrease although cough can continue for months.

Many factors can alter the usual course of pertussis, causing an atypical presentation. Previously vaccinated adolescents and adults may have less severe paroxysmal symptoms.

Species	**Disease**	**Symptoms (e.g.)**	**Transmission route**
Bordetella pertussis	Pertussis whooping cough	1. Stadium catarrhale: symptoms of a cold with slight fever (1-2 weeks) 2. Stadium convulsivum: severe, spasmodic coughing; after deep inspiration follows a coughing staccato (2-6 weeks) 3. Stadium decrementi: Ease of disease with symptoms of a bronchitis (up to 6 weeks)	Highly contagious droplet infection

Infection or presence of pathogen may be identified by:

- Microscopy
- PCR
- Serology: e.g. ELISA

2. INTENDED USE

The Bordetella pertussis IgM ELISA is intended for the qualitative determination of IgM class antibodies against Bordetella pertussis in human serum or plasma (citrate, heparin).

3. PRINCIPLE OF THE ASSAY

The qualitative immunoenzymatic determination of specific antibodies is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique.
Microtiterplates are coated with specific antigens to bind corresponding antibodies of the sample. After washing the wells to remove all unbound sample material a horseradish peroxidase (HRP) labelled conjugate is added. This conjugate binds to the captured antibodies. In a second washing step unbound conjugate is removed. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product.
The intensity of this product is proportional to the amount of specific antibodies in the sample. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450/620 nm is read using an ELISA Microtiterplate reader.

4. MATERIALS

4.1. Reagents supplied

- **Microtiterplate:** 12 break-apart 8-well snap-off strips coated with Bordetella pertussis antigens; in resealable aluminium foil.
- **IgM Sample Dilution Buffer:** 1 bottle containing 100 mL of phosphate buffer (10 mM) for sample dilution; pH 7.2 ± 0.2; anti-human IgG (RF Absorbent); coloured green; ready to use; white cap; ≤ 0.0015% (v/v) CMIT/ MIT (3:1).
- **Stop Solution:** 1 bottle containing 15 mL sulphuric acid, 0.2 mol/L; ready to use; red cap.
- **Washing Buffer (20x conc.):** 1 bottle containing 50 mL of a 20-fold concentrated phosphate buffer (0.2 M), pH 7.2 ± 0.2, for washing the wells; white cap.
- **Conjugate:** 1 bottle containing 20 mL of peroxidase labelled antibody to human IgM in phosphate buffer (10 mM); coloured red; ready to use; black cap.
- **TMB Substrate Solution:** 1 bottle containing 15 mL 3,3',5,5'-tetramethylbenzidine (TMB), < 0.1 %; ready to use; yellow cap.
- **Positive Control:** 1 vial containing 2 mL control; coloured yellow; ready to use; red cap; ≤ 0.02% (v/v) MIT.
- **Cut-off Control:** 1 vial containing 3 mL control; coloured yellow; ready to use; green cap; ≤ 0.02% (v/v) MIT.
- **Negative Control:** 1 vial containing 2 mL control; coloured yellow; ready to use; blue cap; ≤ 0.0015% (v/v) CMIT/ MIT (3:1).

For hazard and precautionary statements see 12.1
For potential hazardous substances please check the safety data sheet.

4.2. Materials supplied

- 1 Cover foil
- 1 Instruction for use (IFU)
- 1 Plate layout

4.3. Materials and Equipment needed

- ELISA Microtiterplate reader, equipped for the measurement of absorbance at 450/620 nm
- Incubator 37 °C
- Manual or automatic equipment for rinsing Microtiterplates
- Pipettes to deliver volumes between 10 and 1000 µL
- Vortex tube mixer
- Distilled water
- Disposable tubes

5. STABILITY AND STORAGE

Store the kit at 2...8 °C. The opened reagents are stable up to the expiry date stated on the label when stored at 2...8 °C.

6. REAGENT PREPARATION

It is very important to bring all reagents and samples to room temperature (20…25 °C) and mix them before starting the test run!

6.1. Microtiterplate

The break-apart snap-off strips are coated with Bordetella pertussis antigens. Immediately after removal of the strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2...8 °C.

6.2. Washing Buffer (20x conc.)

Dilute Washing Buffer 1 + 19; e. g. 10 mL Washing Buffer + 190 mL distilled water. The diluted buffer is stable for 5 days at room temperature (20…25 °C). In case crystals appear in the concentrate, warm up the solution to 37°C e.g. in a water bath. Mix well before dilution.

6.3. TMB Substrate Solution

The reagent is ready to use and has to be stored at 2...8 °C, away from the light. The solution should be colourless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away.

7. SAMPLE COLLECTION AND PREPARATION

Use human serum or plasma (citrate, heparin) samples with this assay. If the assay is performed within 5 days after sample collection, the samples should be kept at 2...8 °C; otherwise they should be aliquoted and stored deep-frozen (-70…-20 °C). If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing.
Heat inactivation of samples is not recommended.

7.1. Sample Dilution

Before assaying, all samples should be diluted 1+100 with IgM Sample Dilution Buffer. Dispense 10 µL sample and 1 mL IgM Sample Dilution Buffer into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

8. ASSAY PROCEDURE

Please read the instruction for use carefully before performing the assay. Result reliability depends on strict adherence to the instruction for use as described. The following test procedure is only validated for manual procedure. If performing the test on ELISA automatic systems we recommend increasing the washing steps from three up to five and the volume of Washing Buffer from 300 µL to 350 µL to avoid washing effects. Pay attention to chapter 12. Prior to commencing the assay, the distribution and identification plan for all samples and standards/controls (duplicates recommended) should be carefully established on the plate layout supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder.

Perform all assay steps in the order given and without any delays.

A clean, disposable tip should be used for dispensing each standard/control and sample.

Adjust the incubator to 37 ± 1 °C.

1. Dispense 100 µL standards/controls and diluted samples into their respective wells. Leave well A1 for the Substrate Blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 1 hour ± 5 min at 37 ± 1 °C.**
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µL of Washing Buffer. Avoid overflows from the reaction wells. The interval between washing and aspiration should be > 5 sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!

   Note: Washing is important! Insufficient washing results in poor precision and false results.
5. Dispense 100 µL Conjugate into all wells except for the Substrate Blank well A1.
6. **Incubate for 30 min at room temperature (20...25 °C).** Do not expose to direct sunlight.
7. Repeat step 4.
8. Dispense 100 µL TMB Substrate Solution into all wells.
9. **Incubate for exactly 15 min at room temperature (20...25 °C) in the dark.** A blue colour occurs due to an enzymatic reaction.
10. Dispense 100 µL Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution, thereby a colour change from blue to yellow occurs.
11. Measure the absorbance at 450/620 nm within 30 min after addition of the Stop Solution.

8.1. Measurement

Adjust the ELISA Microtiterplate reader **to zero** using the **Substrate Blank**.

If - due to technical reasons - the ELISA Microtiterplate reader cannot be adjusted to zero using the Substrate Blank, subtract its absorbance value from all other absorbance values measured in order to obtain reliable results!

Measure the absorbance of all wells at **450 nm** and record the absorbance values for each standard/control and sample in the plate layout.

Bichromatic measurement using a reference wavelength of 620 nm is recommended.

Where applicable calculate the mean absorbance values of all duplicates.

9. RESULTS

9.1. Run Validation Criteria

In order for an assay run to be considered valid, these Instructions for Use have to be strictly followed and the following criteria must be met:

- **Substrate Blank**: Absorbance value **< 0.100**
- **Negative Control**: Absorbance value **< 0.200 and < Cut-off**
- **Cut-off Control**: Absorbance value **0.150 – 1.300**
- **Positive Control**: Absorbance value **> Cut-off**

If these criteria are not met, the test is not valid and must be repeated.

9.2. Calculation of Results

The Cut-off is the mean absorbance value of the Cut-off Control determinations.

Example: Absorbance value Cut-off Control 0.44 + absorbance value Cut-off control 0.42 = 0.86 / 2 = 0.43
Cut-off = 0.43

9.2.1. Results in Units [NTU]

$$\frac{\text{Sample (mean) absorbance value} \times 10}{\text{Cut-off}} = \text{[NovaTec Units = NTU]}$$

Example: $\frac{1.591 \times 10}{0.43} = 37$ NTU (Units)

9.3. Interpretation of Results

Cut-off	10 NTU	-
Positive	> 11 NTU	Antibodies against the pathogen are present. There has been a contact with the antigen (pathogen resp. vaccine).
Equivocal	9 – 11 NTU	Antibodies against the pathogen could not be detected clearly. It is recommended to repeat the test with a fresh sample in 2 to 4 weeks. If the result is equivocal again the sample is judged as **negative**.
Negative	< 9 NTU	The sample contains no antibodies against the pathogen. A previous contact with the antigen (pathogen resp. vaccine) is unlikely.
Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as serological data. In immunocompromised patients and newborns serological data only have restricted value.		

9.3.1. Antibody Isotypes and State of Infection

Serology	Significance
IgM	Characteristic of the primary antibody response High IgM titer with low IgG titer: → suggests a current or very recent infection Rare: → persisting IgM
IgG	Characteristic of the secondary antibody response May persist for several years High IgG titer with low IgM titer: → may indicate a past infection
IgA	Produced in mucosal linings throughout the body (⇒ protective barrier) Usually produced early in the course of the infection

10. SPECIFIC PERFORMANCE CHARACTERISTICS

The results refer to the groups of samples investigated; these are not guaranteed specifications.

For further information about the specific performance characteristics please contact NovaTec Immundiagnostica GmbH.

10.1. Precision

Intraassay	**n**	**Mean (E)**	**CV (%)**
#1	24	0.363	2.51
#2	24	0.790	2.04
#3	24	0.609	2.28
Interassay	**n**	**Mean (NTU)**	**CV (%)**
#1	12	20.82	3.57
#2	12	14.81	4.63
#3	12	6.95	5.53

10.2. Diagnostic Specificity

The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte.
It is 100 % (95% confidence interval: 96.19% - 100%).

10.3. Diagnostic Sensitivity

The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte.
It is 89.19 % (95% confidence interval: 74.58% - 96.97%).

10.4. Interferences

Interferences with hemolytic, lipemic or icteric samples are not observed up to a concentration of 10 mg/mL hemoglobin, 5 mg/mL triglycerides and 0.5 mg/mL bilirubin.

10.5. Cross Reactivity

Investigation of a sample panel with antibody activities to potentially cross-reacting parameters did not reveal evidence of false-positive results due to cross-reactions.

11. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or repeated freeze-thaw cycles of the sample may affect the absorbance values.

12. PRECAUTIONS AND WARNINGS

- The test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the testkits with analyzers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.
- Only for in-vitro diagnostic use.
- All materials of human or animal origin should be regarded and handled as potentially infectious.
- All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive.
- Do not interchange reagents or Microtiterplates of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- Use only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and standard/control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense reagents without splashing accurately into the wells.
- The ELISA is only designed for qualified personnel following the standards of good laboratory practice (GLP).
- For further internal quality control each laboratory should additionally use known samples.

12.1. Safety note for reagents containing hazardous substances

Reagents may contain CMIT/MIT (3:1) or MIT (refer to 4.1)
Therefore, the following hazard and precautionary statements apply.

Warning

H317	May cause an allergic skin reaction.
P261	Avoid breathing spray
P280	Wear protective gloves/ protective clothing.
P302+P352	IF ON SKIN: Wash with plenty of soap and water.
P333+P313	If skin irritation or rash occurs: Get medical advice/ attention.
P362+P364	Take off contaminated and Wash it before reuse.

Further information can be found in the safety data sheet

12.2. Disposal Considerations

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

13. ORDERING INFORMATION

Prod. No.: BOPM0030 Bordetella pertussis IgM ELISA (96 Determinations)

NovaTec Immundiagnostica GmbH

NovaLisa®

Bordetella pertussis IgG

ELISA

CE

Only for in-vitro diagnostic use

Product Number: BOPG0030 (96 Determinations)

ENGLISH

1. INTRODUCTION

Bordetella pertussis is a respiratory pathogen that causes pertussis, commonly known as whooping cough, a localized infection of the ciliated epithelium of the bronchial tree. Pertussis is characterized by a prolonged paroxysmal cough often accompanied by an inspiratory whoop.

The disease affects mainly children, but adults have also been increasingly reported to be affected. The pathogen produces toxins which cause local damage to the cilia of epithelial cells, which leads to prolonged illness and pertussis. Disease presentation varies with age and history of previous exposure or vaccination. Severe disease is infrequent in healthy, vaccinated persons. Infants, particularly those who have not received the primary vaccination series against pertussis, are at risk for complications and mortality.

In addition to B. pertussis, three other Bordetella species can cause disease in humans: B. parapertussis, B. holmesii, and B. bronchiseptica. B. parapertussis causes a pertussis-like illness that is generally milder than pertussis because the bacteria do not produce pertussis toxin. Co-infection of B. pertussis and B. parapertussis is not unusual.

B. pertussis is of worldwide prevalence. Globally, 20-40 million cases of pertussis occur each year, 90 % of which are in developing countries, and there are up to 400,000 fatalities each year, mostly in young infants.

Transmission of B. pertussis occurs primarily via close direct contact with an infected person or inhalation of airborne droplets. Symptoms develop following inhalation of the airborne pathogen. The organism is highly contagious, with up to 90 % of household contacts developing the disease. Infected persons are most contagious in the catarrhal and the paroxysmal stages.

The incubation period is usually seven to 10 days, with a range of 4-21 days.

Typical pertussis symptoms occur in three different stages: catarrhal, paroxysmal, and convalescent.

The catarrhal stage lasts for about 1-2 weeks, and is characterized by non-specific symptoms such as rhinorrhea, sneezing, low-grade fever and cough. The second stage is the paroxysmal stage, lasting for about 4-6 weeks, and is characterized by various pathognomonic symptoms of pertussis such as episodes of paroxysmal cough with a characteristic whooping sound. The final stage is the convalescent stage. During this stage, the respiratory symptoms gradually decrease although cough can continue for months.

Many factors can alter the usual course of pertussis, causing an atypical presentation. Previously vaccinated adolescents and adults may have less severe paroxysmal symptoms.

Species	**Disease**	**Symptoms (e.g.)**	**Transmission route**
Bordetella pertussis	Pertussis whooping cough	1. Stadium catarrhale: symptoms of a cold with slight fever (1-2 weeks) 2. Stadium convulsivum: severe, spasmodic coughing; after deep inspiration follows a coughing staccato (2-6 weeks) 3. Stadium decrementi: Ease of disease with symptoms of a bronchitis (up to 6 weeks)	Highly contagious droplet infection

Infection or presence of pathogen may be identified by:

- Microscopy
- PCR
- Serology: e.g. ELISA

2. INTENDED USE

The Bordetella pertussis IgG ELISA is intended for the qualitative determination of IgG class antibodies against Bordetella pertussis in human serum or plasma (citrate, heparin).

3. PRINCIPLE OF THE ASSAY

The qualitative immunoenzymatic determination of specific antibodies is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique.
Microtiterplates are coated with specific antigens to bind corresponding antibodies of the sample. After washing the wells to remove all unbound sample material a horseradish peroxidase (HRP) labelled conjugate is added. This conjugate binds to the captured antibodies. In a second washing step unbound conjugate is removed. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product.
The intensity of this product is proportional to the amount of specific antibodies in the sample. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450/620 nm is read using an ELISA Microtiterplate reader.

4. MATERIALS

4.1. Reagents supplied

- **Microtiterplate:** 12 break-apart 8-well snap-off strips coated with Bordetella pertussis antigens; in resealable aluminium foil.
- **IgG Sample Dilution Buffer:** 1 bottle containing 100 mL of phosphate buffer (10 mM) for sample dilution; pH 7.2 ± 0.2; coloured yellow; ready to use; white cap; ≤ 0.0015% (v/v) CMIT/ MIT (3:1).
- **Stop Solution:** 1 bottle containing 15 mL sulphuric acid, 0.2 mol/L; ready to use; red cap.
- **Washing Buffer (20x conc.):** 1 bottle containing 50 mL of a 20-fold concentrated phosphate buffer (0.2 M), pH 7.2 ± 0.2, for washing the wells; white cap.
- **Conjugate:** 1 bottle containing 20 mL of peroxidase labelled antibody to human IgG in phosphate buffer (10 mM); coloured blue; ready to use; black cap.
- **TMB Substrate Solution:** 1 bottle containing 15 mL 3,3',5,5'-tetramethylbenzidine (TMB), < 0.1 %; ready to use; yellow cap.
- **Positive Control:** 1 vial containing 2 mL control; coloured yellow; ready to use; red cap; ≤ 0.02% (v/v) MIT.
- **Cut-off Control:** 1 vial containing 3 mL control; coloured yellow; ready to use; green cap; ≤ 0.02% (v/v) MIT.
- **Negative Control:** 1 vial containing 2 mL control; coloured yellow; ready to use; blue cap; ≤ 0.0015% (v/v) CMIT/ MIT (3:1).

For hazard and precautionary statements see 12.1
For potential hazardous substances please check the safety data sheet.

4.2. Materials supplied

- 1 Cover foil
- 1 Instruction for use (IFU)
- 1 Plate layout

4.3. Materials and Equipment needed

- ELISA Microtiterplate reader, equipped for the measurement of absorbance at 450/620 nm
- Incubator 37 °C
- Manual or automatic equipment for rinsing Microtiterplates
- Pipettes to deliver volumes between 10 and 1000 µL
- Vortex tube mixer
- Distilled water
- Disposable tubes

5. STABILITY AND STORAGE

Store the kit at 2...8 °C. The opened reagents are stable up to the expiry date stated on the label when stored at 2...8 °C.

6. REAGENT PREPARATION

It is very important to bring all reagents and samples to room temperature (20…25 °C) and mix them before starting the test run!

6.1. Microtiterplate

The break-apart snap-off strips are coated with Bordetella pertussis antigens. Immediately after removal of the strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2...8 °C.

6.2. Washing Buffer (20x conc.)

Dilute Washing Buffer 1 + 19; e. g. 10 mL Washing Buffer + 190 mL distilled water. The diluted buffer is stable for 5 days at room temperature (20…25 °C). In case crystals appear in the concentrate, warm up the solution to 37 °C e.g. in a water bath. Mix well before dilution.

6.3. TMB Substrate Solution

The reagent is ready to use and has to be stored at 2...8 °C, away from the light. The solution should be colourless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away.

7. SAMPLE COLLECTION AND PREPARATION

Use human serum or plasma (citrate, heparin) samples with this assay. If the assay is performed within 5 days after sample collection, the samples should be kept at 2...8 °C; otherwise they should be aliquoted and stored deep-frozen (-70…-20 °C). If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing.
Heat inactivation of samples is not recommended.

7.1. Sample Dilution

Before assaying, all samples should be diluted 1+100 with IgG Sample Dilution Buffer. Dispense 10 µL sample and 1 mL IgG Sample Dilution Buffer into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

8. ASSAY PROCEDURE

Please read the instruction for use carefully **before** performing the assay. Result reliability depends on strict adherence to the instruction for use as described. The following test procedure is only validated for manual procedure. If performing the test on ELISA automatic systems we recommend increasing the washing steps from three up to five and the volume of Washing Buffer from 300 µL to 350 µL to avoid washing effects. Pay attention to chapter 12. Prior to commencing the assay, the distribution and identification plan for all samples and standards/controls (duplicates recommended) should be carefully established on the plate layout supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder.

Perform all assay steps in the order given and without any delays.

A clean, disposable tip should be used for dispensing each standard/control and sample.

Adjust the incubator to 37 ± 1 °C.

1. Dispense 100 µL standards/controls and diluted samples into their respective wells. Leave well A1 for the Substrate Blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 1 hour ± 5 min at 37 ± 1 °C.**
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µL of Washing Buffer. Avoid overflows from the reaction wells The interval between washing and aspiration should be > 5 sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!

   Note: Washing is important! Insufficient washing results in poor precision and false results.
5. Dispense 100 µL Conjugate into all wells except for the Substrate Blank well A1.
6. **Incubate for 30 min at room temperature (20...25 °C).** Do not expose to direct sunlight.
7. Repeat step 4.
8. Dispense 100 µL TMB Substrate Solution into all wells.
9. **Incubate for exactly 15 min at room temperature (20...25 °C) in the dark.** A blue colour occurs due to an enzymatic reaction.
10. Dispense 100 µL Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution, thereby a colour change from blue to yellow occurs.
11. Measure the absorbance at 450/620 nm within 30 min after addition of the Stop Solution.

8.1. Measurement

Adjust the ELISA Microtiterplate reader **to zero** using the **Substrate Blank**.

If - due to technical reasons - the ELISA Microtiterplate reader cannot be adjusted to zero using the Substrate Blank, subtract its absorbance value from all other absorbance values measured in order to obtain reliable results!

Measure the absorbance of all wells at **450 nm** and record the absorbance values for each standard/control and sample in the plate layout.

Bichromatic measurement using a reference wavelength of 620 nm is recommended.

Where applicable calculate the mean absorbance values of all duplicates.

9. RESULTS

9.1. Run Validation Criteria

In order for an assay run to be considered valid, these Instructions for Use have to be strictly followed and the following criteria must be met:

- **Substrate Blank**: Absorbance value **< 0.100**
- **Negative Control**: Absorbance value **< 0.200 and < Cut-off**
- **Cut-off Control**: Absorbance value **0.150 – 1.300**
- **Positive Control**: Absorbance value **> Cut-off**

If these criteria are not met, the test is not valid and must be repeated.

9.2. Calculation of Results

The Cut-off is the mean absorbance value of the Cut-off Control determinations.

Example: Absorbance value Cut-off Control 0.44 + absorbance value Cut-off control 0.42 = 0.86 / 2 = 0.43
Cut-off = 0.43

9.2.1. Results in Units [NTU]

$$\frac{\text{Sample (mean) absorbance value} \times 10}{\text{Cut-off}} = \text{[NovaTec Units = NTU]}$$

Example: $\frac{1.591 \times 10}{0.43} = 37$ NTU (Units)

9.3. Interpretation of Results

Cut-off	10 NTU	-
Positive	> 11 NTU	Antibodies against the pathogen are present. There has been a contact with the antigen (pathogen resp. vaccine).
Equivocal	9 – 11 NTU	Antibodies against the pathogen could not be detected clearly. It is recommended to repeat the test with a fresh sample in 2 to 4 weeks. If the result is equivocal again the sample is judged as **negative**.
Negative	< 9 NTU	The sample contains no antibodies against the pathogen. A previous contact with the antigen (pathogen resp. vaccine) is unlikely.
Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as serological data. In immunocompromised patients and newborns serological data only have restricted value.		

9.3.1. Antibody Isotypes and State of Infection

Serology	Significance
IgM	Characteristic of the primary antibody response High IgM titer with low IgG titer: → suggests a current or very recent infection Rare: → persisting IgM
IgG	Characteristic of the secondary antibody response May persist for several years High IgG titer with low IgM titer: → may indicate a past infection
IgA	Produced in mucosal linings throughout the body (⇒ protective barrier) Usually produced early in the course of the infection

10. SPECIFIC PERFORMANCE CHARACTERISTICS

The results refer to the groups of samples investigated; these are not guaranteed specifications.

For further information about the specific performance characteristics please contact NovaTec Immundiagnostica GmbH.

10.1. Precision

Intraassay	**n**	**Mean (E)**	**CV (%)**
#1	24	0.455	3.55
#2	24	0.940	2.58
#3	24	0.528	2.74
Interassay	**n**	**Mean (NTU)**	**CV (%)**
#1	12	22.48	8.09
#2	12	11.12	13.56
#3	12	1.18	14.08

10.2. Diagnostic Specificity

The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte.

It is 93.02% (95% confidence interval: 80.94% - 98.54%).

10.3. Diagnostic Sensitivity

The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte.

It is 98.31% (95% confidence interval: 90.91% - 99.96%).

10.4. Interferences

Interferences with hemolytic, lipemic or icteric samples are not observed up to a concentration of 10 mg/mL hemoglobin, 5 mg/mL triglycerides and 0.5 mg/mL bilirubin.

10.5. Cross Reactivity

Investigation of a sample panel with antibody activities to potentially cross-reacting parameters did not reveal evidence of false-positive results due to cross-reactions.

11. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or repeated freeze-thaw cycles of the sample may affect the absorbance values.

12. PRECAUTIONS AND WARNINGS

- The test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the testkits with analyzers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.
- Only for in-vitro diagnostic use.
- All materials of human or animal origin should be regarded and handled as potentially infectious.
- All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive.
- Do not interchange reagents or Microtiterplates of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- Use only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and standard/control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense reagents without splashing accurately into the wells.
- The ELISA is only designed for qualified personnel following the standards of good laboratory practice (GLP).
- For further internal quality control each laboratory should additionally use known samples.

12.1. Safety note for reagents containing hazardous substances

Reagents may contain CMIT/MIT (3:1) or MIT (refer to 4.1)
Therefore, the following hazard and precautionary statements apply.

Warning	H317	May cause an allergic skin reaction.
	P261	Avoid breathing spray.
	P280	Wear protective gloves/ protective clothing.
	P302+P352	IF ON SKIN: Wash with plenty of soap and water.
	P333+P313	If skin irritation or rash occurs: Get medical advice/ attention.
	P362+P364	Take off contaminated and Wash it before reuse.

Further information can be found in the safety data sheet

12.2. Disposal Considerations

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

13. ORDERING INFORMATION

Prod. No.: BOPG0030 Bordetella pertussis IgG ELISA (96 Determinations)

NovaTec Immundiagnostica GmbH

NovaLisa®

Corynebacterium diphtheriae toxin IgG

ELISA

CE

Only for in-vitro diagnostic use

Product Number: CORG0090 (96 Determinations)

ENGLISH

1. INTRODUCTION

Corynebacteria are aerobic non spore-forming gram-positive rods of irregular shape (0.5 –1 µm thick and 2-6 µm long). They comprise skin commensals, opportunist pathogens and several major pathogens, including Corynebacterium diphtheriae. In general, they are isolated from throat swabs on selective media containing tellurite. The bacterial infection caused by C. diphtheriae, Diphtheria, has two forms. Respiratory diphtheria is typically caused by toxin-producing (toxigenic) strains; cutaneous disease can be caused by either toxigenic or nontoxigenic strains. In the respiratory form of the disease, a membrane is formed; this membrane is usually visible on the throat or tonsils. Persons may die from asphyxiation when the membrane obstructs breathing. Other complications are caused by remote effects of the diphtheria toxin (myocarditis, nerve paralysis) Cutaneous diphtheria is usually mild, typically consisting of non-distinctive sores or shallow ulcers and only rarely involving toxic complications (1-2% of infections with toxigenic strains). Diphtheria was one of the most common causes of death among children during the prevaccine era.

Since the introduction and widespread use of diphtheria toxoid vaccine (formalin-inactivated diphtheria toxin) in most industrialized countries the disease is now characterized by sporadic cases and intermittent outbreaks of low intensity. But recent large epidemics of diphtheria in several eastern European countries have again drawn attention to this „forgotten“ disease – and, the majority of these cases have occurred among adolescents and adults instead of children.

The only effective way to control diphtheria is by prophylactic immunization with diphtheria toxoid. Antibody to the toxoid protects against the action of the toxin; immunized persons can be infected by toxin-producing strains of diphtheria, but the systemic manifestations of diphtheria do not occur. The outcome of the disease improves with early, appropriate treatment. Prompt recognition of the disease is important to assure early, appropriate treatment with diphtheria anti-toxin

Species	**Disease**	**Symptoms (e.g.)**	**Transmission route**
Corynebacterium diphtheriae	Diphtheria (respiratory)	with malaise, sore throat, anorexia, low-grade fever and swelling of the neck (“bull neck”) from inflammation. Complications: exotoxin-induced damage to other organs.	Transmission from person to person through close physical and respiratory contact Transmission is increased in overcrowded and poor socio-economic conditions

Infection or presence of pathogen may be identified by:

- Microscopy
- Serology: e.g. by ELISA

2. INTENDED USE

The Corynebacterium diphtheriae toxin IgG ELISA is intended for the quantitative determination of IgG class antibodies against Corynebacterium diphtheriae toxin in human serum or plasma (citrate, heparin).

3. PRINCIPLE OF THE ASSAY

The quantitative immunoenzymatic determination of specific antibodies is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique.
Microtiterplates are coated with specific antigens to bind corresponding antibodies of the sample. After washing the wells to remove all unbound sample material a horseradish peroxidase (HRP) labelled conjugate is added. This conjugate binds to the captured antibodies. In a second washing step unbound conjugate is removed. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product.
The intensity of this product is proportional to the amount of specific antibodies in the sample. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450/620 nm is read using an ELISA Microtiterplate reader.

4. MATERIALS

4.1. Reagents supplied

- **Microtiterplate:** 12 break apart 8-well snap-off strips coated with Corynebacterium diphtheriae toxin (toxoid) antigens; in resealable aluminium foil.
- **IgG Sample Dilution Buffer:** 1 bottle containing 100 mL of phosphate buffer (10 mM) for sample dilution; pH 7.2 ± 0.2; coloured yellow; ready to use; white cap; ≤ 0.0015% (v/v) CMIT/ MIT (3:1).
- **Stop Solution:** 1 bottle containing 15 mL sulphuric acid, 0.2 mol/L; ready to use; red cap.
- **Washing Buffer (20x conc.):** 1 bottle containing 50 mL of a 20-fold concentrated phosphate buffer (0.2 M), pH 7.2 ± 0.2, for washing the wells; white cap.
- **Conjugate:** 1 bottle containing 20 mL of peroxidase labelled antibody to human IgG in phosphate buffer (10 mM); coloured blue; ready to use; black cap.
- **TMB Substrate Solution:** 1 bottle containing 15 mL 3,3',5,5'-tetramethylbenzidine (TMB), < 0,1 %; ready to use; yellow cap.
- **Standards:** 4 vials, each containing 2 mL standard; coloured yellow; ready to use; ≤ 0.02% (v/v) MIT.

  Standard A: 0.000 IU/mL; blue cap

  Standard B: 0.015 IU/mL; green cap

  Standard C: 0.075 IU/mL; yellow cap

  Standard D: 0.150 IU/mL; red cap

  The standards are calibrated in accordance with the ‘‘1st International Standard for Diphtheria Antitoxin Human IgG (WHO, 2012).

For hazard and precautionary statements see 12.1

For potential hazardous substances please check the safety data sheet.

4.2. Materials supplied

- 1 Cover foil
- 1 Instruction for use (IFU)
- 1 Plate layout

4.3. Materials and Equipment needed

- ELISA Microtiterplate reader, equipped for the measurement of absorbance at 450/620 nm
- Incubator 37 °C
- Manual or automatic equipment for rinsing Microtiterplates
- Pipettes to deliver volumes between 10 and 1000 µL
- Vortex tube mixer
- Distilled water
- Disposable tubes

5. STABILITY AND STORAGE

Store the kit at 2...8 °C. The opened reagents are stable up to the expiry date stated on the label when stored at 2...8 °C.

6. REAGENT PREPARATION

It is very important to bring all reagents and samples to room temperature (20…25 °C) and mix them before starting the test run!

6.1. Microtiterplate

The break-apart snap-off strips are coated with Corynebacterium diphtheriae toxin (toxoid) antigens. Immediately after removal of the strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2...8 °C.

6.2. Washing Buffer (20x conc.)

Dilute Washing Buffer 1 + 19; e. g. 10 mL Washing Buffer + 190 mL distilled water. The diluted buffer is stable for 5 days at room temperature (20…25 °C). In case crystals appear in the concentrate, warm up the solution to 37 °C e.g. in a water bath. Mix well before dilution.

6.3. TMB Substrate Solution

The reagent is ready to use and has to be stored at 2...8 °C, away from the light. The solution should be colourless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away.

7. SAMPLE COLLECTION AND PREPARATION

Use human serum or plasma (citrate, heparin) samples with this assay. If the assay is performed within 5 days after sample collection, the samples should be kept at 2...8 °C; otherwise they should be aliquoted and stored deep-frozen (-70…-20 °C). If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing.
Heat inactivation of samples is not recommended.

7.1. Sample Dilution

Before assaying, all samples should be diluted 1+100 with IgG Sample Dilution Buffer. Dispense 10 µL sample and 1 mL IgG Sample Dilution Buffer into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

8. ASSAY PROCEDURE

Please read the instruction for use carefully before performing the assay. Result reliability depends on strict adherence to the instruction for use as described. The following test procedure is only validated for manual procedure. If performing the test on ELISA automatic systems we recommend increasing the washing steps from three up to five and the volume of Washing Buffer from 300 µL to 350 µL to avoid washing effects. Pay attention to chapter 12. Prior to commencing the assay, the distribution and identification plan for all samples and standards/controls (duplicates recommended) should be carefully established on the plate layout supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder.

Perform all assay steps in the order given and without any delays.

A clean, disposable tip should be used for dispensing each standard/control and sample.

Adjust the incubator to 37 ± 1 °C.

1. Dispense 100 µL standards/controls and diluted samples into their respective wells. Leave well A1 for the Substrate Blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 1 hour ± 5 min at 37 ± 1 °C.**
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µL of Washing Buffer. Avoid overflows from the reaction wells. The interval between washing and aspiration should be > 5 sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!

   Note: Washing is important! Insufficient washing results in poor precision and false results.
5. Dispense 100 µL Conjugate into all wells except for the Substrate Blank well A1.
6. **Incubate for 30 min at room temperature(20...25 °C).** Do not expose to direct sunlight.
7. Repeat step 4.
8. Dispense 100 µL TMB Substrate Solution into all wells.
9. **Incubate for exactly 15 min at room temperature (20...25 °C) in the dark.** A blue colour occurs due to an enzymatic reaction.
10. Dispense 100 µL Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution, thereby a colour change from blue to yellow occurs.
11. Measure the absorbance at 450/620 nm within 30 min after addition of the Stop Solution.

8.1. Measurement

Adjust the ELISA Microtiterplate reader **to zero** using the **Substrate Blank**.

If - due to technical reasons - the ELISA Microtiterplate reader cannot be adjusted to zero using the Substrate Blank, subtract its absorbance value from all other absorbance values measured in order to obtain reliable results!

Measure the absorbance of all wells at **450 nm** and record the absorbance values for each standard/control and sample in the plate layout.

Bichromatic measurement using a reference wavelength of 620 nm is recommended.

Where applicable calculate the mean absorbance values of all duplicates.

9. RESULTS

9.1. Run Validation Criteria

In order for an assay run to be considered valid, these Instructions for Use have to be strictly followed and the following criteria must be met:

- **Substrate blank: Absorbance value < 0.100**
- **Standard A: Absorbance value < 0.200**
- **Standard B: Absorbance value > 0.100**
- **Standard C: Absorbance value > 0.500**
- **Standard D: Absorbance value > 1.000**

Standard A < Standard B < Standard C < Standard D

If these criteria are not met, the test is not valid and must be repeated.

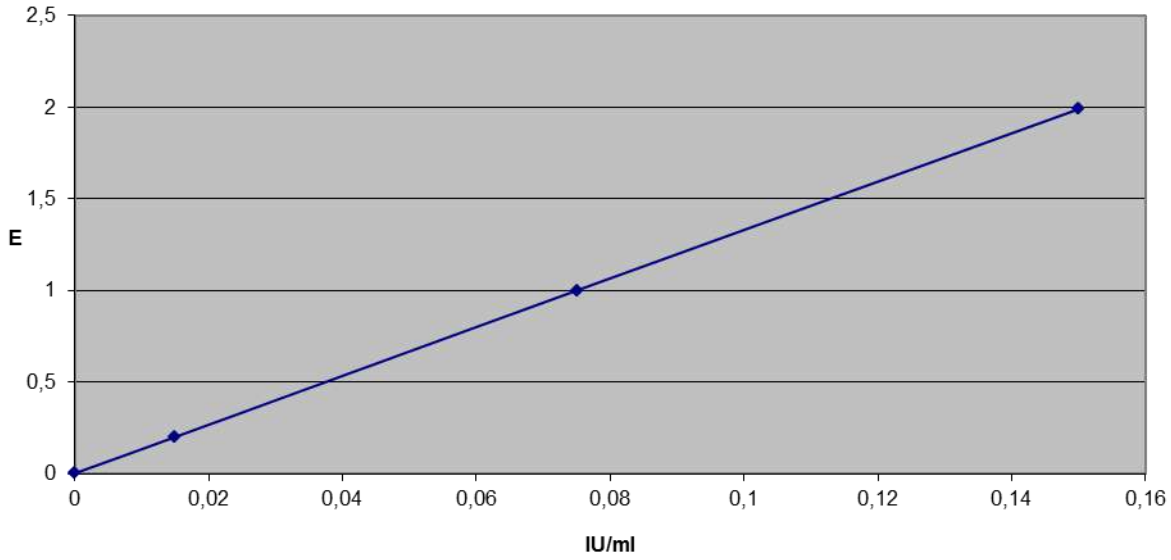
9.2. Calculation of Results

In order to obtain **quantitative results in IU/mL** plot the (mean) absorbance values of the 4 Standards A, B, C and D on (linear/linear) graph paper in a system of coordinates against their corresponding concentrations (0.000, 0.015, 0.075, 0.150 IU/mL) and draw a standard calibration curve (absorbance values on the y-axis, concentrations on the x-axis).

Read results from this standard curve employing the (mean) absorbance values of each patient sample.

For the calculation of the standard-curve mathematical Point to Point function should be used.

9.3. Typical standard Curve



9.4. Interpretation of Results

according to: RKI 1999

< 0.01 IU/mL	No protective antibody level! Immediate full course of basic immunization is recommended!
0.01 - 0.09 IU/mL	No reliable protection! Immediate booster injection is recommended.
0.1 – 1.0 IU/mL	Reliable protection!
> 1.0 IU/mL	Reliable long term protection: After about 10 years after last booster control and booster injection is recommended. It is recommended that the basic immunisation or booster is checked 4-6 weeks after immunisation and to record the data on the certificate of vaccination.

Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as serological data.

In immunocompromised patients and newborns serological data only have restricted value.

10. SPECIFIC PERFORMANCE CHARACTERISTICS

The results refer to the groups of samples investigated; these are not guaranteed specifications.

For further information about the specific performance characteristics please contact NovaTec Immundiagnostica GmbH.

10.1. Precision

Intraassay	n	Mean value (E)	CV (%)
#1	24	1,347	3,85
#2	24	1,843	3,86
#3	24	0,527	3,02

Interassay	n	Mean value (IU/mL)	CV (%)
#1	12	7,83	12,95
#2	12	34,47	6,99
#3	12	35,39	6,86

10.2. Diagnostic Specificity

The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte.
It is 100% (95% confidence interval: 89.42% - 100%).

10.3. Diagnostic Sensitivity

The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte.
It is 100% (95% confidence interval: 95.44% - 100%).

10.4. Analytical Sensitivity

The analytical sensitivity (according to CLSI EP17-A) is defined as the apparent concentration of the analyte that can be distinguished from the zero calibrator. It is 0.00092 IU/mL.

10.5. Interferences

Interferences with hemolytic, lipemic or icteric samples are not observed up to a concentration of 10 mg/mL hemoglobin, 5 mg/mL triglycerides and 0.5 mg/mL bilirubin.

10.6. Cross Reactivity

Investigation of a sample panel with antibody activities to potentially cross-reacting parameters did not reveal evidence of false-positive results due to cross-reactions.

10.7. Measurement range

The measurement range is 0.00092 IU/mL – 0.15 IU/mL.

11. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or repeated freeze-thaw cycles of the sample may affect the absorbance values.

12. PRECAUTIONS AND WARNINGS

- The test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the testkits with analyzers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.
- Only for in-vitro diagnostic use.
- All materials of human or animal origin should be regarded and handled as potentially infectious.
- All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive.
- Do not interchange reagents or Microtiterplates of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- Use only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and standard/control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense reagents without splashing accurately into the wells.
- The ELISA is only designed for qualified personnel following the standards of good laboratory practice (GLP).
- For further internal quality control each laboratory should additionally use known samples.

12.1. Safety note for reagents containing hazardous substances

Reagents may contain CMIT/MIT (3:1) or MIT (refer to 4.1)
Therefore, the following hazard and precautionary statements apply.

Warning

H317	May cause an allergic skin reaction.
P261	Avoid breathing spray
P280	Wear protective gloves/ protective clothing.
P302+P352	IF ON SKIN: Wash with plenty of soap and water.
P333+P313	If skin irritation or rash occurs: Get medical advice/ attention.
P362+P364	Take off contaminated and Wash it before reuse.

Further information can be found in the safety data sheet.

12.2. Disposal Considerations

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

13. ORDERING INFORMATION

Prod. No.: CORG0090 Corynebacterium diphtheriae toxin IgG ELISA (96 Determinations)

NovaTec Immundiagnostica GmbH

NovaLisa®

Echinococcus IgG

ELISA

CE

Only for in-vitro diagnostic use

Product Number: ECHG0130 (96 Determinations)

ENGLISH

1. INTRODUCTION

Echinococci are microscopic cestodes (tapeworms) of 1-6 mm which are dependent on their genus found either in dogs or other canids (E. granulosus) or in foxes, coyotes and wolves (E. multilocularis). In their larval stage they are the causative agent of human echinococcosis (Hydatiosis, or hydatic disease). The adult tapeworms reside in the small bowel of the definitive hosts, and gravid proglottids release eggs that are passed in the feces. After ingestion of a suitable intermediate host, the egg hatches in the small bowel and releases an oncosphere that penetrates the intestinal wall and through the circulatory system into various organs, especially the liver and lungs, where it develops into a cyst. Echinococcus infections remain silent for years before the enlarging cysts cause symptoms in the affected organs (liver, lung, and less commonly other organs as brain, bone, heart). E. granulosus occurs practically worldwide, and more frequently in rural, grazing areas where dogs ingest organs from infected animals. E. multilocularis occurs in the northern hemisphere, including central Europe and the northern parts of Europe, Asia, and North America. Although human cases are rare, infection in humans causes parasitic tumors to form in the liver, the lungs, and less commonly, the brain, and other organs. If left untreated, infection can be fatal.

Species	Disease	Symptoms (e.g.)	Transmission route
E. granulosus	Cystic chinococcosis (Cystic Hydatid Disease, CHD)	(Depends on localization size, and number of cysts) Liver: Upper abdominal pain, hepatomegaly, cholestasis, jaundice, etc.	"hand-to-mouth" transmission. Infection by oral uptake of eggs. e.g.: contaminated wild berries.
E. multilocularis	Alveolar Echinococcosis (Alveolar Hydatid Disease, AHD)	Lungs: Thoracic pains, cough, expectoration, dyspnea, etc. CNS: Neurological symptoms	

Infection or presence of pathogen may be identified by:

- Microscopy
- Serology: e.g by ELISA

2. INTENDED USE

The Echinococcus IgG ELISA is intended for the qualitative determination of IgG class antibodies against Echinococcus in human serum or plasma (citrate, heparin).

3. PRINCIPLE OF THE ASSAY

The qualitative immunoenzymatic determination of specific antibodies is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique.
Microtiterplates are coated with specific antigens to bind corresponding antibodies of the sample. After washing the wells to remove all unbound sample material a horseradish peroxidase (HRP) labelled conjugate is added. This conjugate binds to the captured antibodies. In a second washing step unbound conjugate is removed. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product.
The intensity of this product is proportional to the amount of specific antibodies in the sample. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450/620 nm is read using an ELISA Microtiterplate reader.

4. MATERIALS

4.1. Reagents supplied

- **Microtiterplate:** 12 break-apart 8-well snap-off strips coated with Echinococcus antigens; in resealable aluminium foil.
- **IgG Sample Dilution Buffer:** 1 bottle containing 100 mL of phosphate buffer (10 mM) for sample dilution; pH 7.2 ± 0.2; coloured yellow; ready to use; white cap; ≤ 0.0015% (v/v) CMIT/ MIT (3:1).
- **Stop Solution:** 1 bottle containing 15 mL sulphuric acid, 0.2 mol/L; ready to use; red cap.
- **Washing Buffer (20x conc.):** 1 bottle containing 50 mL of a 20-fold concentrated phosphate buffer (0.2 M), pH 7.2 ± 0.2, for washing the wells; white cap.
- **Conjugate:** 1 bottle containing 20 mL of peroxidase labelled antibody to human IgG in phosphate buffer (10 mM); coloured blue; ready to use; black cap.
- **TMB Substrate Solution:** 1 bottle containing 15 mL 3,3',5,5'-tetramethylbenzidine (TMB), < 0.1 %; ready to use; yellow cap;
- **Positive Control:** 1 vial containing 2 mL control; coloured yellow; ready to use; red cap; ≤ 0.02% (v/v) MIT.
- **Cut-off Control:** 1 vial containing 3 mL control; coloured yellow; ready to use; green cap; ≤ 0.02% (v/v) MIT.
- **Negative Control:** 1 vial containing 2 mL control; coloured yellow; ready to use; blue cap; ≤ 0.0015% (v/v) CMIT/ MIT (3:1).

For hazard and precautionary statements see 12.1
For potential hazardous substances please check the safety data sheet.

4.2. Materials supplied

- 1 Cover foil
- 1 Instruction for use (IFU)
- 1 Plate layout

4.3. Materials and Equipment needed

- ELISA Microtiterplate reader, equipped for the measurement of absorbance at 450/620 nm
- Incubator 37°C
- Manual or automatic equipment for rinsing Microtiterplates
- Pipettes to deliver volumes between 10 and 1000 µL
- Vortex tube mixer
- Distilled water
- Disposable tubes

5. STABILITY AND STORAGE

Store the kit at 2...8 °C. The opened reagents are stable up to the expiry date stated on the label when stored at 2...8 °C.

6. REAGENT PREPARATION

It is very important to bring all reagents and samples to room temperature (20…25 °C) and mix them before starting the test run!

6.1. Microtiterplate

The break-apart snap-off strips are coated with Echinococcus antigens. Immediately after removal of the strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2...8 °C.

6.2. Washing Buffer (20x conc.)

Dilute Washing Buffer 1 + 19; e. g. 10 mL Washing Buffer + 190 mL distilled water. The diluted buffer is stable for 5 days at room temperature (20…25 °C). In case crystals appear in the concentrate, warm up the solution to 37°C e.g. in a water bath. Mix well before dilution.

6.3. TMB Substrate Solution

The reagent is ready to use and has to be stored at 2...8 °C, away from the light. The solution should be colourless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away.

7. SAMPLE COLLECTION AND PREPARATION

Use human serum or plasma (citrate, heparin) samples with this assay. If the assay is performed within 5 days after sample collection, the samples should be kept at 2...8 °C; otherwise they should be aliquoted and stored deep-frozen (-70…-20 °C). If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing.
Heat inactivation of samples is not recommended.

7.1. Sample Dilution

Before assaying, all samples should be diluted 1+100 with IgG Sample Dilution Buffer. Dispense 10 µL sample and 1 mL IgG Sample Dilution Buffer into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

8. ASSAY PROCEDURE

Please read the instruction for use carefully **before** performing the assay. Result reliability depends on strict adherence to the instruction for use as described. The following test procedure is only validated for manual procedure. If performing the test on ELISA automatic systems we recommend increasing the washing steps from three up to five and the volume of Washing Buffer from 300 µL to 350 µL to avoid washing effects. Pay attention to chapter 12. Prior to commencing the assay, the distribution and identification plan for all samples and standards/controls (duplicates recommended) should be carefully established on the plate layout supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder.

Perform all assay steps in the order given and without any delays.

A clean, disposable tip should be used for dispensing each standard/control and sample.

Adjust the incubator to 37 ± 1 °C.

1. Dispense 100 µL standards/controls and diluted samples into their respective wells. Leave well A1 for the Substrate Blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 1 hour ± 5 min at 37 ± 1 °C.**
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µL of Washing Buffer. Avoid overflows from the reaction wells. The interval between washing and aspiration should be > 5 sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!

   Note: Washing is important! Insufficient washing results in poor precision and false results.
5. Dispense 100 µL Conjugate into all wells except for the Substrate Blank well A1.
6. **Incubate for 30 min at room temperature (20...25 °C).** Do not expose to direct sunlight.
7. Repeat step 4.
8. Dispense 100 µL TMB Substrate Solution into all wells.
9. **Incubate for exactly 15 min at room temperature (20...25 °C) in the dark.** A blue colour occurs due to an enzymatic reaction.
10. Dispense 100 µL Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution, thereby a colour change from blue to yellow occurs.
11. Measure the absorbance at 450/620 nm within 30 min after addition of the Stop Solution.

8.1. Measurement

Adjust the ELISA Microtiterplate reader **to zero** using the **Substrate Blank**.

If - due to technical reasons - the ELISA Microtiterplate reader cannot be adjusted to zero using the Substrate Blank, subtract its absorbance value from all other absorbance values measured in order to obtain reliable results!

Measure the absorbance of all wells at **450 nm** and record the absorbance values for each standard/control and sample in the plate layout.

Bichromatic measurement using a reference wavelength of 620 nm is recommended.

Where applicable calculate the mean absorbance values of all duplicates.

9. RESULTS

9.1. Run Validation Criteria

In order for an assay run to be considered valid, these Instructions for Use have to be strictly followed and the following criteria must be met:

- **Substrate Blank**: Absorbance value **< 0.100**
- **Negative Control**: Absorbance value **< 0.200 and < Cut-off**
- **Cut-off Control**: Absorbance value **0.150 – 1.300**
- **Positive Control**: Absorbance value **> Cut-off**

If these criteria are not met, the test is not valid and must be repeated.

9.2. Calculation of Results

The Cut-off is the mean absorbance value of the Cut-off Control determinations.

Example: Absorbance value Cut-off Control 0.44 + absorbance value Cut-off control 0.42 = 0.86 / 2 = 0.43
Cut-off = 0.43

9.2.1. Results in Units [NTU]

$$\frac{\text{Sample (mean) absorbance value} \times 10}{\text{Cut-off}} = \text{[NovaTec Units = NTU]}$$

Example: $\frac{1.591 \times 10}{0.43} = 37$ NTU (Units)

9.3. Interpretation of Results

Cut-off	10 NTU	-
Positive	> 11 NTU	Antibodies against the pathogen are present. There has been a contact with the antigen (pathogen resp. vaccine).
Equivocal	9 – 11 NTU	Antibodies against the pathogen could not be detected clearly. It is recommended to repeat the test with a fresh sample in 2 to 4 weeks. If the result is equivocal again the sample is judged as **negative**.
Negative	< 9 NTU	The sample contains no antibodies against the pathogen. A previous contact with the antigen (pathogen resp. vaccine) is unlikely.
Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as serological data. In immunocompromised patients and newborns serological data only have restricted value.		

10. SPECIFIC PERFORMANCE CHARACTERISTICS

The results refer to the groups of samples investigated; these are not guaranteed specifications.

For further information about the specific performance characteristics please contact NovaTec Immundiagnostica GmbH.

10.1. Precision

Intraassay	n	Mean (E)	CV (%)
#1	24	0.479	8.00
#2	24	0.863	3.43
#3	24	0.657	3.33
Interassay	**n**	**Mean (NTU)**	**CV (%)**
#1	12	17.88	3.87
#2	12	13.28	4.74
#3	12	6.02	6.61

10.2. Diagnostic Specificity

The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte.

It is 98.82% (95% confidence interval: 95.81% - 99.86%).

10.3. Diagnostic Sensitivity

The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte.

It is 97.22% (95% confidence interval: 85.47% - 99.93%).

10.4. Interferences

Interferences with hemolytic, lipemic or icteric samples are not observed up to a concentration of 10 mg/mL hemoglobin, 5 mg/mL triglycerides and 0.5 mg/mL bilirubin.

10.5. Cross Reactivity

Investigation of a sample panel with antibody activities to potentially cross-reacting parameters did not reveal evidence of false-positive results due to cross-reactions.

11. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or repeated freeze-thaw cycles of the sample may affect the absorbance values.

12. PRECAUTIONS AND WARNINGS

- The test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the testkits with analyzers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.
- Only for in-vitro diagnostic use.
- All materials of human or animal origin should be regarded and handled as potentially infectious.
- All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive.
- Do not interchange reagents or Microtiterplates of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- Use only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and standard/control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense reagents without splashing accurately into the wells.
- The ELISA is only designed for qualified personnel following the standards of good laboratory practice (GLP).
- For further internal quality control each laboratory should additionally use known samples.

12.1. Safety note for reagents containing hazardous substances

Reagents may contain CMIT/MIT (3:1) or MIT (refer to 4.1)
Therefore, the following hazard and precautionary statements apply.

Warning	H317	May cause an allergic skin reaction.
	P261	Avoid breathing spray.
	P280	Wear protective gloves/ protective clothing.
	P302+P352	IF ON SKIN: Wash with plenty of soap and water.
	P333+P313	If skin irritation or rash occurs: Get medical advice/ attention.
	P362+P364	Take off contaminated and Wash it before reuse.

Further information can be found in the safety data sheet.

12.2. Disposal Considerations

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

13. ORDERING INFORMATION

Prod. No.: ECHG0130 Echinococcus IgG ELISA (96 Determinations)

NovaTec Immundiagnostica GmbH

NovaLisa®

Measles Virus IgM

ELISA

CE

Only for in-vitro diagnostic use

Product Number: MEAM0330 (96 Determinations)

ENGLISH

1. INTRODUCTION

Measles or morbilli virus belongs to the RNA viruses of the family Paramyxoviridae. The virions are spherical particles of 150-250 nm in diameter consisting of the ribonucleoprotein with helical symmetry and an envelope with spikes containing the strain-specific and hemagglutinating antigens. Morbilli viruses have no neuraminidase activity. Measles is a classic childhood disease. The virus is endemic: at the age of 20 about 90% of the population has had immunological experience with it. Newborns are protected by maternal antibodies for the first 3-4 months of life; the active disease leaves lifelong immunity. The measles virus has a contagiousity index of about 96%, is worldwide distributed, and can be serious. Bacterial superinfection was a serious threat in the preantibiotic era, but the prognosis of uncomplicated measles is now good. CNS complications such as encephalomyelitis (0.1%) which may occur after the acute phase of measles infection subsides, however still have a high mortality (10%). Prognosis of recovery in these patients is poor. Between 10-30% of all cases are fatal; 20-50% develop significant damages. Subacute sclerosing panencephalitis (SSPE) is a rare (1:1000) degenerative disease of the CNS which is thought to be a slow virus infection.

Species	Disease	Symptoms (e.g.)	Transmission route
Measles Virus	Measles	Fever, malaise, productive cough and runny nose, headache, abdominal pain, typical lesions in the mouth (Koplik spots), characteristic exanthema	By air or contact with saliva or nasal secretions
	SSPE (Subacute sclerosing panencephalitis)	Complications: Encephalomyelitis, decrease in intellectual skills that progresses to an almost complete loss of brain function and death	

Infection or presence of pathogen may be identified by:

- PCR
- Serology: e.g. ELISA

2. INTENDED USE

The Measles Virus IgM ELISA is intended for the qualitative determination of IgM class antibodies against Measles Virus in human serum or plasma (citrate, heparin).

3. PRINCIPLE OF THE ASSAY

The qualitative immunoenzymatic determination of specific antibodies is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique.
Microtiterplates are coated with specific antigens to bind corresponding antibodies of the sample. After washing the wells to remove all unbound sample material a horseradish peroxidase (HRP) labelled conjugate is added. This conjugate binds to the captured antibodies. In a second washing step unbound conjugate is removed. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product.
The intensity of this product is proportional to the amount of specific antibodies in the sample. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450/620 nm is read using an ELISA Microtiterplate reader.

4. MATERIALS

4.1. Reagents supplied

- **Microtiterplate (IgM):** 12 break-apart 8-well snap-off strips coated with Measles Virus antigens; in resealable aluminium foil.
- **IgM Sample Dilution Buffer:** 1 bottle containing 100 mL of phosphate buffer (10 mM) for sample dilution; pH 7.2 ± 0.2; anti-human IgG (RF Absorbent); coloured green; ready to use; white cap; ≤ 0.0015% (v/v) CMIT/ MIT (3:1).
- **Stop Solution:** 1 bottle containing 15 mL sulphuric acid, 0.2 mol/L; ready to use; red cap.
- **Washing Buffer (20x conc.):** 1 bottle containing 50 mL of a 20-fold concentrated phosphate buffer (0.2 M), pH 7.2 ± 0.2, for washing the wells; white cap.
- **Conjugate:** 1 bottle containing 20 mL of peroxidase labelled antibody to human IgM in phosphate buffer (10 mM); coloured red; ready to use; black cap.
- **TMB Substrate Solution:** 1 bottle containing 15 mL 3,3',5,5'-tetramethylbenzidine (TMB), < 0.1 %; ready to use; yellow cap.
- **Positive Control:** 1 vial containing 2 mL control; coloured yellow; ready to use; red cap; ≤ 0.02% (v/v) MIT.
- **Cut-off Control:** 1 vial containing 3 mL control; coloured yellow; ready to use; green cap; ≤ 0.02% (v/v) MIT.
- **Negative Control:** 1 vial containing 2 mL control; coloured yellow; ready to use; blue cap; ≤ 0.0015% (v/v) CMIT/ MIT (3:1).

For hazard and precautionary statements see 12.1
For potential hazardous substances please check the safety data sheet.

4.2. Materials supplied

- 1 Cover foil
- 1 Instruction for use (IFU)
- 1 Plate layout

4.3. Materials and Equipment needed

- ELISA Microtiterplate reader, equipped for the measurement of absorbance at 450/620 nm
- Incubator 37 °C
- Manual or automatic equipment for rinsing Microtiterplates
- Pipettes to deliver volumes between 10 and 1000 µL
- Vortex tube mixer
- Distilled water
- Disposable tubes

5. STABILITY AND STORAGE

Store the kit at 2...8 °C. The opened reagents are stable up to the expiry date stated on the label when stored at 2...8 °C.

6. REAGENT PREPARATION

It is very important to bring all reagents and samples to room temperature (20…25 °C) and mix them before starting the test run!

6.1. Microtiterplate

The break-apart snap-off strips are coated with Measles Virus antigens. Immediately after removal of the strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2...8 °C.

6.2. Washing Buffer (20x conc.)

Dilute Washing Buffer 1 + 19; e. g. 10 mL Washing Buffer + 190 mL distilled water. The diluted buffer is stable for 5 days at room temperature (20…25 °C). In case crystals appear in the concentrate, warm up the solution to 37 °C e.g. in a water bath. Mix well before dilution.

6.3. TMB Substrate Solution

The reagent is ready to use and has to be stored at 2...8 °C, away from the light. The solution should be colourless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away.

7. SAMPLE COLLECTION AND PREPARATION

Use human serum or plasma (citrate, heparin) samples with this assay. If the assay is performed within 5 days after sample collection, the samples should be kept at 2...8 °C; otherwise they should be aliquoted and stored deep-frozen (-70…-20 °C). If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing.
Heat inactivation of samples is not recommended.

7.1. Sample Dilution

Before assaying, all samples should be diluted 1+100 with IgM Sample Dilution Buffer. Dispense 10 µL sample and 1 mL IgM Sample Dilution Buffer into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

8. ASSAY PROCEDURE

Please read the instruction for use carefully **before** performing the assay. Result reliability depends on strict adherence to the instruction for use as described. The following test procedure is only validated for manual procedure. If performing the test on ELISA automatic systems we recommend increasing the washing steps from three up to five and the volume of Washing Buffer from 300 µL to 350 µL to avoid washing effects. Pay attention to chapter 12. Prior to commencing the assay, the distribution and identification plan for all samples and standards/controls (duplicates recommended) should be carefully established on the plate layout supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder.

Perform all assay steps in the order given and without any delays.

A clean, disposable tip should be used for dispensing each standard/control and sample.

Adjust the incubator to 37 ± 1 °C.

1. Dispense 100 µL standards/controls and diluted samples into their respective wells. Leave well A1 for the Substrate Blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 1 hour ± 5 min at 37 ± 1 °C.**
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µL of Washing Buffer. Avoid overflows from the reaction wells. The interval between washing and aspiration should be > 5 sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!

   Note: Washing is important! Insufficient washing results in poor precision and false results.
5. Dispense 100 µL Conjugate into all wells except for the Substrate Blank well A1.
6. **Incubate for 30 min at room temperature (20...25 °C).** Do not expose to direct sunlight.
7. Repeat step 4.
8. Dispense 100 µL TMB Substrate Solution into all wells.
9. **Incubate for exactly 15 min at room temperature (20...25 °C) in the dark.** A blue colour occurs due to an enzymatic reaction.
10. Dispense 100 µL Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate, thereby a colour change from blue to yellow occurs.
11. Measure the absorbance at 450/620 nm within 30 min after addition of the Stop Solution.

8.1. Measurement

Adjust the ELISA Microtiterplate reader **to zero** using the **Substrate Blank**.

If - due to technical reasons - the ELISA Microtiterplate reader cannot be adjusted to zero using the Substrate Blank, subtract its absorbance value from all other absorbance values measured in order to obtain reliable results!

Measure the absorbance of all wells at **450 nm** and record the absorbance values for each standard/control and sample in the plate layout.

Bichromatic measurement using a reference wavelength of 620 nm is recommended.

Where applicable calculate the mean absorbance values of all duplicates.

9. RESULTS

9.1. Run Validation Criteria

In order for an assay run to be considered valid, these Instructions for Use have to be strictly followed and the following criteria must be met:

- **Substrate Blank**: Absorbance value **< 0.100**
- **Negative Control**: Absorbance value **< 0.200 and < Cut-off**
- **Cut-off Control**: Absorbance value **0.150 – 1.300**
- **Positive Control**: Absorbance value **> Cut-off**

If these criteria are not met, the test is not valid and must be repeated.

9.2. Calculation of Results

The Cut-off is the mean absorbance value of the Cut-off Control determinations.

Example: Absorbance value Cut-off Control 0.44 + absorbance value Cut-off control 0.42 = 0.86 / 2 = 0.43
Cut-off = 0.43

9.2.1. Results in Units [NTU]

$$\frac{\text{Sample (mean) absorbance value} \times 10}{\text{Cut-off}} = \text{[NovaTec Units = NTU]}$$

Example: $\frac{1.591 \times 10}{0.43} = 37$ NTU (Units)

9.3. Interpretation of Results

Cut-off	10 NTU	-
Positive	> 11 NTU	Antibodies against the pathogen are present. There has been a contact with the antigen (pathogen resp. vaccine).
Equivocal	9 – 11 NTU	Antibodies against the pathogen could not be detected clearly. It is recommended to repeat the test with a fresh sample in 2 to 4 weeks. If the result is equivocal again the sample is judged as **negative**.
Negative	< 9 NTU	The sample contains no antibodies against the pathogen. A previous contact with the antigen (pathogen resp. vaccine) is unlikely.
Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as serological data. In immunocompromised patients and newborns serological data only have restricted value.		

9.3.1. Antibody Isotypes and State of Infection

Serology	Significance
IgM	Characteristic of the primary antibody response High IgM titer with low IgG titer: → suggests a current or very recent infection Rare: → persisting IgM
IgG	Characteristic of the secondary antibody response May persist for several years High IgG titer with low IgM titer: → may indicate a past infection

10. SPECIFIC PERFORMANCE CHARACTERISTICS

The results refer to the groups of samples investigated; these are not guaranteed specifications.

For further information about the specific performance characteristics please contact NovaTec Immundiagnostica GmbH.

10.1. Precision

Intraassay	**n**	**Mean (E)**	**CV (%)**
#1	24	0.417	6.68
#2	24	0.995	2.99
#3	24	1.987	3.20
Interassay	**n**	**Mean (NTU)**	**CV (%)**
#1	12	24.29	4.31
#2	12	52.75	6.65
#3	12	4.70	9.87

10.2. Diagnostic Specificity

The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte.
It is 100% (95% confidence interval: 98.71% - 100%).

10.3. Diagnostic Sensitivity

The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte.
It is 100% (95% confidence interval: 91.19% - 100%).

10.4. Interferences

Interferences with hemolytic, lipemic or icteric samples are not observed up to a concentration of 10 mg/mL hemoglobin, 5 mg/mL triglycerides and 0.5 mg/mL bilirubin.

10.5. Cross Reactivity

Investigation of a sample panel with antibody activities to potentially cross-reacting parameters did not reveal evidence of false-positive results due to cross-reactions.

11. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or repeated freeze-thaw cycles of the sample may affect the absorbance values.

12. PRECAUTIONS AND WARNINGS

- The test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the testkits with analyzers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.
- Only for in-vitro diagnostic use.
- All materials of human or animal origin should be regarded and handled as potentially infectious.
- All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive.
- Do not interchange reagents or Microtiterplates of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- Use only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and standard/control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense reagents without splashing accurately into the wells.
- The ELISA is only designed for qualified personnel following the standards of good laboratory practice (GLP).
- For further internal quality control each laboratory should additionally use known samples.

12.1. Safety note for reagents containing hazardous substances

Reagents may contain CMIT/MIT (3:1) or MIT (refer to 4.1)
Therefore, the following hazard and precautionary statements apply.

Warning		
	H317	May cause an allergic skin reaction.
	P261	Avoid breathing spray
	P280	Wear protective gloves/ protective clothing.
	P302+P352	IF ON SKIN: Wash with plenty of soap and water.
	P333+P313	If skin irritation or rash occurs: Get medical advice/ attention.
	P362+P364	Take off contaminated and Wash it before reuse.

Further information can be found in the safety data sheet.

12.2. Disposal Considerations

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

13. ORDERING INFORMATION

Prod. No.: MEAM0330 Measles Virus IgM ELISA (96 Determinations)

DEUTSCH

1. EINLEITUNG

Das Masern- oder Morbillivirus ist ein RNA-Virus der Familie der Paramyxoviridae. Masernviren sind antigenisch stabil und bilden einen Serotyp. Bei Untersuchungen mit Hilfe molekularbiologischer Methoden werden mehrere Genotypen unterschieden (in Mitteleuropa kommen gegenwärtig C2 und D6 vor). Das Masernvirus ist sehr empfindlich gegenüber äußeren Einflüssen wie erhöhten Temperaturen, Licht, UV-Strahlen, Fettlösungs- und Desinfektionsmitteln. Masernvirusinfektionen treten typischerweise in der Kindheit auf. Der einzige Wirt für das hochkontagiöse Virus ist der Mensch. Die Übertragung erfolgt aerogen durch Tröpfcheninfektion und führt bereits bei kurzer Exposition nicht-immuner Personen zur Erkrankung (Kontagionsindex nahe 100%).

Das Virus ist sehr lymphotrop und verursacht nach Replikation in den lymphatischen Geweben eine transiente Lymphopenie mit begleitender Immunsuppression. Nach hämatogener Aussaat erreicht das Virus die Haut und die oberen Atemwege. Die einsetzende zelluläre Immunantwort führt schließlich zur Ausbildung des typischen Exanthems. Nach einer Inkubationszeit von etwa 2 Wochen entwickelt sich eine unspezifische katarrhalische Symptomatik. Es entwickelt sich eine Entzündung der oberen Atemwege (Rhinitis, Pharyngitis, Laryngitis, Tracheitis, Bronchitis), sowie häufig auch eine Konjunktivitis. Pneumonien sind möglich, jedoch selten. Unter Fieberanstieg bis 41°C entsteht schließlich das makulopapulöse Masernexanthem (beginnend am Kopf mit kraniokaudaler Ausbreitung). Besonders komplikationsreich sind die Masernenzephalitiden, die nach einer akuten Infektion auftreten können. Man unterscheidet drei Verlaufsformen:

die akute postinfektiöse Form (bei etwa 10-20 % der Betroffenen endet sie tödlich, bei etwa 20-30 % muss mit Residualschäden am ZNS gerechnet werden)

die akute, progressive Form (Auftreten gilt als infaust; Komplikation bei Patienten mit eingeschränkter Immunkompetenz) und

die subakute sklerosierende Panenzephalitis (SSPE) als sehr seltene Spätkomplikation (1-5 Fälle pro 1 Mio. Erkr.), die sich nach durchschnittlich 6-8 Jahren manifestiert. Beginnend mit psychischen und intellektuellen Veränderungen entwickelt sich ein progredienter Verlauf mit neurologischen Störungen und Ausfällen bis zum Verlust zerebraler Funktionen. Die Prognose ist stets infaust.

Eine Masernerkrankung kann vorübergehend die zelluläre Immunität so unterdrücken, dass eine Tuberkulose exazerbiert.

Zur Prophylaxe steht ein Lebendimpfstoff, sowohl als Monopräparat als auch als Kombinationsimpfstoff, zur Verfügung.

Spezies	**Erkrankung**	**Symptome (z.B.)**	**Infektionsweg**
Masernvirus (Morbillivirus) **(auf English Measles Virus)**	Masern	Fieber, Malaise, produktive Husten und laufende Nase, Kopfschmerzen, Bauchschmerzen, Koplik-Flecken (kalkspritzerartige, weiße Flecken in der Wangenschleimhaut), charakteristisches Exanthem	aerogen durch Tröpfchen oder Kontakt mit Speichel und Nasensekret
	Subakute sklerosierende Panenzephalitis	Komplikationen: Enzephalomyelitis, Abnahme der intellektuellen Fähigkeiten bis zu einem fast vollständigen Verlust der Hirnfunktion und Tod	

Nachweis des Erregers bzw. der Infektion durch:

- PCR
- Serologie: z.B. ELISA

2. VERWENDUNGSZWECK

Der Measles Virus IgM ELISA ist für den qualitativen Nachweis spezifischer IgM-Antikörper gegen Measles Virus **(Masernviren)** in humanem Serum oder Plasma (Citrat, Heparin) bestimmt.

3. TESTPRINZIP

Die qualitative immunenzymatische Bestimmung von spezifischen Antikörpern beruht auf der ELISA (Enzyme-linked Immunosorbent Assay) Technik.
Die Mikrotiterplatten sind mit spezifischen Antigenen beschichtet, an welche die korrespondierenden Antikörper aus der Probe binden. Ungebundenes Probenmaterial wird durch Waschen entfernt. Anschließend erfolgt die Zugabe eines Meerettich-Peroxidase (HRP) Konjugates. Dieses Konjugat bindet an die an der Mikrotiterplatte gebundenen spezifischen Antikörper. In einem zweiten Waschschritt wird ungebundenes Konjugat entfernt. Die Immunkomplexe, die durch die Bindung des Konjugates entstanden sind, werden durch die Zugabe von Tetramethylbenzidin (TMB)-Substratlösung und eine resultierende Blaufärbung nachgewiesen.
Die Intensität des Reaktionsproduktes ist proportional zur Menge der spezifischen Antikörper in der Probe. Die Reaktion wird mit Schwefelsäure gestoppt, wodurch ein Farbumschlag von blau nach gelb erfolgt. Die Absorption wird bei 450/620 nm mit einem Mikrotiterplatten-Photometer gemessen.

NovaTec Immundiagnostica GmbH

NovaLisa®

Measles Virus IgG

ELISA

CE

Only for in-vitro diagnostic use

Product Number: MEAG0330 (96 Determinations)

ENGLISH

1. INTRODUCTION

Measles or morbilli virus belongs to the RNA viruses of the family Paramyxoviridae. The virions are spherical particles of 150-250 nm in diameter consisting of the ribonucleoprotein with helical symmetry and an envelope with spikes containing the strain-specific and hemagglutinating antigens. Morbilli viruses have no neuraminidase activity. Measles is a classic childhood disease. The virus is endemic: at the age of 20 about 90% of the population has had immunological experience with it. Newborns are protected by maternal antibodies for the first 3-4 months of life; the active disease leaves lifelong immunity. The measles virus has a contagiousity index of about 96%, is worldwide distributed, and can be serious. Bacterial superinfection was a serious threat in the preantibiotic era, but the prognosis of uncomplicated measles is now good. CNS complications such as encephalomyelitis (0.1%) which may occur after the acute phase of measles infection subsides, however still have a high mortality (10%). Prognosis of recovery in these patients is poor. Between 10-30% of all cases are fatal; 20-50% develop significant damages. Subacute sclerosing panencephalitis (SSPE) is a rare (1:1000) degenerative disease of the CNS which is thought to be a slow virus infection.

Species	Disease	Symptoms (e.g.)	Transmission route
Measles Virus	Measles	Fever, malaise, productive cough and runny nose, headache, abdominal pain, typical lesions in the mouth (Koplik spots),characteristic exanthema	By air or contact with saliva or nasal secretions
	SSPE (Subacute sclerosing panencephalitis)	Complications: Encephalomyelitis, decrease in intellectual skills that progresses to an almost complete loss of brain function and death	

Infection or presence of pathogen may be identified by:

- PCR
- Serology: e.g. by ELISA

2. INTENDED USE

The Measles Virus IgG ELISA is intended for the qualitative determination of IgG class antibodies against Measles Virus in human serum or plasma (citrate, heparin).
Measles Virus IgG avidity can be determined with assay Avidity Measles Virus IgG (Product code: AMEA7330).

3. PRINCIPLE OF THE ASSAY

The qualitative immunoenzymatic determination of specific antibodies is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique.
Microtiterplates are coated with specific antigens to bind corresponding antibodies of the sample. After washing the wells to remove all unbound sample material a horseradish peroxidase (HRP) labelled conjugate is added. This conjugate binds to the captured antibodies. In a second washing step unbound conjugate is removed. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product.
The intensity of this product is proportional to the amount of specific antibodies in the sample. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450/620 nm is read using an ELISA Microtiterplate reader.

4. MATERIALS

4.1. Reagents supplied

- **Microtiterplate:** 12 break-apart 8-well snap-off strips coated with Measles Virus antigens; in resealable aluminium foil.
- **IgG Sample Dilution Buffer:** 1 bottle containing 100 mL of phosphate buffer (10 mM) for sample dilution; pH 7.2 ± 0.2; coloured yellow; ready to use; white cap; ≤ 0.0015% (v/v) CMIT/ MIT (3:1).
- **Stop Solution:** 1 bottle containing 15 mL sulphuric acid, 0.2 mol/L; ready to use; red cap.
- **Washing Buffer (20x conc.):** 1 bottle containing 50 mL of a 20-fold concentrated phosphate buffer (0.2 M), pH 7.2 ± 0.2, for washing the wells; white cap.
- **Conjugate:** 1 bottle containing 20 mL of peroxidase labelled antibody to human in phosphate buffer (10 mM); coloured blue; ready to use; black cap.
- **TMB Substrate Solution:** 1 bottle containing 15 mL 3,3',5,5'-tetramethylbenzidine (TMB), < 0.1 %; ready to use; yellow cap.
- **Positive Control:** 1 vial containing 2 mL control; coloured yellow; ready to use; red cap; ≤ 0.02% (v/v) MIT.
- **Cut-off Control:** 1 vial containing 3 mL control; coloured yellow; ready to use; green cap; ≤ 0.02% (v/v) MIT.
- **Negative Control:** 1 vial containing 2 mL control; coloured yellow; ready to use; blue cap; ≤ 0.0015% (v/v) CMIT/ MIT (3:1).

For hazard and precautionary statements see 12.1
For potential hazardous substances please check the safety data sheet.

4.2. Materials supplied

- 1 Cover foil
- 1 Instruction for use (IFU)
- 1 Plate layout

4.3. Materials and Equipment needed

- ELISA Microtiterplate reader, equipped for the measurement of absorbance at 450/620 nm
- Incubator 37 °C
- Manual or automatic equipment for rinsing Microtiterplate
- Pipettes to deliver volumes between 10 and 1000 µL
- Vortex tube mixer
- Distilled water
- Disposable tubes

5. STABILITY AND STORAGE

Store the kit at 2...8 °C. The opened reagents are stable up to the expiry date stated on the label when stored at 2...8 °C.

6. REAGENT PREPARATION

It is very important to bring all reagents and samples to room temperature (20…25 °C) and mix them before starting the test run!

6.1. Microtiterplate

The break-apart snap-off strips are coated with Measles Virus antigens. Immediately after removal of the strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2...8 °C.

6.2. Washing Buffer (20x conc.)

Dilute Washing Buffer 1 + 19; e. g. 10 mL Washing Buffer + 190 mL distilled water. The diluted buffer is stable for 5 days at room temperature (20…25 °C). In case crystals appear in the concentrate, warm up the solution to 37 °C e.g. in a water bath. Mix well before dilution.

6.3. TMB Substrate Solution

The reagent is ready to use and has to be stored at 2...8 °C, away from the light. The solution should be colourless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away.

7. SAMPLE COLLECTION AND PREPARATION

Use human serum or plasma (citrate, heparin) samples with this assay. If the assay is performed within 5 days after sample collection, the samples should be kept at 2...8 °C; otherwise they should be aliquoted and stored deep-frozen (-70…-20 °C). If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing.
Heat inactivation of samples is not recommended.

7.1. Sample Dilution

Before assaying, all samples should be diluted 1+100 with IgG Sample Dilution Buffer. Dispense 10 µL sample and 1 mL IgG Sample Dilution Buffer into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

8. ASSAY PROCEDURE

Please read the instruction for use carefully **before** performing the assay. Result reliability depends on strict adherence to the instruction for use as described. The following test procedure is only validated for manual procedure. If performing the test on ELISA automatic systems we recommend increasing the washing steps from three up to five and the volume of Washing Buffer from 300 µL to 350 µL to avoid washing effects. Pay attention to chapter 12. Prior to commencing the assay, the distribution and identification plan for all samples and standards/controls (duplicates recommended) should be carefully established on the plate layout supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder.

Perform all assay steps in the order given and without any delays.

A clean, disposable tip should be used for dispensing each standard/control and sample.

Adjust the incubator to 37 ± 1 °C.

1. Dispense 100 µL standards/controls and diluted samples into their respective wells. Leave well A1 for the Substrate Blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 1 hour ± 5 min at 37 ± 1 °C.**
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µL of Washing Buffer. Avoid overflows from the reaction wells. The interval between washing and aspiration should be > 5 sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!

   Note: Washing is important! Insufficient washing results in poor precision and false results.
5. Dispense 100 µL Conjugate into all wells except for the Substrate Blank well A1.
6. **Incubate for 30 min at room temperature (20...25 °C).** Do not expose to direct sunlight.
7. Repeat step 4.
8. Dispense 100 µL TMB Substrate Solution into all wells.
9. **Incubate for exactly 15 min at room temperature (20...25 °C) in the dark.** A blue colour occurs due to an enzymatic reaction.
10. Dispense 100 µL Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution, thereby a colour change from blue to yellow occurs.
11. Measure the absorbance at 450/620 nm within 30 min after addition of the Stop Solution.

8.1. Measurement

Adjust the ELISA Microtiterplate reader **to zero** using the **Substrate Blank**.

If - due to technical reasons - the ELISA Microtiterplate reader cannot be adjusted to zero using the Substrate Blank, subtract its absorbance value from all other absorbance values measured in order to obtain reliable results!

Measure the absorbance of all wells at **450 nm** and record the absorbance values for each standard/control and sample in the plate layout.

Bichromatic measurement using a reference wavelength of 620 nm is recommended.

Where applicable calculate the mean absorbance values of all duplicates.

9. RESULTS

9.1. Run Validation Criteria

In order for an assay run to be considered valid, these Instructions for Use have to be strictly followed and the following criteria must be met:

- **Substrate Blank**: Absorbance value **< 0.100**
- **Negative Control**: Absorbance value **< 0.200 and < Cut-off**
- **Cut-off Control**: Absorbance value **0.150 – 1.300**
- **Positive Control**: Absorbance value **> Cut-off**

If these criteria are not met, the test is not valid and must be repeated.

9.2. Calculation of Results

The Cut-off is the mean absorbance value of the Cut-off Control determinations.

Example: Absorbance value Cut-off Control 0.44 + absorbance value Cut-off control 0.42 = 0.86 / 2 = 0.43

Cut-off = 0.43

9.2.1. Results in Units [NTU]

$$\frac{\text{Sample (mean) absorbance value} \times 10}{\text{Cut-off}} = \text{[NovaTec Units = NTU]}$$

Example: $\frac{1.591 \times 10}{0.43} = 37$ NTU (Units)

9.3. Interpretation of Results

	NTU	**mIU/mL** (3rd International Standard)	
Cut-off	10	-	-
Positive	> 11	> 220	Antibodies against the pathogen are present. There has been a contact with the antigen (pathogen resp. vaccine).
Equivocal	9 – 11	120 – 220	Antibodies against the pathogen could not be detected clearly. It is recommended to repeat the test with a fresh sample in 2 to 4 weeks. If the result is equivocal again the sample is judged as **negative**.
Negative	< 9	< 120	The sample contains no antibodies against the pathogen. A previous contact with the antigen (pathogen resp. vaccine) is unlikely.
Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as serological data. In immunocompromised patients and newborns serological data only have restricted value.			

9.3.1. Antibody Isotypes and State of Infection

Serology	Significance
IgM	Characteristic of the primary antibody response High IgM titer with low IgG titer: → suggests a current or very recent infection Rare: → persisting IgM
IgG	Characteristic of the secondary antibody response May persist for several years High IgG titer with low IgM titer: → may indicate a past infection

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The results refer to the groups of samples investigated; these are not guaranteed specifications.

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10.1. Precision

Intraassay	**n**	**Mean (E)**	**CV (%)**
#1	24	0.314	6.93
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#3	24	0.718	9.98
Interassay	**n**	**Mean (NTU)**	**CV (%)**
#1	12	33.93	2.76
#2	12	28.09	7.46
#3	12	3.85	12.11

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The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte.
It is 100% (95% confidence interval: 90.0% - 100%).

10.3. Diagnostic Sensitivity

The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte.
It is 97.01% (95% confidence interval: 93.93% - 98.79%).

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Interferences with hemolytic, lipemic or icteric samples are not observed up to a concentration of 10 mg/mL hemoglobin, 5 mg/mL triglycerides and 0.5 mg/mL bilirubin.

10.5. Cross Reactivity

Investigation of a sample panel with antibody activities to potentially cross-reacting parameters did not reveal evidence of false-positive results due to cross-reactions.

11. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or repeated freeze-thaw cycles of the sample may affect the absorbance values.

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- All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive.
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Further information can be found in the safety data sheet.

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13. ORDERING INFORMATION

Prod. No.:	MEAG0330	Measles Virus IgG ELISA (96 Determinations)

For avidity testing:

AMEA7330	Avidity Measles Virus IgG ELISA (48 Determinations)

DEUTSCH

1. EINLEITUNG

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- die akute, progressive Form (Auftreten gilt als infaust; Komplikation bei Patienten mit eingeschränkter Immunkompetenz) und
- die subakute sklerosierende Panenzephalitis (SSPE) als sehr seltene Spätkomplikation (1-5 Fälle pro 1 Mio. Erkr.), die sich nach durchschnittlich 6-8 Jahren manifestiert. Beginnend mit psychischen und intellektuellen Veränderungen entwickelt sich ein progredienter Verlauf mit neurologischen Störungen und Ausfällen bis zum Verlust zerebraler Funktionen. Die Prognose ist stets infaust.

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Nachweis des Erregers bzw. der Infektion durch:

- PCR
- Serologie: z.B. ELISA

2. VERWENDUNGSZWECK

Der Measles Virus IgG ELISA ist für den qualitativen Nachweis spezifischer IgG-Antikörper gegen Measles Virus **(Masernviren)** in humanem Serum oder Plasma (Citrat, Heparin) bestimmt.
Die Masernvirus IgG Avidität kann bestimmt werden mit dem Test: Avidity Measles Virus IgG (Produktnummer: AMEA7330).

3. TESTPRINZIP

Die qualitative immunenzymatische Bestimmung von spezifischen Antikörpern beruht auf der ELISA (Enzyme-linked Immunosorbent Assay) Technik.
Die Mikrotiterplatten sind mit spezifischen Antigenen beschichtet, an welche die korrespondierenden Antikörper aus der Probe binden. Ungebundenes Probenmaterial wird durch Waschen entfernt. Anschließend erfolgt die Zugabe eines Meerettich-Peroxidase (HRP) Konjugates. Dieses Konjugat bindet an die an der Mikrotiterplatte gebundenen spezifischen Antikörper. In einem zweiten Waschschritt wird ungebundenes Konjugat entfernt. Die Immunkomplexe, die durch die Bindung des Konjugates entstanden sind, werden durch die Zugabe von Tetramethylbenzidin (TMB)-Substratlösung und eine resultierende Blaufärbung nachgewiesen.
Die Intensität des Reaktionsproduktes ist proportional zur Menge der spezifischen Antikörper in der Probe. Die Reaktion wird mit Schwefelsäure gestoppt, wodurch ein Farbumschlag von blau nach gelb erfolgt. Die Absorption wird bei 450/620 nm mit einem Mikrotiterplatten-Photometer gemessen.

NovaTec Immundiagnostica GmbH

NovaLisa®

Avidity Measles Virus IgG

ELISA Supplementary Instruction

CE

Only for in-vitro diagnostic use

Product Number: AMEA7330 (48 Determinations)

ENGLISH

1. INTRODUCTION

The presence of IgG antibodies to Measles virus indicates the occurrence of the infection but does not distinguish between recent and past infection. Specific IgM antibodies are first detected approximately in ten days and peak at about four weeks post infection. They may persist for several months after acute infections. Based on the evidence that antibody avidity gradually increases after exposure to an immunogen, avidity of IgG antibodies can be used as a marker for distinguishing recent primary from long-term infections. Avidity describes the binding strength of a specific antibody to its antigen. Low-avidity IgG antibodies indicate a primary infection, whereas the presence of IgG antibodies with high avidity points to persistency or reactivation of infection.

2. INTENDED USE

The Avidity Measles Virus IgG ELISA is intended to indicate the Measles-specific IgG avidity in human serum or plasma (citrate, heparin) to differentiate between acute and past infection.

3. PRINCIPLE OF THE ASSAY

The qualitative immunoenzymatic determination of specific antibodies is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique.
Microplates are coated with specific antigens to bind corresponding antibodies of the sample (dual pipetting). After washing the wells to remove all unbound sample material, one well is incubated with avidity reagent and the corresponding well with washing buffer. The avidity reagent removes the low-avidity antibodies from the antigens whereas the high-avidity ones are still bound to the specific antigens. After second washing step to remove the rest of avidity reagent and low-avidity antibodies, a horseradish peroxidase (HRP) labelled conjugate is added. This conjugate binds to the captured antibodies. In a third washing step unbound conjugate is removed. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product.
The intensity of this product is proportional to the amount of specific antibodies in the sample. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450/620 nm is read using an ELISA microwell plate reader.

4. MATERIALS

4.1. Reagents supplied

- **Avidity Reagent:** 1 bottle containing 15 mL of an urea solution, coloured blue, ready to use; black cap.
- **Control Low:** 1 vial containing 2 mL control; coloured yellow; ready to use; blue cap; ≤ 0.02% (v/v) MIT.
- **Control High:** 1 vial containing 2 mL control; coloured yellow; ready to use; red cap; ≤ 0.02% (v/v) MIT.

For hazard and precautionary statements see 12.1
For potential hazardous substances please check the safety data sheet.

4.2. Materials supplied

- 1 instruction for use Avidity Measles Virus IgG ELISA (Product Number: AMEA7330)
- 1 Instruction for use Measles Virus IgG ELISA (Product Number: MEAG0330)
- 1 empty labelled bottle (white with white cap) for ready to use Washing Buffer

5. STABILITY AND STORAGE

Store the kit at 2...8 °C. The opened reagents are stable up to the expiry date stated on the label when stored at 2...8 °C.

6. REAGENT PREPARATION

It is very important to bring all reagents and samples to room temperature (20…25 °C) and mix them before starting the test run!

6.1. Avidity Reagent

If crystals have formed in the reagent warm up to 37 °C e.g. in a water bath and mix gently until they disappear.

6.2. Washing Buffer

It is recommended to fill 15 mL ready to use Washing Buffer into supplied bottle (s. 4.2) to use it in step Step 5 of the test preparation.
Note: Ready to use Washing Buffer is stable for 5 days at room temperature (20…25 °C).

7. SPECIMEN COLLECTION AND PREPARATION

Use human serum or plasma (citrate, heparin) **Measles Virus IgG positive** samples with this assay.
Note: For samples with high absorbance values (OD > 2.000) appropriate higher dilutions should be used.

7.1. Sample Dilution

Before assaying, all samples should be diluted 1+100 with IgG Sample Dilution Buffer. Dispense 10 µL sample and 1 mL IgG Sample Dilution Buffer into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

8. ASSAY PROCEDURE

Please read the instruction for use carefully **before** performing the assay. Result reliability depends on strict adherence to the instruction for use as described. The following test procedure is only validated for manual procedure. If performing the test on ELISA automatic systems we recommend increasing the washing steps from three up to five and the volume of Washing Buffer from 300 µL to 350 µL to avoid washing effects. Pay attention to chapter 12. Prior to commencing the assay, the distribution and identification plan for all samples and standards/controls (duplicates recommended) should be carefully established on the plate layout supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder.

Perform all assay steps in the order given and without any delays.

A clean, disposable tip should be used for dispensing each standard/control and sample.

Adjust the incubator to 37 ± 1 °C.

For avidity determination dual pipetting of standards/controls and diluted samples is needed.

1. Dispense 100 µL standards/controls and diluted samples into their respective wells. Leave wells A1/A2 for the Substrate Blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 1 hour ± 5 min at 37 ± 1 °C.**
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µL of Washing Buffer. Avoid overflows from the reaction wells. The interval between washing and aspiration should be > 5 sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!

   Note: Washing is important! Insufficient washing results in poor precision and false results.
5. Dispense 100 µL of Avidity Reagent in wells B1, C1, D1, E1 etc, except for the Substrate Blank well A1.

   Dispense 100 µL of Washing Buffer in wells B2, C2, D2, E2 etc, except for the Substrate Blank well A2.
6. **Incubate for exactly 10 min at 37 ± 1 °C.**
7. Repeat step 4.
8. Dispense 100 µL Conjugate into all wells except in the blank wells (A1/A2).
9. **Incubate for 30 min at room temperature (20...25 °C).** Do not expose to direct sunlight.
10. Repeat step 4.
11. Dispense 100 µL TMB Substrate Solution into all wells.
12. **Incubate for exactly 15 min at room temperature (20...25 °C) in the dark.** A blue colour occurs due to an enzymatic reaction.
13. Dispense 100 µL Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate, thereby a colour change from blue to yellow occurs.
14. Measure the absorbance at 450/620 nm within 30 min after addition of the Stop Solution.

8.1. Measurement

Adjust the ELISA Microtiterplate reader **to zero** using the **Substrate Blank**.

If - due to technical reasons - the ELISA Microtiterplate reader cannot be adjusted to zero using the Substrate Blank, subtract its absorbance value from all other absorbance values measured in order to obtain reliable results!

Measure the absorbance of all wells at **450 nm** and record the absorbance values for each standard/control and sample in the plate layout.

Bichromatic measurement using a reference wavelength of 620 nm is recommended.

Where applicable calculate the mean absorbance values of all duplicates.

9. RESULTS

9.1. Run Validation Criteria

In order for an assay run to be considered valid, these Instructions for Use have to be strictly followed and the following criteria must be met:

- **Substrate Blank**: Absorbance value **< 0.100**
- **Control Low**: Avidity (%): **< 45 %**
- **Control High**: Avidity (%): **> 55 %**

If these criteria are not met, the test is not valid and must be repeated.

9.2. Calculation of Results

For each patient sample or control calculate the ratio between the absorbance of the well dispensed with Avidity Reagent and the absorbance of the well dispensed with Washing Buffer multiplied by 100:

$$\frac{\text{Absorbance (sample or control) Avidity Reagent}}{\text{Absorbance (sample or control) Washing Buffer (diluted 1+19)}} \times 100 = \text{Avidity (\%)}$$

Note: For samples with high absorbance values (OD > 2.000) appropriate higher dilutions should be used.

9.3. Interpretation of Results

Result	Avidity	Interpretation
Low-avidity IgG	< 45 %	An avidity index of less than 45 % indicates a primary infection acquired within the past 2 months.
Equivocal	45 – 55 %	No clinical interpretation can be deduced from an equivocal result. It is recommended to take a second sample within an appropriate period of time (e.g. 2 weeks) and repeat testing. If the result of the repeated test is still equivocal, precise statements regarding the time of infection cannot be made.
High-avidity IgG	> 55 %	The presence of high-avidity IgG indicates a past infection or reinfection.
Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as serological data. In immunocompromised patients and newborns serological data only have restricted value. A result of high avidity can not exclude the possibility of a recent infection.		

9.3.1. Antibody Isotypes and State of Infection

IgG	IgM	IgG-Avidity	Probable result
+	-	low	Vague, further investigation necessary
+	-	high	Indicatives of a past infection
+	+	low	Suggests a current or very recent infection
+	+	high	Suggests a past infection with persisting IgM or reactivation of infection

10. SPECIFIC PERFORMANCE CHARACTERISTICS

The results refer to the groups of samples investigated; these are not guaranteed specifications.
For further information about the specific performance characteristics please contact NovaTec Immundiagnostica GmbH.

10.1. Diagnostic Performance

The evaluation of the diagnostic performance of the Avidity Measles Virus IgG test was performed in comparison to well defined samples. The resulting relative agreement was 100 %.

11. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or repeated freeze-thaw cycles of the sample may affect the absorbance values.

12. PRECAUTIONS AND WARNINGS

- The test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the testkits with analyzers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.
- Only for in-vitro diagnostic use.
- All materials of human or animal origin should be regarded and handled as potentially infectious.
- All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive.
- Do not interchange reagents or Microtiterplates of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- Use only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and standard/control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense reagents without splashing accurately into the wells.
- The ELISA is only designed for qualified personnel following the standards of good laboratory practice (GLP).
- For further internal quality control each laboratory should additionally use known samples.

12.1. Safety note for reagents containing hazardous substances

Reagents may contain CMIT/MIT (3:1) or MIT (refer to 4.1)
Therefore, the following hazard and precautionary statements apply.

Warning	H317	May cause an allergic skin reaction.
	P261	Avoid breathing spray.
	P280	Wear protective gloves/ protective clothing.
	P302+P352	IF ON SKIN: Wash with plenty of soap and water.
	P333+P313	If skin irritation or rash occurs: Get medical advice/ attention.
	P362+P364	Take off contaminated and Wash it before reuse.

Further information can be found in the safety data sheet.

12.2. Disposal Considerations

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

13. ORDERING INFORMATION

Prod. No.: AMEA7330 Avidity Measles Virus IgG (48 Determinations)

DEUTSCH

1. EINLEITUNG

Obwohl die Anwesenheit von IgG-Antikörpern gegen das Masernvirus auf das Vorliegen einer Infektion hindeutet, kann man nicht zwischen einer akuten und einer bereits abgelaufenen Infektion unterscheiden. Virus-spezifische IgM-Antikörper sind ca. zehn Tage nach der Infektion erstmals nachweisbar und erreichen nach ungefähr vier Wochen ihre höchste Konzentration. Sie können für mehrere Monate nach der akuten Infektion im Blut persistent sein. Aufgrund der Tatsache, dass die Antikörper-Avidität nach der Exposition zum Antigen allmählich zunimmt, kann die Avidität von IgG Antikörpern als Marker benutzt werden, um zwischen einer akuten oder einer bereits länger zurückliegenden Infektion zu unterscheiden. Avidität beschreibt die Bindungsstärke der spezifischen Antikörper an das Antigen. Niedrig-avide IgG-Antikörper deuten auf eine frische Infektion hin, während hoch-avide IgG Antikörper ein Hinweis auf eine länger zurückliegende Infektion oder eine Reaktivierung sind.

2. VERWENDUNGSZWECK

Der Avidity Measles Virus IgG ELISA ist für die Aviditätsbestimmung der spezifischen IgG-Antikörper gegen das Masernvirus in humanem Serum oder Plasma (Citrat, Heparin), zur Differenzierung zwischen akuten und zurückliegenden Infektionen bestimmt.

3. TESTPRINZIP

Die qualitative immunenzymatische Bestimmung von spezifischen Antikörpern beruht auf der ELISA (Enzyme-linked Immunosorbent Assay) Technik.
Die Mikrotiterplatten sind mit spezifischen Antigenen beschichtet, an welche die korrespondierenden Antikörper aus der Probe (zweifaches Pipettieren) binden. Nach dem Waschen, wodurch das ungebundene Probenmaterial entfernt wird, wird eine Vertiefung mit dem Aviditätsreagenz und die andere dazugehörige Vertiefung mit dem Waschpuffer inkubiert. Durch das Aviditätsreagenz wird die Bindung zwischen den niedrig-aviden Antikörpern und den Antigenen gelöst, während die hoch-aviden Antikörper noch an den spezifischen Antigenen gebunden bleiben. Nach dem zweiten Waschschritt werden die Reste des Aviditätsreagenzes sowie niedrig-avide Antikörper entfernt. Anschließend erfolgt die Zugabe eines Meerettich-Peroxidase (HRP) Konjugates. Dieses Konjugat bindet an die an der Mikrotiterplatte gebundenen spezifischen Antikörper. In einem dritten Waschschritt wird ungebundenes Konjugat entfernt. Die Immunkomplexe, die durch die Bindung des Konjugates entstanden sind, werden durch die Zugabe von Tetramethylbenzidin (TMB)-Substratlösung und eine resultierende Blaufärbung nachgewiesen.
Die Intensität des Reaktionsproduktes ist proportional zur Menge der spezifischen Antikörper in der Probe. Die Reaktion wird mit Schwefelsäure gestoppt, wodurch ein Farbumschlag von blau nach gelb erfolgt. Die Absorption wird bei 450/620 nm mit einem Mikrotiterplatten-Photometer gemessen.

4. MATERIALIEN

4.1. Mitgelieferte Reagenzien

- **Aviditätsreagenz:** 1 Flasche mit 15 mL Harnstofflösung; blau gefärbt; gebrauchsfertig; schwarze Verschlusskappe.
- **Kontrolle Niedrig:** 1 Fläschchen mit 2 mL Kontrolle; gelb gefärbt; gebrauchsfertig; blaue Verschlusskappe; ≤ 0,02% (v/v) MIT.
- **Kontrolle Hoch:** 1 Fläschchen mit 2 mL Kontrolle; gelb gefärbt; gebrauchsfertig; rote Verschlusskappe; ≤ 0,02% (v/v) MIT.

Für Gefahren- und Sicherheitshinweise siehe 12.1.
Für potenzielle Gefahrstoffe überprüfen Sie bitte das Sicherheitsdatenblatt.

4.2. Mitgeliefertes Zubehör

- 1 Arbeitsanleitung Avidity Measles Virus IgG ELISA (Produktnummer: AMEA7330)
- 1 Arbeitsanleitung Measles Virus IgG ELISA (Produktnummer: MEAG0330)
- 1 leere, etikettierte Flasche (weiß mit weißem Deckel) für den gebrauchsfertigen Waschpuffer

5. STABILITÄT UND LAGERUNG

Testkit bei 2...8 °C lagern. Die geöffneten Reagenzien sind bis zu den auf den Etiketten angegebenen Verfallsdaten verwendbar, wenn sie bei 2...8 °C gelagert werden.

6. VORBEREITUNG DER REAGENZIEN

Es ist sehr wichtig, alle Reagenzien und Proben vor ihrer Verwendung auf Raumtemperatur (20...25 °C) zu bringen und zu mischen!

6.1. Aviditätsreagenz

Sollte eine Kristallisation im Aviditätsreagenz auftreten, das Reagenz z. B. in einem Wasserbad auf 37 °C erwärmen und vor der Verwendung gut mischen.

6.2. Waschpuffer

Es wird empfohlen 15 mL des gebrauchsfertigen Waschpuffers in die mitgelieferte Flasche (s. 4.2) zu überführen, um diese in dem Schritt 5 der Testvorbereitung zu verwenden.
Beachte: Der gebrauchsfertige Waschpuffer ist bei Raumtemperatur (20…25 °C) 5 Tage haltbar.

NovaTec Immundiagnostica GmbH

NovaLisa®

Mumps Virus IgG

ELISA

CE

Only for in-vitro diagnostic use

Product Number: MUMG0340 (96 Determinations)

ENGLISH

1. INTRODUCTION

Mumps viruses are RNA viruses of the family Paramyxoviridae. The virions are spherical particles of 150-250 nm in diameter consisting of a ribonucleoprotein with helical symmetry and enveloped by matrix protein and a lipid bilayer which contains two spikeline structures: viral hemagglutinin (H) and viral neuraminidase (N). Mumps virus involves primarily the parotid and related salivary glands; however infection can lead to CNS disease and accumulation of the virus in CSF. Mumps (Epidemic Parotitis) is an acute contagious viral disease mostly occuring in children. Nearly 50% of all infections are subclinical. The highest incidence of clinical manifestations is found in the age group of 4 to 15 years. Secondary infections are rare because of long-lasting immunity.
10 to 35 % of mumps cases develop orchitis which occurs nearly always after puberty. The process is mostly unilateral and the prognosis usually good. Mumps virus has been one of the most important causes of viral CNS disease (meningitis and encephalitis) in USA; vaccine administration has greatly reduced its incidence

Species	Disease	Symptoms (e.g.)	Transmission route
Mumps Virus	Mumps	Fever and unilateral or bilateral swelling of the parotid gland; the sublingual and submaxillary glands may also be involved Complications: Orchitis, Meningoencephalitis, Pancreatitis	Virus transmission occurs by droplet infection

Infection or presence of pathogen may be identified by:

- PCR
- Serology: e.g. ELISA

2. INTENDED USE

The Mumps Virus IgG ELISA is intended for the qualitative determination of IgG class antibodies against Mumps Virus in human serum or plasma (citrate, heparin).

3. PRINCIPLE OF THE ASSAY

The qualitative immunoenzymatic determination of specific antibodies is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique.
Microtiterplates are coated with specific antigens to bind corresponding antibodies of the sample. After washing the wells to remove all unbound sample material a horseradish peroxidase (HRP) labelled conjugate is added. This conjugate binds to the captured antibodies. In a second washing step unbound conjugate is removed. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product.
The intensity of this product is proportional to the amount of specific antibodies in the sample. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450/620 nm is read using an ELISA Microtiterplate reader.

4. MATERIALS

4.1. Reagents supplied

- **Microtiterplate:** 12 break-apart 8-well snap-off strips coated with Mumps Virus antigens; in resealable aluminium foil.
- **IgG Sample Dilution Buffer:** 1 bottle containing 100 mL of phosphate buffer (10 mM) for sample dilution; pH 7.2 ± 0.2; coloured yellow; ready to use; white cap; ≤ 0.0015% (v/v) CMIT/ MIT (3:1).
- **Stop Solution:** 1 bottle containing 15 mL sulphuric acid, 0.2 mol/L; ready to use; red cap.
- **Washing Buffer (20x conc.):** 1 bottle containing 50 mL of a 20-fold concentrated phosphate buffer (0.2 M), pH 7.2 ± 0.2, for washing the wells; white cap.
- **Conjugate:** 1 bottle containing 20 mL of peroxidase labelled antibody to human IgG in phosphate buffer (10 mM); coloured blue; ready to use; black cap.
- **TMB Substrate Solution:** 1 bottle containing 15 mL 3,3',5,5'-tetramethylbenzidine (TMB), < 0.1 %; ready to use; yellow cap.
- **Positive Control:** 1 vial containing 2 mL control; coloured yellow; ready to use; red cap; ≤ 0.02% (v/v) MIT.
- **Cut-ssoff Control:** 1 vial containing 3 mL control; coloured yellow; ready to use; green cap; ≤ 0.02% (v/v) MIT.
- **Negative Control:** 1 vial containing 2 mL control; coloured yellow; ready to use; blue cap; ≤ 0.0015% (v/v) CMIT/ MIT (3:1).

For hazard and precautionary statements see 12.1
For potential hazardous substances please check the safety data sheet.

4.2. Materials supplied

- 1 Cover foil
- 1 Instruction for use (IFU)
- 1 Plate layout

4.3. Materials and Equipment needed

- ELISA Microtiterplate reader, equipped for the measurement of absorbance at 450/620 nm
- Incubator 37 °C
- Manual or automatic equipment for rinsing Microtiterplates
- Pipettes to deliver volumes between 10 and 1000 µL
- Vortex tube mixer
- Distilled water
- Disposable tubes

5. STABILITY AND STORAGE

Store the kit at 2...8 °C. The opened reagents are stable up to the expiry date stated on the label when stored at 2...8 °C.

6. REAGENT PREPARATION

It is very important to bring all reagents and samples to room temperature (20…25 °C) and mix them before starting the test run!

6.1. Microtiterplate

The break-apart snap-off strips are coated with Mumps Virus antigens. Immediately after removal of the strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2...8 °C.

6.2. Washing Buffer (20x conc.)

Dilute Washing Buffer 1 + 19; e. g. 10 mL Washing Buffer + 190 mL distilled water. The diluted buffer is stable for 5 days at room temperature (20…25 °C). In case crystals appear in the concentrate, warm up the solution to 37 °C e.g. in a water bath. Mix well before dilution.

6.3. TMB Substrate Solution

The reagent is ready to use and has to be stored at 2...8 °C, away from the light. The solution should be colourless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away.

7. SAMPLE COLLECTION AND PREPARATION

Use human serum or plasma (citrate, heparin) samples with this assay. If the assay is performed within 5 days after sample collection, the samples should be kept at 2...8 °C; otherwise they should be aliquoted and stored deep-frozen (-70…-20 °C). If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing.
Heat inactivation of samples is not recommended.

7.1. Sample Dilution

Before assaying, all samples should be diluted 1+100 with IgG Sample Dilution Buffer. Dispense 10 µL sample and 1 mL IgG Sample Dilution Buffer into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

8. ASSAY PROCEDURE

Please read the instruction for use carefully **before** performing the assay. Result reliability depends on strict adherence to the instruction for use as described. The following test procedure is only validated for manual procedure. If performing the test on ELISA automatic systems we recommend increasing the washing steps from three up to five and the volume of Washing Buffer from 300 µL to 350 µL to avoid washing effects. Pay attention to chapter 12. Prior to commencing the assay, the distribution and identification plan for all samples and standards/controls (duplicates recommended) should be carefully established on the plate layout supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder.

Perform all assay steps in the order given and without any delays.

A clean, disposable tip should be used for dispensing each standard/control and sample.

Adjust the incubator to 37 ± 1 °C.

1. Dispense 100 µL standards/controls and diluted samples into their respective wells. Leave well A1 for the Substrate Blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 1 hour ± 5 min at 37 ± 1 °C.**
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µL of Washing Buffer. Avoid overflows from the reaction wells. The interval between washing and aspiration should be > 5 sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!

   Note: Washing is important! Insufficient washing results in poor precision and false results.
5. Dispense 100 µL Conjugate into all wells except for the Substrate Blank well A1.
6. **Incubate for 30 min at room temperature (20...25 °C).** Do not expose to direct sunlight.
7. Repeat step 4.
8. Dispense 100 µL TMB Substrate Solution into all wells.
9. **Incubate for exactly 15 min at room temperature (20...25 °C) in the dark.** A blue colour occurs due to an enzymatic reaction.
10. Dispense 100 µL Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution, thereby a colour change from blue to yellow occurs.
11. Measure the absorbance at 450/620 nm within 30 min after addition of the Stop Solution.

8.1. Measurement

Adjust the ELISA Microtiterplate reader **to zero** using the **Substrate Blank**.

If - due to technical reasons - the ELISA Microtiterplate reader cannot be adjusted to zero using the Substrate Blank, subtract its absorbance value from all other absorbance values measured in order to obtain reliable results!

Measure the absorbance of all wells at **450 nm** and record the absorbance values for each standard/control and sample in the plate layout.

Bichromatic measurement using a reference wavelength of 620 nm is recommended.

Where applicable calculate the mean absorbance values of all duplicates.

9. RESULTS

9.1. Run Validation Criteria

In order for an assay run to be considered valid, these Instructions for Use have to be strictly followed and the following criteria must be met:

- **Substrate Blank**: Absorbance value **< 0.100**
- **Negative Control**: Absorbance value **< 0.200 and < Cut-off**
- **Cut-off Control**: Absorbance value **0.150 – 1.300**
- **Positive Control**: Absorbance value **> Cut-off**

If these criteria are not met, the test is not valid and must be repeated.

9.2. Calculation of Results

The Cut-off is the mean absorbance value of the Cut-off Control determinations.

Example: Absorbance value Cut-off Control 0.44 + absorbance value Cut-off control 0.42 = 0.86 / 2 = 0.43
Cut-off = 0.43

9.2.1. Results in Units [NTU]

$$\frac{\text{Sample (mean) absorbance value} \times 10}{\text{Cut-off}} = \text{[NovaTec Units = NTU]}$$

Example: $\frac{1.591 \times 10}{0.43} = 37$ NTU (Units)

9.3. Interpretation of Results

Cut-off	10 NTU	-
Positive	> 11 NTU	Antibodies against the pathogen are present. There has been a contact with the antigen (pathogen resp. vaccine).
Equivocal	9 – 11 NTU	Antibodies against the pathogen could not be detected clearly. It is recommended to repeat the test with a fresh sample in 2 to 4 weeks. If the result is equivocal again the sample is judged as **negative**.
Negative	< 9 NTU	The sample contains no antibodies against the pathogen. A previous contact with the antigen (pathogen resp. vaccine) is unlikely.
Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as serological data. In immunocompromised patients and newborns serological data only have restricted value.		

9.3.1. Antibody Isotypes and State of Infection

Serology	Significance
IgM	Characteristic of the primary antibody response High IgM titer with low IgG titer: → suggests a current or very recent infection Rare: → persisting IgM
IgG	Characteristic of the secondary antibody response May persist for several years High IgG titer with low IgM titer: → may indicate a past infection

10. SPECIFIC PERFORMANCE CHARACTERISTICS

The results refer to the groups of samples investigated; these are not guaranteed specifications.

For further information about the specific performance characteristics please contact NovaTec Immundiagnostica GmbH.

10.1. Precision

Intraassay	**n**	**Mean (E)**	**CV (%)**
#1	24	0.411	4.14
#2	24	1.173	4.32
#3	24	1.338	1.41

Interassay	**n**	**Mean (NTU)**	**CV (%)**
#1	12	30.07	4.58
#2	12	31.66	5.52
#3	12	2.87	9.27

10.2. Diagnostic Specificity

The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte.
It is 95.83% (95% confidence interval: 85.75% - 99.49%).

10.3. Diagnostic Sensitivity

The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte.
It is 93.55% (95% confidence interval: 89.41% - 96.43%).

10.4. Interferences

Interferences with hemolytic, lipemic or icteric samples are not observed up to a concentration of 10 mg/mL hemoglobin, 5 mg/mL triglycerides and 0.5 mg/mL bilirubin.

10.5. Cross Reactivity

Investigation of a sample panel with antibody activities to potentially cross-reacting parameters did not reveal evidence of false-positive results due to cross-reactions.

11. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or repeated freeze-thaw cycles of the sample may affect the absorbance values.

12. PRECAUTIONS AND WARNINGS

- The test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the testkits with analyzers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.
- Only for in-vitro diagnostic use.
- All materials of human or animal origin should be regarded and handled as potentially infectious.
- All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive.
- Do not interchange reagents or Microtiterplates of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- Use only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and standard/control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense reagents without splashing accurately into the wells.
- The ELISA is only designed for qualified personnel following the standards of good laboratory practice (GLP).
- For further internal quality control each laboratory should additionally use known samples.

12.1. Safety note for reagents containing hazardous substances

Reagents may contain CMIT/MIT (3:1) or MIT (refer to 4.1)
Therefore, the following hazard and precautionary statements apply.

Warning

H317	May cause an allergic skin reaction.
P261	Avoid breathing spray.
P280	Wear protective gloves/ protective clothing.
P302+P352	IF ON SKIN: Wash with plenty of soap and water.
P333+P313	If skin irritation or rash occurs: Get medical advice/ attention.
P362+P364	Take off contaminated and Wash it before reuse.

Further information can be found in the safety data sheet.

12.2. Disposal Considerations

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

13. ORDERING INFORMATION

Prod. No.: MUMG0340 Mumps Virus IgG ELISA (96 Determinations)

NovaTec Immundiagnostica GmbH

NovaLisa®

Mumps Virus IgM

ELISA

CE

Only for in-vitro diagnostic use

Product Number: MUMM0340 (96 Determinations)

ENGLISH

1. INTRODUCTION

Mumps viruses are RNA viruses of the family Paramyxoviridae. The virions are spherical particles of 150-250 nm in diameter consisting of a ribonucleoprotein with helical symmetry and enveloped by matrix protein and a lipid bilayer which contains two spikeline structures: viral hemagglutinin (H) and viral neuraminidase (N). Mumps virus involves primarily the parotid and related salivary glands; however infection can lead to CNS disease and accumulation of the virus in CSF. Mumps (Epidemic Parotitis) is an acute contagious viral disease mostly occuring in children. Nearly 50% of all infections are subclinical. The highest incidence of clinical manifestations is found in the age group of 4 to 15 years. Secondary infections are rare because of long-lasting immunity.
10 to 35 % of mumps cases develop orchitis which occurs nearly always after puberty. The process is mostly unilateral and the prognosis usually good. Mumps virus has been one of the most important causes of viral CNS disease (meningitis and encephalitis) in USA; vaccine administration has greatly reduced its incidence.

Species	Disease	Symptoms (e.g.)	Transmission route
Mumps Virus	Mumps	Fever and unilateral or bilateral swelling of the parotid gland; the sublingual and submaxillary glands may also be involved Complications: Orchitis, Meningoencephalitis, Pancreatitis	Virus transmission occurs by droplet infection

Infection or presence of pathogen may be identified by:

- PCR
- Serology: e.g. ELISA

2. INTENDED USE

The Mumps Virus IgM ELISA is intended for the qualitative determination of IgM class antibodies against Mumps Virus in human serum or plasma (citrate, heparin).

3. PRINCIPLE OF THE ASSAY

The qualitative immunoenzymatic determination of specific antibodies is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique.
Microtiterplates are coated with specific antigens to bind corresponding antibodies of the sample. After washing the wells to remove all unbound sample material a horseradish peroxidase (HRP) labelled conjugate is added. This conjugate binds to the captured antibodies. In a second washing step unbound conjugate is removed. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product.
The intensity of this product is proportional to the amount of specific antibodies in the sample. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450/620 nm is read using an ELISA Microtiterplate reader.

4. MATERIALS

4.1. Reagents supplied

- **Microtiterplate:** 12 break-apart 8-well snap-off strips coated with Mumps Virus antigens; in resealable aluminium foil.
- **IgM Sample Dilution Buffer:** 1 bottle containing 100 mL of phosphate buffer (10 mM) for sample dilution; pH 7.2 ± 0.2; anti-human IgG (RF Absorbent); coloured green; ready to use; white cap; ≤ 0.0015% (v/v) CMIT/ MIT (3:1).
- **Stop Solution:** 1 bottle containing 15 mL sulphuric acid, 0.2 mol/L; ready to use; red cap.
- **Washing Buffer (20x conc.):** 1 bottle containing 50 mL of a 20-fold concentrated phosphate buffer (0.2 M), pH 7.2 ± 0.2, for washing the wells; white cap.
- **Conjugate:** 1 bottle containing 20 mL of peroxidase labelled antibody to human IgM in phosphate buffer (10 mM); coloured red; ready to use; black cap.
- **TMB Substrate Solution:** 1 bottle containing 15 mL 3,3',5,5'-tetramethylbenzidine (TMB), < 0.1 %; ready to use; yellow cap.
- **Positive Control:** 1 vial containing 2 mL control; coloured yellow; ready to use; red cap; ≤ 0.02% (v/v) MIT.
- **Cut-off Control:** 1 vial containing 3 mL control; coloured yellow; ready to use; green cap; ≤ 0.02% (v/v) MIT.
- **Negative Control:** 1 vial containing 2 mL control; coloured yellow; ready to use; blue cap; ≤ 0.0015% (v/v) CMIT/ MIT (3:1).

For hazard and precautionary statements see 12.1
For potential hazardous substances please check the safety data sheet.

4.2. Materials supplied

- 1 Cover foil
- 1 Instruction for use (IFU)
- 1 Plate layout

4.3. Materials and Equipment needed

- ELISA Microtiterplate reader, equipped for the measurement of absorbance at 450/620 nm
- Incubator 37 °C
- Manual or automatic equipment for rinsing Microtiterplates
- Pipettes to deliver volumes between 10 and 1000 µL
- Vortex tube mixer
- Distilled water
- Disposable tubes

5. STABILITY AND STORAGE

Store the kit at 2...8 °C. The opened reagents are stable up to the expiry date stated on the label when stored at 2...8 °C.

6. REAGENT PREPARATION

It is very important to bring all reagents and samples to room temperature (20…25 °C) and mix them before starting the test run!

6.1. Microtiterplate

The break-apart snap-off strips are coated with Mumps Virus antigens. Immediately after removal of the strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2...8 °C.

6.2. Washing Buffer (20x conc.)

Dilute Washing Buffer 1 + 19; e. g. 10 mL Washing Buffer + 190 mL distilled water. The diluted buffer is stable for 5 days at room temperature (20…25 °C). In case crystals appear in the concentrate, warm up the solution to 37 °C e.g. in a water bath. Mix well before dilution.

6.3. TMB Substrate Solution

The reagent is ready to use and has to be stored at 2...8 °C, away from the light. The solution should be colourless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away.

7. SAMPLE COLLECTION AND PREPARATION

Use human serum or plasma (citrate, heparin) samples with this assay. If the assay is performed within 5 days after sample collection, the samples should be kept at 2...8 °C; otherwise they should be aliquoted and stored deep-frozen (-70…-20 °C). If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing.
Heat inactivation of samples is not recommended.

7.1. Sample Dilution

Before assaying, all samples should be diluted 1+100 with IgM Sample Dilution Buffer. Dispense 10 µL sample and 1 mL IgM Sample Dilution Buffer into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

8. ASSAY PROCEDURE

Please read the instruction for use carefully **before** performing the assay. Result reliability depends on strict adherence to the instruction for use as described. The following test procedure is only validated for manual procedure. If performing the test on ELISA automatic systems we recommend increasing the washing steps from three up to five and the volume of Washing Buffer from 300 µL to 350 µL to avoid washing effects. Pay attention to chapter 12. Prior to commencing the assay, the distribution and identification plan for all samples and standards/controls (duplicates recommended) should be carefully established on the plate layout supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder.

Perform all assay steps in the order given and without any delays.

A clean, disposable tip should be used for dispensing each standard/control and sample.

Adjust the incubator to 37 ± 1 °C.

1. Dispense 100 µL standards/controls and diluted samples into their respective wells. Leave well A1 for the Substrate Blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 1 hour ± 5 min at 37 ± 1 °C.**
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µL of Washing Buffer. Avoid overflows from the reaction wells. The interval between washing and aspiration should be > 5 sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!

   Note: Washing is important! Insufficient washing results in poor precision and false results.
5. Dispense 100 µL Conjugate into all wells except for the Substrate Blank well A1.
6. **Incubate for 30 min at room temperature (20...25 °C).** Do not expose to direct sunlight.
7. Repeat step 4.
8. Dispense 100 µL TMB Substrate Solution into all wells.
9. **Incubate for exactly 15 min at room temperature (20...25 °C) in the dark.** A blue colour occurs due to an enzymatic reaction.
10. Dispense 100 µL Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution, thereby a colour change from blue to yellow occurs.
11. Measure the absorbance at 450/620 nm within 30 min after addition of the Stop Solution.

8.1. Measurement

Adjust the ELISA Microtiterplate reader **to zero** using the **Substrate Blank**.

If - due to technical reasons - the ELISA Microtiterplate reader cannot be adjusted to zero using the Substrate Blank, subtract its absorbance value from all other absorbance values measured in order to obtain reliable results!

Measure the absorbance of all wells at **450 nm** and record the absorbance values for each standard/control and sample in the plate layout.

Bichromatic measurement using a reference wavelength of 620 nm is recommended.

Where applicable calculate the mean absorbance values of all duplicates.

9. RESULTS

9.1. Run Validation Criteria

In order for an assay run to be considered valid, these Instructions for Use have to be strictly followed and the following criteria must be met:

- **Substrate Blank**: Absorbance value **< 0.100**
- **Negative Control**: Absorbance value **< 0.200 and < Cut-off**
- **Cut-off Control**: Absorbance value **0.150 – 1.300**
- **Positive Control**: Absorbance value **> Cut-off**

If these criteria are not met, the test is not valid and must be repeated.

9.2. Calculation of Results

The Cut-off is the mean absorbance value of the Cut-off Control determinations.

Example: Absorbance value Cut-off Control 0.44 + absorbance value Cut-off control 0.42 = 0.86 / 2 = 0.43
Cut-off = 0.43

9.2.1. Results in Units [NTU]

$$\frac{\text{Sample (mean) absorbance value} \times 10}{\text{Cut-off}} = \text{[NovaTec Units = NTU]}$$

Example: $\frac{1.591 \times 10}{0.43} = 37$ NTU (Units)

9.3. Interpretation of Results

Cut-off	10 NTU	-
Positive	> 11 NTU	Antibodies against the pathogen are present. There has been a contact with the antigen (pathogen resp. vaccine).
Equivocal	9 – 11 NTU	Antibodies against the pathogen could not be detected clearly. It is recommended to repeat the test with a fresh sample in 2 to 4 weeks. If the result is equivocal again the sample is judged as **negative**.
Negative	< 9 NTU	The sample contains no antibodies against the pathogen. A previous contact with the antigen (pathogen resp. vaccine) is unlikely.
Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as serological data. In immunocompromised patients and newborns serological data only have restricted value.		

9.3.1. Antibody Isotypes and State of Infection

Serology	Significance
IgM	Characteristic of the primary antibody response High IgM titer with low IgG titer: → suggests a current or very recent infection Rare: → persisting IgM
IgG	Characteristic of the secondary antibody response May persist for several years High IgG titer with low IgM titer: → may indicate a past infection

10. SPECIFIC PERFORMANCE CHARACTERISTICS

The results refer to the groups of samples investigated; these are not guaranteed specifications.

For further information about the specific performance characteristics please contact NovaTec Immundiagnostica GmbH.

10.1. Precision

Intraassay	**n**	**Mean (E)**	**CV (%)**
#1	24	0.600	6.05
#2	24	1.039	4.81
#3	24	0.639	3.71

Interassay	**n**	**Mean (NTU)**	**CV (%)**
#1	12	21.38	7.50
#2	12	13.88	8.95
#3	12	1.39	10.35

10.2. Diagnostic Specificity

The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte.
It is 97.68% (95% confidence interval: 95.03% - 99.15%).

10.3. Diagnostic Sensitivity

The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte.
It is 94.44% (95% confidence interval: (72.71% - 99.86%).

10.4. Interferences

Interferences with hemolytic, lipemic or icteric samples are not observed up to a concentration of 10 mg/mL hemoglobin, 5 mg/mL triglycerides and 0.5 mg/mL bilirubin.

10.5. Cross Reactivity

Investigation of a sample panel with antibody activities to potentially cross-reacting parameters did not reveal evidence of false-positive results due to cross-reactions.

11. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or repeated freeze-thaw cycles of the sample may affect the absorbance values.

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Reagents may contain CMIT/MIT (3:1) or MIT (refer to 4.1)
Therefore, the following hazard and precautionary statements apply.

Warning

H317	May cause an allergic skin reaction.
P261	Avoid breathing spray.
P280	Wear protective gloves/ protective clothing.
P302+P352	IF ON SKIN: Wash with plenty of soap and water.
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P362+P364	Take off contaminated and Wash it before reuse.

Further information can be found in the safety data sheet.

12.2. Disposal Considerations

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

13. ORDERING INFORMATION

Prod. No.: MUMM0340 Mumps Virus IgM ELISA (96 Determinations)

Certificate

TÜVRheinland®

Quality Management System
EN ISO 13485:2016

Registration No.: SX 1624072-1

Organization: Seramun Diagnostica GmbH
Spreenhagener Str. 1
15754 Heidesee
Germany

Scope: Design and development, manufacture and distribution of in-vitro diagnostics and laboratory systems, consisting of laboratory equipment and associated analysis software, for the detection of autoimmune diseases and infectious diseases

Design and development, manufacture and distribution of substrates and protein stabilizers for in-vitro diagnostics, as well as antibody production and contract development

The Certification Body of TÜV Rheinland LGA Products GmbH certifies that the organization has established and applies a quality management system for medical devices.
Proof has been furnished that the requirements specified in the abovementioned standard are fulfilled. The quality management system is subject to yearly surveillance.

Report No.: 3324901-90
Effective date: 2021-03-10
Expiry date: 2024-03-09
Issue date: 2021-03-03

TÜV Rheinland LGA Products GmbH Zertifizierungsstelle TÜVRheinland

Dipl.-Ing. A. Fechner
TÜV Rheinland LGA Products GmbH
Tillystraße 2 · 90431 Nürnberg · Germany

DAkkS
Deutsche
Akkreditierungsstelle
D-ZM-14169-01-02

Serazym® Clostridium difficile Toxin A+B

Enzyme immunoassay for detection of *Clostridium difficile* Toxin A and B
in stool specimens and culture suspensions

REF E-040 96 REF E-040-A2 2x 96 IVD *In-vitro*- diagnostic medical device CE

Seramun Diagnostica GmbH · Spreenhagener Straße 1 · 15754 Heidesee · Germany · **www.seramun.com**
phone +49 (0) 33767 79110 · fax +49 (0) 33767 79199 · info@seramun.com

Introduction

Clostridium difficile is a bacterium causing nosocomial diarrhea in adults during or after the treatment with antibiotics such as 3rd generation cephalosporines (1). Although 2 - 3% of healthy adults and 20 - 50% of healthy children are colonized with *Clostridium difficile*, the infection is usually of exogenous origin and results from the contact either to hospital staff or to *Clostridium difficile* spores which may contaminate toilets, bed clothes etc. Both exotoxins A and B of this spore-forming bacteria cause the depolymerisation of actin filaments due to the intracellular enzymatic modification of rho-proteins. Consequently, the permeability of cell membrane is raised and neutrophils may invade leading to expression of the clinical picture of the so-called Clostridium difficile-associated diarrhea and colitis or finally the pseudomembraneous colitis (PMC) (1). As the production of toxins and the outbreak of disease is correlated, diagnosis of *Clostridium difficile* infection is based mainly on a direct detection of the toxins in stool specimens. To date the cytotoxicity test has been considered as the gold standard for detection of *Clostridium difficile* toxins. Recently it has been replaced to a large extent by immunological tests such as enzyme immunoassay (2).

References:

1. Rambaud J-C., LaMont J-T. (Hrsg.): “Ökosystem Darm Special - Updates on Clostridium difficile” Springer Verlag 1995
2. Wilkins T.D. and Lyerly D.M. (2003): „Clostridium difficile Testing: after 20 Years, Still Challenging“ Journal Of Clinical Microbiology, Vol. 41, No. 2, p. 531-534

Intended Use

The *Serazym*® Clostridium difficile Toxin A+B is an *in-vitro*-diagnostic medical device for direct detection of the toxins A and B of *Clostridium difficile* in stool specimens and culture suspensions.

Principle of The Test

Serazym® Clostridium difficile Toxin A + B is an indirect two-site-immunoassay for the qualitative determination of both *Clostridium difficile* toxins A and B based on polyclonal and monoclonal antibodies. *Clostridium difficile* toxins of stool specimens or culture suspensions and the positive control react with monoclonal anti-toxin A and polyclonal anti-toxin B antibodies coated on the solid phase of the microplate. After incubation non-bound material is removed by a washing step. Subsequently bound toxins specifically react with biotinylated polyclonal anti-toxin A and monoclonal anti-toxin B antibodies during a second incubation period. Non-bound material is separated from the solid-phase immune complexes by a subsequent washing step. During the next incubation period horseradish peroxidase (HRP) conjugated streptavidin reacts with the bound biotinylated antibodies. Unbound conjugate is removed by a washing step. HRP converts the subsequently added colourless substrate solution of 3,3',5,5'-tetramethylbenzidine (TMB) into a blue product. The enzyme reaction is terminated by sulphuric acid dispensed into the wells turning the solution from blue to yellow. The optical density (OD) of the solution read at 450 / ≥ 620 nm is directly proportional to the specifically bound amount of *Clostridium difficile* toxin A and B. After consideration of the cut-off value, results are interpreted as positive or negative.

Test Components

			For 96 Wells	For 2x 96 Wells
1	WELLS	**Microtitration plate** coated with monoclonal anti-Toxin A- (mouse) and polyclonal anti-Toxin B-antibodies (rabbit)	12 single breakable 8-well strips colour coding red vacuum-sealed with desiccant	2x 12 single breakable 8-well strips colour coding red vacuum-sealed with desiccant
2	WASHBUF CONC 10x	**Wash buffer** 10-fold	100 ml concentrate for 1000 ml solution white cap	2x 100 ml concentrate for 2x 1000 ml solution white cap
3	DIL	**Sample diluent**	100 ml · ready to use coloured yellow black cap	2x 100 ml · ready to use coloured yellow black cap
4	CONTROL +	**Positive control** *C. difficile* Toxin reactive sample	2.0 ml · ready to use coloured blue red cap	4.0 ml · ready to use coloured blue red cap
5	CONTROL –	**Negative control** *C. difficile* Toxin negative sample	2.0 ml · ready to use coloured blue green cap	4.0 ml · ready to use coloured blue green cap
6/1	CONJ BIOTIN	**Biotin-conjugate** Biotinylated, polyclonal anti-Toxin A- (rabbit) and monoclonal anti-Toxin B-antibodies (mouse)	15 ml · ready to use coloured green white cap	30 ml · ready to use coloured green white cap
6/2	CONJ STREPT	**Streptavidin-HRP-conjugate**	15 ml · ready to use coloured red brown cap	30 ml · ready to use coloured red brown cap
7	SUBSTR TMB	**Substrate** 3,3',5,5'-Tetramethylbenzidine and hydrogen peroxide	15 ml · ready to use blue cap	28 ml · ready to use blue cap
8	STOP	**Stop solution** 0.25 M sulphuric acid	15 ml · ready to use yellow cap	28 ml · ready to use yellow cap

Preparation And Storage Of Samples

Toxin detection from stool specimens
Collection and storage
Stool samples should be stored at 2…8°C immediately after collection and processed within 72 hours or frozen at at least –20°C. Storage at -20°C as well as repeated freezing and thawing of samples should be avoided.
Formalin-preserved stool samples should not be used in this assay. Stool samples already diluted with the *Serazym*® sample diluent can be stored for up to 72 h at 2…8°C before testing in the ELISA.
Preparation
Warm samples to room temperature and mix thouroughly. Pipette 1000 µl of sample diluent into a clean tube. Using a disposable stirring rod tansfer about 200 mg (diameter about 4 - 6 mm) of faeces if solid or pipette 200 µl if liquid into the tube and suspend thoroughly. If necessary spin down floating particles in a micro centrifuge at maximum speed for 1 min.

Toxin detection from culture suspensions (toxigenic culture)
Colonies of *Clostridium difficile* grown on blood or CCFA agar for 48 hours can be tested directly in the *Serazym*® Clostridium difficile Toxin A+B. Prepare a bacterial suspension according to Mc Farland standard 1 (OD value at 600 nm: 0.20 - 0.25 after zero compensation to the yellow coloured *Serazym*® sample diluent):
Pipette 1000 µl of sample diluent into a clean tube.
Transfer 2 - 4 inoculating loops of a *C. difficile* culture into the sample diluent and suspend on a vortex mixer. Read OD value at 600 nm as described above where required.
Use 100 µl for ELISA testing. If selective culture media are used the detectable amount of toxins may be reduced due to inhibitory components of such media resulting in decreased OD values in the ELISA. Therefore using selective media for toxigenic culture requires the preparation of a bacterial suspension at least according to Mc Farland standard 4 (OD 600 nm > 1.0 after zero compensation to the yellow coloured *Serazym*® sample diluent). In this case the *Clostridium difficile* colonies of at least half of a densely grown agar plate have to be harvested. Where required the recommendations and instructions of the medium manufacturers are to be observed.

Materials Required But Not Provided

Micropipettes · multi-channel pipette or multi-pipette · Reagent container for multi-channel pipette · 8-channel wash comb with vacuum pump and waste bottle or microplate washer or 8-channel pipette · microplate reader with optical filters for 450 nm for measurement and ≥ 620 nm for reference · distilled or deionized water · glassware · tubes (2 ml) for sample preparation · orbital shaker for performance of test variant 2

Preparation And Storage Of Reagents

Kit size and expiry
One kit is designed for 1x 96 or 2x 96 determinations. The expiry date of each component is reported on its respective label, that of the complete kit on the outer box label. Upon receipt, all test components have to be kept at 2…8°C, preferably in the original kit box. After opening all kit components are stable for at least 2 months, provided proper storage. The ready to use wash solution can be used for at least one month when stored at 2…8°C.

Reagent preparation
Allow all components to reach room temperature prior to use in the assay. The microtitration plate is vacuum-sealed in a foil with desiccant. The plate consists of a frame and strips with breakable wells. Allow the sealed plate to reach room temperature before opening. Unused wells should be stored refrigerated and protected from moisture in the original cover carefully resealed.
Prepare a sufficient amount of wash solution by diluting the 10-fold concentrated wash buffer 1 + 9 with distilled or deionized water.
For Example: 10 ml wash buffer concentrate *(2)* + 90 ml distilled or deionized water.

Assay Procedure

The *Serazym*® Clostridium difficile Toxin A+B can be performed in two ways:

1. Incubation without shaking; complete test duration 2 hours and 15 minutes
2. Incubation with shaking; complete test duration 1 hour and 15 minutes

Dilute samples with sample diluent *(3)* 1 : 6, e.g. 200 mg or 200 µl stool + 1.0 ml sample diluent *(3)* or transfer 2 - 4 inoculation loops of a *C. difficile* colony into a tube with 1.0 ml sample diluent *(3)* and mix thoroughly on a vortex.
Avoid any time shift during dispensing of reagents and samples.
Make sure the soak time of the wash buffer in the wells is at least 5 seconds per wash cycle!
Avoid direct light exposure of the TMB substrate solution!

Working steps variant 1: without shaking

1. Warm all reagents to room temperature (RT) before use. Mix gently without causing foam.
2. Pipette: 100 µl **CONTROL +** positive control *(4)*
   100 µl **CONTROL –** negative control *(5)*
   100 µl **diluted stool specimen** or **culture suspension**.
3. Cover plate and incubate for 60 min at RT.
4. Decant, then wash each well 5x with 300 µl wash solution (diluted from *(2)*) and tap dry onto absorbent paper if necessary.
5. Dispense 3 drops (or 120 µl) **CONJ BIOTIN** biotin-conjugate *(6/1)* per well.
6. Cover plate and incubate for 30 min at RT.
7. Decant, then wash each well 5x with 300 µl wash solution (diluted from *(2)*) and tap dry onto absorbent paper if necessary.
8. Dispense 3 drops (or 120 µl) **CONJ STREPT** streptavidin-HRP-conjugate *(6/2)* per well.
9. Cover plate and incubate for 30 min at RT
10. Decant, then wash each well 5x with 300 µl wash solution (diluted from *(2)*) and tap dry onto absorbent paper if necessary.
11. Dispense 3 drops (or 120 µl) **SUBSTR TMB** substrate *(7)* per well.
12. Incubate for 15 min at RT protected from light.
13. Dispense 3 drops (or 120 µl) **STOP** stop solution *(8)* per well, mix gently.
14. Read OD at 450 nm / ≥ 620 nm with a microplate reader within 30 min after reaction stop.

Working steps variant 2: with shaking

1. Warm all reagents to room temperature (RT) before use. Mix gently without causing foam.
2. Pipette: 100 µl **CONTROL +** positive control *(4)*
   100 µl **CONTROL –** negative control *(5)*
   100 µl **diluted stool specimen** or **culture suspension**.
3. Cover plate and incubate for 30 min at RT on an orbital shaker with a frequency of 500-700/min.
4. Decant, then wash each well 5x with 300 µl wash solution (diluted from *(2)*) and tap dry onto absorbent paper if necessary.
5. Dispense 3 drops (or 120 µl) **CONJ BIOTIN** biotin-conjugate *(6/1)* per well.
6. Cover plate and incubate for 15 min at RT on an orbital shaker with a frequency of 500-700/min.
7. Decant, then wash each well 5x with 300 µl wash solution (diluted from *(2)*) and tap dry onto absorbent paper if necessary.
8. Dispense 3 drops (or 120 µl) **CONJ STREPT** streptavidin-HRP-conjugate *(6/2)* per well.
9. Cover plate and incubate for 15 min at RT on an orbital shaker with a frequency of 500-700/min.
10. Decant, then wash each well 5x with 300 µl wash solution (diluted from *(2)*) and tap dry onto absorbent paper if necessary.
11. Dispense 3 drops (or 120 µl) **SUBSTR TMB** substrate *(7)* per well.
12. Incubate for 15 min at RT without shaking protected from light.
13. Dispense 3 drops (or 120 µl) **STOP** stop solution *(8)* per well, mix gently.
14. Read OD at 450 nm / ≥ 620 nm with a microplate reader within 30 min after reaction stop.

Result Interpretation

Qualitative evaluation
Cut-off determination: OD negative control + 0.20
Samples with absorbances higher than the cut-off value are considered positive, samples with absorbances 10% below the cut-off value are considered negative for *Clostridium difficile* toxin A and B antigen. Samples within 10 % below the cut-off up to the cut-off value have to be considered borderline and should be repeatedly tested. In case of repeated borderline result a second sample of the corresponding patient should be investigated.

Reference Values

***Serazym*® C. difficile Toxin A+B**	
Positive	> Cut-off
Borderline	0,9 x Cut-off – Cut-off
Negative	< 0,9 x Cut-off

It is recommended that each laboratory establishes its own normal and pathological reference ranges as usually done for other diagnostic parameters too. Therefore, the mentioned reference values provide a guide only to values which might be expected.

Test validity

The test run is valid if:

- the mean OD of the negative control is ≤ 0.20 (manual performance)
  ≤ 0.30 (automatic performance)
- the mean OD of the positive control is ≥ 1.00

If the above mentioned quality criteria are not met, repeat the test and make sure that the test procedure is followed correctly (incubation times and temperatures, sample and wash buffer dilution, wash steps etc.). In case of repeated failure of the quality criteria contact your supplier.

Limitations of the procedure

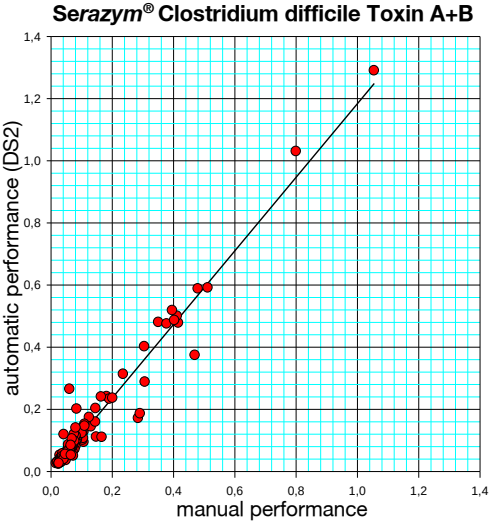
There is no correlation between measured absorbance and seriousness of the infection. It is also not allowed to correlate absorbances of the samples with that of the positive control. Cross contamination of reagents and samples can produce false positive results. Incorrect dilutions, not sufficiently homogenized samples or solid particles after centrifugation of the suspension can cause false negative as well as false positive results. Formalin treated samples may cause false positive results. A negative test result in the *Serazym*® Clostridium difficile Toxin A+B does not exclude an infection: The overall interpretation of the ELISA results should always consider the microbiological examination as well as clinical findings.

Automatic Processing

Performing the *Serazym*® Clostridium difficile Toxin A+B on fully automated microplate processors (e.g. DS2, DSX) may cause elevated absorbances in comparison to the manual procedure due to individual differences concerning wash procedures and general technical specifications of the equipment. In these cases a maximum value of 0.3 absorbance units is permissible for the negative control. It is recommended to use a wash procedure including 10 seconds soak time per strip and wash step followed by a wash step with distilled or deionized water with 10 seconds of soak time after the final wash step of each wash cycle. If necessary the number of washing steps can be enhanced from 5x to 7x-8x.

Correlation: manual – automatic processing

A panel of 125 stool specimens was investigated in parallel by manual and automatic processing method (DS2, Dynex Technologies) resp. The correlation was calculated with r = 0.976.



Performance Characteristics

Precision

Intra-assay coefficient of variation (CV) in the *Serazym*® Clostridium difficile Toxin A+B calculated from 8-fold determinations of samples:

sample	mean OD	standard deviation	CV (%)
1	1.386	0.042	3.0
2	0.506	0.017	3.3
3	0.332	0.028	8.5

Inter-assay coefficient of variation (CV) in the *Serazym*® Clostridium difficile Toxin A+B in 5 different test runs on 2 different days from 8-fold determinations of samples:

sample	mean OD	standard deviation	CV (%)
1	1.321	0.102	7.7
2	0.485	0.034	6.9
3	0.345	0.037	10.8

Specificity and sensitivity

A total of 154 stool specimens were tested in parallel with the *Serazym*® Clostridium difficile Toxin A+B and another commercially available ELISA.

	comparative ELISA positive	comparative ELISA negative
Serazym® ELISA positive	103	4
Serazym® ELISA negative	2	45

Specificity: 91.8% · Sensitivity: 98.0%

Cross reactivity

Faecal samples positive for one of the following intestinal bacteria did not show any cross reaction in the *Serazym*® Clostridium difficile Toxin A+B:
Staphylococcus aureus, enterotoxin negative*; Staphylococcus aureus,* enterotoxin positive*;* EHEC*; Pseudomonas aeruginosa; Salmonella typhimurium; Salmonella enteritidis; Salmonella spec. Aeromonas hydrophila; Aeromonas caviae; Campylobacter spec.; Hafnia alvei; Yersinia enterocolitica* O:3*.*

Negative stool specimens have been spiked with ≥ 10^8 colony forming units of the following microorganisms and tested negative with the *Serazym*® ELISA (OD 450 / 620 nm < Cut-Off):

Aeromonas hydrophila	(ATCC 7966)
Bacillus cereus	(ATCC 11778)
Bacillus subtilis	(ATCC 6633)
Bacteroides fragilis	(ATCC 25285)
Candida albicans	(ATCC 10231)
Campylobacter coli	(ATCC 33559)
Campylobacter jejuni	(ATCC 33291)
Citrobacter freundii	(ATCC 8090)
Clostridium sordellii	(ATCC 9714)
Enterobacter aerogenes	(ATCC 13048)
Enterobacter cloacae	(ATCC 13047)
Enterococcus faecalis	(ATCC 29212)

Escherichia coli	(ATCC 25922)
Klebsiella pneumoniae	(ATCC 13883)
Peptostreptococcus anaerobius	(ATCC 27337)
Proteus vulgaris	(ATCC 8427)
Pseudomonas aeruginosa	(ATCC 10145)
Salmonella enterica Serovar enteritidis	(ATCC 13076)
Salmonella enterica Serovar typhimurium	(ATCC 14028)
Shigella flexneri	(ATCC 12022)
Shigella sonnei	(ATCC 25931)
Staphylococcus aureus	(ATCC 25923)
Staphylococcus epidermidis	(ATCC 12228)
Vibrio parahaemolyticus	(ATCC 17802)

The *C. sordellii* strain ATCC 9714 did not cross react in the *Serazym*® Clostridium difficile Toxin A+B although some publications describe cross reactivities of toxins of some *C. sordellii* strains with anti-C. difficile toxin antibodies.

Common Advices And Precautions

This kit is for *in-vitro* use only. Follow the working instructions carefully. The kit should be performed by trained technical staff only. Do not use reagents from damaged packages or bottles. The expiration dates stated on the respective labels are to be observed. **Do not use or mix reagents from different lots except for sample diluent, wash buffer, TMB/substrate solution and stop solution.**
The sample diluent, wash buffer, TMB/substrate solution and stop solution is universally applicable for the *Serazym*® stool ELISA Adenovirus (E-017), Rotavirus (E-020), Astrovirus (E-045), Norovirus (E-061), Clostridium difficile Toxin A+B (E-040), Clostridium difficile GDH (E-107), Campylobacter (E-093), H. pylori 2nd Gen. (E-114), Entamoeba histolytica (E-018), Cryptosporidium parvum (E-039), Giardia lamblia (E-038) and Giardia (E-106).
Do not use reagents from other manufacturers. Avoid time shift during dispensing of reagents. All reagents should be kept at 2…8°C before use. Some of the reagents may contain biocides as preservative. Further information can be found in the safety data sheet. They must not be swallowed or allowed to come into contact with skin or mucous membranes. Handle all components and all patient samples as if potentially hazardous. Since the kit contains potentially hazardous materials, the following precautions should generally be observed:

Do not smoke, eat or drink while handling kit material!
Always use protective gloves!
Never pipette material by mouth!
Note safety precautions of the single test components!

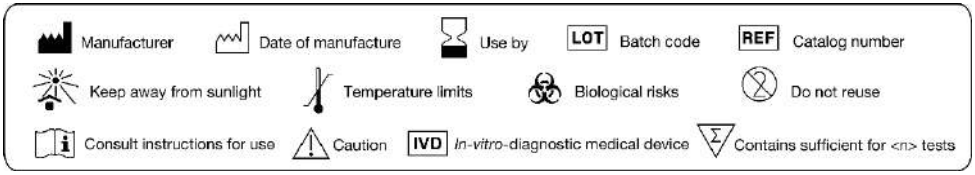
History Of Changes

Version	Section	Modifications
2020-07-21	Intended Use	Correction
	Test Components	Correction
	Preparation And Storage Of Samples	Update
	Assay Procedure	Update
	Common Advices And Precautions	Update
	History of Changes	New section “History of Changes“

Incubation Scheme *Serazym*® Clostridium difficile Toxin A+B (E-040)

1.		pipette	
		100 µl	CONTROL + *(4)*
		100 µl	CONTROL – *(5)*
		100 µl	**diluted stool specimen** or **culture suspension**
		60 min	incubation (RT) **alternatively 30 min while shaking**
		5 x wash	with wash solution
2.		3 drops (or 120 µl)	CONJ BIOTIN *(6/1)*
		30 min	incubation (RT) **alternatively 15 min while shaking**
		5 x wash	with wash solution
3.		3 drops (or 120 µl)	CONJ STREPT *(6/2)*
		30 min	incubation (RT) **alternatively 15 min while shaking**
		5 x wash	with wash solution
4.		3 drops (or 120 µl)	SUBSTR TMB *(7)*
		15 min	incubation protected from light (RT) **without shaking**
5.		3 drops (or 120 µl)	STOP *(8)*

Read OD at 450 / ≥ 620 nm



Serazym ® Clostridium difficile GDH

Enzyme immunoassay for detection of *Clostridium difficile* GDH in faecal specimens

REF E-107 96 REF E-107-A2 2x 96 IVD *In-vitro*-diagnostic medical device CE

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Introduction

The spore forming anaerobic bacterium *Clostridium difficile* is the most common cause of nosocomial diarrheas predominantly developing in the course of antibiotic therapy. The severity of the disease in dependence of the health status of the patient but also of the pathogenicity of the *C. difficile* strain may range from bloody diarrhea to Colitis and pseudomembranous Colitis up to toxic megacolon. The two exotoxins A and B are responsible for the development of the symptoms. Most *Clostridium difficile* strains produce both, toxin A and B, but some are characterized by isolated toxin B production. Atoxigenic strains are generally considered as nonpathogenic and therefore diagnostically irrelevant. The *Clostridium difficile* specific enzyme Glutamatdehydrogenase, also described as “common antigen” is produced by both: toxigenic and atoxigenic strains.
The human intestine is colonized with *Clostridium difficile* in 2 - 3% of healthy adults and in up to 50% of children < 2 years of age. Therefore, the diagnosis of a *Clostridium difficile* associated diarrhea (CDAD) has to be confirmed by the detection of the toxins A and B.
Changes in the epidemiological situation are observed since several years: Increasingly also young adults and not hospitalized patients get sick developing the symptoms of CDAD. An increasing number of severe developments of the disease caused by highly virulent strains also occur. In this context testing of patient´s status as carrier seems reasonable.
Different approaches have developed in laboratory diagnosis of *C. difficile* infections. Usually direct toxin A and B detection from stool or enrichment cultures is performed by enzyme immunoassay. In addition, a new two-step procedure has been established in several laboratories that comprise a first screening of *C. difficile* GDH and the subsequent testing for toxins A and B of GDH positive samples. The isolated detection of GDH is neither sufficient for diagnosing a CDAD nor suitable for a therapeutic decision, but always needs to be completed by the testing for toxins A and B.
References:

1. Wilkins TD and Lyerly DM (2003): „Clostridium difficile Testing: after 20 Years, Still Challenging“ Journal Of Clinical Microbiology, Vol. 41, No.2, p531-534
2. Von Eichel-Streiber C und Braun V (2008): “Das difficile Clostridium” Journal Of Laboratory Medicine, Vol. 32, No. 4, p219-234
3. Lyerly DM, Barroso LA, Wilkins TD (1991): “Identification of the latex test-reactive protein of Clostridium difficile as glutamate dehydrogenase.” Journal Of Clinical Microbiology, Vol. 29, p2639-2642.
4. Zhen L, Keller SF, Lyerly DM et al. (2004): „Multicenter Evaluation of a New Screening Test That Detects Clostridium difficile in Faecal Specimens“ Journal Of Clinical Microbiology, Vol. 42, No. 8, p3837-3840.
5. Peterson LR and Robicsek A (2009): “Does My Patient Have Clostridium difficile Infection?” Annals of Internal Medicine, Vol. 151, No. 3, p176-180.

Intended Use

***Serazym*® Clostridium difficile GDH is an *in-vitro* diagnostic medical device for direct detection of Glutamatdehydrogenase (GDH) of *Clostridium difficile* in faecal specimens.**

Principle of the Test

Serazym® Clostridium difficile GDH is a one-step enzyme immunoassay on the basis of polyclonal and monoclonal antibodies to Glutamatdehydrogenase (GDH) of *Clostridium difficile*. Diluted stool specimens and monoclonal anti-GDH antibodies are dispensed simultaneously into the wells of a microtitration plate coated with polyclonal anti-GDH antibodies. After an incubation time of 60 min at room temperature (RT) unbound components are removed by a washing step. Alternatively, the first incubation can be shortened to 30 min by shaking. HRP converts the subsequently added colourless substrate solution of 3,3’,5,5’-Tetramethylbenzidine (TMB) within a 10 min reaction time into a blue product. The enzyme reaction is terminated by sulphuric acid dispensed into the wells turning the solution from blue to yellow. The optical density (OD) of the solution read at 450 / $\geq$ 620 nm is directly proportional to the specifically bound amount of GDH antigen in the respective sample.
Considering the cut-off value results are interpreted as positive or negative.

Test Components

			For 96 Wells	For 2x 96 Wells
1	WELLS	**Microtitration plate** coated with polyclonal anti-C.difficile GDH antibodies (sheep)	12 single breakable 8-well strips colour coding green vacuum-sealed with desiccant	2x 12 single breakable 8-well strips colour coding green vacuum-sealed with desiccant
2	WASHBUF CONC 10x	**Wash buffer** 10-fold	100 ml concentrate for 1000 ml solution white cap	2x 100 ml concentrate for 2x 1000 ml solution white cap
3	DIL	**Sample diluent**	100 ml · ready to use coloured yellow black cap	2x 100 ml · ready to use coloured yellow black cap
4	CONTROL +	**Positive control** Recombinant *C. difficile* GDH	2.0 ml · ready to use coloured blue red cap	4.0 ml · ready to use coloured blue red cap
5	CONTROL –	**Negative control** *C. difficile* GDH negative sample	2.0 ml · ready to use coloured blue green cap	4.0 ml · ready to use coloured blue green cap
6	CONJ HRP	**HRP-conjugate** HRP-labelled, monoclonal anti-C.difficile GDH antibodies (mouse)	15 ml · ready to use coloured green brown cap	25 ml · ready to use coloured green brown cap
7	SUBSTR TMB	**Substrate** 3,3',5,5'-Tetramethylbenzidine and hydrogen peroxide	15 ml · ready to use blue cap	28 ml · ready to use blue cap
8	STOP	**Stop solution** 0.25 M sulphuric acid	15 ml · ready to use yellow cap	28 ml · ready to use yellow cap

Preparation and Storage of Samples

Collection and storage

Stool samples should be stored at 2…8°C immediately after collection and processed within 72 hours. Longer storage is possible at least at-20°C. Repeated (> 3x) freezing and thawing of samples should be avoided. Stool samples already diluted with the *Serazym*® sample diluent can be stored for up to 72 h at 2…8 °C before testing in the ELISA.

Preparation

Mix samples thoroughly. Pipette 1000 µl of sample diluent into a clean tube. Using a disposable stirring rod tansfer about 200 mg (diameter about 4 - 6 mm) of faeces if solid or pipette 200 µl if liquid into the tube and suspend thoroughly. Spin down floating particles in a micro centrifuge at maximum speed for 1 min if necessary.

Materials required but not provided

Micropipettes · multi-channel pipette or multi-pipette · Reagent container for multi-channel pipette · 8-channel wash comb with vacuum pump and waste bottle or microplate washer or 8-channel pipette · microplate reader with optical filters for 450 nm for measurement and ≥ 620 nm for reference · distilled or deionized water · glassware · tubes (2 ml) for sample preparation · orbital shaker for performance of test variant 2

Preparation and Storage of Reagents

Kit size and expiry
One kit is designed for 1x 96 or 2x 96 determinations. The expiry date of each component is reported on its respective label, that of the complete kit on the outer box label. Upon receipt, all test components have to be kept at 2…8°C, preferably in the original kit box. After opening all kit components are stable for at least 2 months, provided proper storage. The ready to use wash solution can be used for at least one month when stored at 2…8°C.

Reagent preparation
Allow all components to reach room temperature prior to use in the assay. The microtitration plate is vacuum-sealed in a foil with desiccant. The plate consists of a frame and strips with breakable wells. Allow the sealed plate to reach room temperature before opening. Unused wells should be stored refrigerated and protected from moisture in the original cover carefully resealed.
Prepare a sufficient amount of wash solution by diluting the 10-fold concentrated wash buffer 1 + 9 with distilled or deionized water.
For Example: 10 ml wash buffer concentrate *(2)* + 90 ml distilled or deionized water.

Assay Procedure

The *Serazym*® Clostridium difficile GDH can be performed in two ways:

1. Incubation without shaking; complete test duration 1 h and 10 minutes
2. Incubation with shaking; complete test duration 40 minutes

Dilute samples with sample diluent *(3)* 1 : 6 e.g. 200 mg or 200 µl stool + 1.0 ml sample diluent *(3)* and mix thoroughly.
Avoid any time shift during dispensing of reagents and samples.
Make sure the soak time of the wash buffer in the wells is at least 5 seconds per wash cycle and that residual fluid is completely drained in every single wash cycle!
Avoid direct light exposure of the TMB substrate solution!

Working steps variant 1: without shaking

1. Warm all reagents to room temperature (RT) before use. Mix gently without causing foam.
2. Dispense 3 drops (or 100 µl) **CONJ HRP** HRP-conjugate *(6)* per well and
3. Pipette: 100 µl **CONTROL +** positive control *(4)*
   100 µl **CONTROL –** negative control *(5)*
   100 µl **diluted sample**, mix gently
4. Cover plate and incubate for 60 min at RT.
5. Decant, then wash each well 5x with 300 µl wash solution (diluted from *(2)*) and tap dry onto absorbent paper if necessary.
6. Dispense 3 drops (or 100 µl) **SUBSTR TMB** substrate *(7)* per well.
7. Incubate for 10 min at RT protected from light.
8. Dispense 3 drops (or 100 µl) **STOP** stop solution *(8)* per well, mix gently.
9. Read OD at 450 nm / ≥ 620 nm with a microplate reader within 30 min after reaction stop.

Working steps variant 2: with shaking

1. Warm all reagents to room temperature (RT) before use. Mix gently without causing foam.
2. Dispense 3 drops (or 100 µl) **CONJ HRP** HRP-conjugate *(6)* per well and
3. Pipette: 100 µl **CONTROL +** positive control *(4)*
   100 µl **CONTROL –** negative control *(5)*
   100 µl **diluted sample**, mix gently
4. Cover plate and incubate for 30 min at RT on an orbital shaker with a frequency of 500 – 700 / min.
5. Decant, then wash each well 5x with 300 µl wash solution (diluted from *(2)*) and tap dry onto absorbent paper if necessary.
6. Dispense 3 drops (or 100 µl) **SUBSTR TMB** substrate *(7)* per well.
7. Incubate for 10 min at RT protected from light.
8. Dispense 3 drops (or 100 µl) **STOP** stop solution *(8)* per well, mix gently.
9. Read OD at 450 nm / ≥ 620 nm with a microplate reader within 30 min after reaction stop.

Result Interpretation

Qualitative evaluation
Cut-off determination: OD negative control + 0.10
Samples with OD values equal to or higher than the cut-off value are considered positive, samples with OD values below the cut-off value are considered negative in the *Serazym*® C. difficile GDH.

Reference Values

***Serazym*® Clostridium difficile GDH**	
Positive	≥ Cut-off
Negative	< Cut-off

It is recommended that each laboratory establishes its own normal and pathological reference ranges as usually done for other diagnostic parameters too. Therefore, the mentioned reference values provide a guide only to values which might be expected.

Test validity
The test run is valid if:

- the mean OD of the negative control is ≤ 0.20 (manual performance)
  ≤ 0.30 (automatic performance)
- the mean OD of the positive control is ≥ 1.00

If the above mentioned quality criteria are not met, repeat the test and make sure that the test procedure is followed correctly (incubation times and temperatures, sample and wash buffer dilution, wash steps etc.). In case of repeated failure of the quality criteria contact your supplier.

Limitations of the procedure

The qualitative determination of *Clostridium difficile* GDH in stool specimens by enzyme immunoassay is not equivalent to the diagnosis of a *C. difficile* associated disease (CDAD). CDADs are caused by the toxins A and B of pathogenic *C. difficile* strains. Therefore, a positive GDH test result has to be supplemented by testing for toxins A and B, in order to confirm or exclude a toxigenic strain. On the other hand a negative GDH test result does not necessarily exclude a *C. difficile* infection. After dilution in sample diluent the samples should be tested by ELISA as quickly as possible, at least within 72 hours, because storage-depending antigen degradation may cause false negative results. More than 3 freeze-thaw cycles may also cause false negative results due to antigen degradation. As a result of inhomogeneous antigen distribution in some stool samples insufficient homogenizing may also cause false negative results. Cross contamination of reagents and samples can produce false positive results. Incorrect dilutions or solid particles after centrifugation of the suspension can cause false negative as well as false positive results. The overall interpretation of the ELISA results should always consider the microbiological examination as well as clinical findings.

Automatic Processing

Performing the *Serazym*® Clostridium difficile GDH on fully automated microplate processors (e.g. DS2 or DSX) may cause elevated absorbances in comparison to the manual procedure due to individual differences concerning wash procedures and general technical specifications of the equipment. In these cases a maximum value of 0.3 absorbance units is permissible for the negative control. It is recommended to use a wash procedure including 10 seconds soak time per strip and wash step followed by a wash step with distilled or deionized water with 10 seconds of soak time after the final wash step of each wash cycle. If necessary the number of washing steps can be enhanced from 5x to 7x - 8x.

Correlation: manual – automatic processing

A panel of 90 stool specimens (45 positive and 45 negative samples) was investigated in parallel by manual and automatic processing method (DS2, Dynex Technologies) resp. The correlation was calculated with r = 0.999.

Performance Characteristics

Precision

Intra-assay coefficient of variation (CV) calculated from 8-fold determinations of samples:

sample	mean OD	standard deviation	CV (%)
1	2.800	0.040	1.52
2	1.960	0.045	2.46
3	0.611	0.041	7.19
4	0.352	0.017	5.15

Inter-assay coefficient of variation (CV) in 5 different test runs calculated from twofold determinations of samples:

sample	mean OD	standard deviation	CV (%)
1	1.707	0.250	14.6
2	1.155	0.148	12.8
3	0.895	0.098	10.9
4	0.381	0.068	17.9

Lot-to-Lot reproducibility in 3 different production lots calculated from 8-fold determinations of samples:

sample	mean OD	standard deviation	CV (%)
1	1.840	0.166	9.0
2	1.285	0.172	13.4
3	0.349	0.093	26.6
4	0.026	0.005	17.6

Lower detection limit
The lower detection limit of Glutamatdehydrogenase (GDH) in the *Serazym*® Clostridium difficile GDH was determined 10 ng/ml by titration of recombinant GDH antigen.

Sensitivity and specificity

Sensitivity in comparison to PCR
Ninety eight out of 102 stool specimens characterized *Clostridium difficile* positive by PCR were tested positive with the *Serazym*® ELISA corresponding to a sensitivity of 96.1%.

Comparative sensitivity and specificity
In two comparative studies 235 and 170 stool samples were tested in parallel in the *Serazym*® Clostridium difficile GDH and 2 other commercially available ELISAs respectively.

Study 1

n = 235	comparative ELISA 1 positive	comparative ELISA 1 negative
***Serazym*® ELISA positive**	101	3**
***Serazym*® ELISA negative**	12*	119

Sensitivity: 89.4% · Specificity: 97.5%

* 10 out of 12 *Serazym*® ELISA negative and comparative ELISA 1 positive samples were tested negative in 2 other commercial ELISAs. Sensitivity amended: 98.1%
** One sample was confirmed true positive by PCR. Specificity amended: 98.3%

Study 2

n = 170	comparative ELISA 2 positive	comparative ELISA 2 negative
***Serazym*® ELISA positive**	69	1*
***Serazym*® ELISA negative**	3	97

Sensitivity: 95.8% · Specificity: 98.9%

* This sample was confirmed true positive by PCR.
Specificity amended: 100%

Cross reactivity
Negative stool specimens have been spiked with $\geq 10^8$ colony forming units of the following microorganisms and tested negative with the *Serazym*® ELISA (OD 450 / 620 nm < Cut-Off):

Aeromonas hydrophila	(ATCC 7966)
Bacillus cereus	(ATCC 11778)
Bacillus subtilis	(ATCC 6633)
Bacteroides fragilis	(ATCC 25285)
Candida albicans	(ATCC 10231)
Campylobacter coli	(ATCC 33559)
Campylobacter jejuni	(ATCC 33291)
Citrobacter freundii	(ATCC 8090)
Clostridium sordellii	(ATCC 9714)
Enterobacter aerogenes	(ATCC 13048)
Enterobacter cloacae	(ATCC 13047)
Enterococcus faecalis	(ATCC 29212)
Escherichia coli	(ATCC 25922)

Klebsiella pneumoniae	(ATCC 13883)
Peptostreptococcus anaerobius	(ATCC 27337)
Proteus vulgaris	(ATCC 8427)
Pseudomonas aeruginosa	(ATCC 10145)
Salmonella enterica Serovar enteritidis	(ATCC 13076)
Salmonella enterica Serovar typhimurium	(ATCC 14028)
Shigella flexneri	(ATCC 12022)
Shigella sonnei	(ATCC 25931)
Staphylococcus aureus	(ATCC 25923)
Staphylococcus epidermidis	(ATCC 12228)
Vibrio parahaemolyticus	(ATCC 17802)
Vibrio cholerae	Clinical isolate
Yersinia enterocolitica Serotyp 03, 09	Clinical isolates

Interference
None of the following substances added to GDH positive and negative stool samples showed a significant impact on the test result:
barium sulphate (5%), Buscopan® (2 mg/ml), cyclamate (5%), Diclofenac (2 mg/ml), haemoglobine (5 mg/ml), Hylak® N (5%), Immodium® akut duo (0.2/12.5 mg/ml), Iberogast® (5%), loperamide (0.2 mg/ml), metronidazole (2 mg/ml), mucin (5 mg/ml), Nexium® (2 mg/ml), palmitic acid (20%), Pentofuryl® (2 mg/ml), Pepto-Bismol (1 mg/ml), Perenterol (2.5 mg/ml), Rennie® (8 mg/ml), Simagel® (2 mg/ml), stearic acid (20%), vancomycin hydrochloride (0.5%).

Common Advices and Precautions

This kit is for *in-vitro* use only. Follow the working instructions carefully. The kit should be performed by trained technical staff only. Do not use reagents from damaged packages or bottles. The expiration dates stated on the respective labels are to be observed. **Do not use or mix reagents from different lots except for sample diluent, wash buffer, TMB/substrate solution and stop solution.**
The sample diluent, wash buffer, TMB/substrate solution and stop solution is universally applicable for the *Serazym*® stool ELISA Adenovirus (E-017), Rotavirus (E-020), Astrovirus (E-045), Norovirus (E-061), Clostridium difficile Toxin A+B (E-040), Clostridium difficile GDH (E-107), Campylobacter (E-093), H. pylori 2nd Gen. (E-114), Entamoeba histolytica (E-018), Cryptosporidium parvum (E-039), Giardia lamblia (E-038) and Giardia (E-106).
Do not use reagents from other manufacturers. Avoid time shift during dispensing of reagents. All reagents should be kept at 2…8°C before use. Some of the reagents may contain biocides as preservative. Further information can be found in the safety data sheet. They must not be swallowed or allowed to come into contact with skin or mucous membranes. Handle all components and all patient samples as if potentially hazardous. Since the kit contains potentially hazardous materials, the following precautions should generally be observed:

Do not smoke, eat or drink while handling kit material!
Always use protective gloves!
Never pipette material by mouth!
Note safety precautions of the single test components!

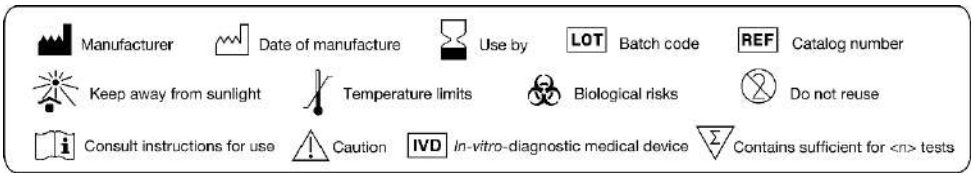
History of Changes

Version	Section	Modifications
2021-02-02	Preparation And Storage Of Samples, Assay Procedure	Update

Incubation Scheme *Serazym*® Clostridium difficile GDH (E-107)

1.		3 drops (or 100 µl) + pipette	CONJ HRP *(6)*
		100 µl	CONTROL + *(4)*
		100 µl	CONTROL – *(5)*
		100 µl	**stool sample**, mix gently
		60 min	incubation (room temperature) **alternatively 30 min while shaking**
		5x wash	with wash solution
2.		3 drops (or 100 µl)	SUBSTR TMB *(7)*
		10 min	incubation protected from light (room temperature) **without shaking**
3.		3 drops (or 100 µl)	STOP *(8)*

Read OD at 450 / ≥ 620 nm



NovaTec Immundiagnostica GmbH

NovaLisa®

Clostridium tetani toxin IgG

ELISA

CE

Only for in-vitro diagnostic use

Product Number: TETG0430 (96 Determinations)

ENGLISH

1. INTRODUCTION

Clostridia are spore-forming gram-positive bacteria. The round spores are build at the terminal end which results in the microscope in a “tennis racket” like shape.

Tetanus develops only when spores of Clostridium tetani germinate under strict anaerobic conditions after gaining access to wounds and small lacerations. The clinical manifestation of the disease is primary not caused by the invasion of the exciter but by the secretion of a powerful neurotoxin (tetanospasmin). This toxin blocks the inhibition of the signal transduction and has a high affinity to the central nervous system. The consequence is hyper excitability of the muscles to external stimuli in combination with a principal increase of the muscle tonus without influence of consciousness. It starts with tonic spasm of muscles (trimus), mimic muscles and gallet muscles. Neck, back and abdominal musculature follow. At the same time the appearance of refectory spasm of whole muscle groups can hamper breathing. Hyper salivation and swallowing problems cause aspiration and pneumonia with the next breath.

Clostridium tetani is ubiquitous present in soil and intestine of humans and animals. Ingestion of bacteria or growth in the intestine of man or animal is without harm. The spores are extremely resistant towards heat and can stay infectious for a long period. The bacteria can get under the skin by even smallest wounds. In Europe tetanus mainly occurs after injuries and sometimes postoperative whereas in developing countries Tetanus is widely disseminated. The WHO assumes that one million people die because of tetanus worldwide per year.

Tetanus toxin is an excellent immunogen in man - only one antigenic type of toxin. The only effective way to control tetanus is by prophylactic active immunization.

Species	**Disease**	**Symptoms (e.g.)**	**Transmission route**
Clostridium tetani	Tetanus	Trismus, dysphagia, severe, painful spasms of whole muscle groups, hypersalivation, aspiration, asphyxia	Injury (Infection of the wound with Clostridium tetani)

The presence of pathogen or infection may be identified by

- Microscopy
- Serology: e.g. ELISA

2. INTENDED USE

The Clostridium tetani toxin IgG ELISA is intended for the quantitative determination of IgG class antibodies against Clostridium tetani toxin in human serum or plasma (citrate, heparin).

3. PRINCIPLE OF THE ASSAY

The quantitative immunoenzymatic determination of specific antibodies is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique.
Microplates are coated with specific antigens to bind corresponding antibodies of the sample. After washing the wells to remove all unbound sample material a horseradish peroxidase (HRP) labelled conjugate is added. This conjugate binds to the captured antibodies. In a second washing step unbound conjugate is removed. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product.
The intensity of this product is proportional to the amount of specific antibodies in the sample. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450/620 nm is read using an ELISA microwell plate reader.

4. MATERIALS

4.1. Reagents supplied

- **Clostridium tetani toxin Coated Microplate (IgG):** 12 break apart 8-well snap-off strips coated with Clostridium tetani toxin (toxoid) antigens; in resealable aluminium foil.
- **IgG Sample Diluent:** 1 bottle containing 100 ml of phosphate buffer (10 mM) for sample dilution; pH 7.2 ± 0.2; coloured yellow; ready to use; white cap.
- **Stop Solution:** 1 bottle containing 15 ml sulphuric acid, 0.2 mol/l; ready to use; red cap.
- **Washing Buffer (20x conc.):** 1 bottle containing 50 ml of a 20-fold concentrated phosphate buffer (0.2 M), pH 7.2 ± 0.2, for washing the wells; white cap.
- **Clostridium tetani toxin anti-IgG Conjugate:** 1 bottle containing 20 ml of peroxidase labelled antibody to human IgG in phosphate buffer (10 mM); coloured blue; ready to use; black cap.
- **TMB Substrate Solution:** 1 bottle containing 15 ml 3,3',5,5'-tetramethylbenzidine (TMB), < 0.1 %; ready to use; yellow cap; < 5% NMP.
- **Clostridium tetani toxin IgG Standards:** 4 vials, each containing 2 ml standard (human serum or plasma); coloured yellow; ready to use.

  Standard A: 0.0 IU/ml; blue cap

  Standard B: 0.1 IU/ml; green cap

  Standard C: 0.5 IU/ml; yellow cap

  Standard D: 1.0 IU/ml; red cap

  The standards are calibrated in accordance with the Who International Standard; ”1st International Standard for Tetanus Immunoglobulin, Human”; NIBSC Code: TE-3.

For potential hazardous substances please check the safety data sheet.

4.2. Materials supplied

- 1 Cover foil
- 1 Instruction for use (IFU)
- 1 Plate layout

4.3. Materials and Equipment needed

- ELISA microwell plate reader, equipped for the measurement of absorbance at 450/620 nm
- Incubator 37°C
- Manual or automatic equipment for rinsing wells
- Pipettes to deliver volumes between 10 and 1000 µl
- Vortex tube mixer
- Distilled water
- Disposable tubes

5. STABILITY AND STORAGE

Store the kit at 2...8 °C. The opened reagents are stable up to the expiry date stated on the label when stored at 2...8 °C.

6. REAGENT PREPARATION

It is very important to bring all reagents and samples to room temperature (20…25 °C) and mix them before starting the test run!

6.1. Coated Microplate

The break-apart snap-off strips are coated with Clostridium tetani toxin (toxoid) antigens. Immediately after removal of the strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2...8 °C.

6.2. Washing Buffer (20x conc.)

Dilute Washing Buffer 1 + 19; e. g. 10 ml Washing Buffer + 190 ml distilled water. The diluted buffer is stable for 5 days at room temperature (20…25 °C). In case crystals appear in the concentrate, warm up the solution to 37°C e.g. in a water bath. Mix well before dilution.

6.3. TMB Substrate Solution

The reagent is ready to use and has to be stored at 2...8 °C, away from the light. The solution should be colourless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away.

7. SAMPLE COLLECTION AND PREPARATION

Use human serum or plasma (citrate, heparin) samples with this assay. For CSF please use the instruction for use ABVL0001. If the assay is performed within 5 days after sample collection, the samples should be kept at 2...8 °C; otherwise they should be aliquoted and stored deep-frozen (-70…-20 °C). If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing.
Heat inactivation of samples is not recommended.

7.1. Sample Dilution

Before assaying, all samples should be diluted 1+100 with IgG Sample Diluent. Dispense 10 µl sample and 1 ml IgG Sample Diluent into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

8. ASSAY PROCEDURE

8.1. Test Preparation

Please read the instruction for use carefully **before** performing the assay. Result reliability depends on strict adherence to the instruction for use as described. The following test procedure is only validated for manual procedure. If performing the test on ELISA automatic systems we recommend increasing the washing steps from three to five and the volume of Washing Buffer from 300 µl to 350 µl to avoid washing effects. Pay attention to chapter 12. Prior to commencing the assay, the distribution and identification plan for all samples and standards/controls (duplicates recommended) should be carefully established on the plate layout supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder.

Perform all assay steps in the order given and without any delays.

A clean, disposable tip should be used for dispensing each standard/control and sample.

Adjust the incubator to 37 ± 1 °C.

1. Dispense 100 µl standards/controls and diluted samples into their respective wells. Leave well A1 for the Substrate Blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 1 hour ± 5 min at 37 ± 1 °C.**
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µl of Washing Buffer. Avoid overflows from the reaction wells. The interval between washing and aspiration should be > 5 sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!

   Note: Washing is important! Insufficient washing results in poor precision and false results.
5. Dispense 100 µl Conjugate into all wells except for the Substrate Blank well A1.
6. **Incubate for 30 min at room temperature (20…25°C).** Do not expose to direct sunlight.
7. Repeat step 4.
8. Dispense 100 µl TMB Substrate Solution into all wells.
9. **Incubate for exactly 15 min at room temperature (20...25 °C) in the dark.** A blue colour occurs due to an enzymatic reaction.
10. Dispense 100 µl Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution, thereby a colour change from blue to yellow occurs.
11. Measure the absorbance at 450/620 nm within 30 min after addition of the Stop Solution.

8.2. Measurement

Adjust the ELISA microwell plate reader **to zero** using the **Substrate Blank**.

If - due to technical reasons - the ELISA microwell plate reader cannot be adjusted to zero using the Substrate Blank, subtract its absorbance value from all other absorbance values measured in order to obtain reliable results!

Measure the absorbance of all wells at **450 nm** and record the absorbance values for each standard/control and sample in the plate layout.

Bichromatic measurement using a reference wavelength of 620 nm is recommended.

Where applicable calculate the **mean absorbance values** of all duplicates.

9. RESULTS

9.1. Run Validation Criteria

In order for an assay to be considered valid, the following criteria must be met:

- **Substrat-Blank: Absorbance value < 0.100**
- **Standard A: Absorbance value < 0.200**
- **Standard B: Absorbance value > 0.150**
- **Standard C: Absorbance value > 0.500**
- **Standard D: Absorbance value > 1.000**

Standard A < Standard B < Standard C < Standard D

If these criteria are not met, the test is not valid and must be repeated.

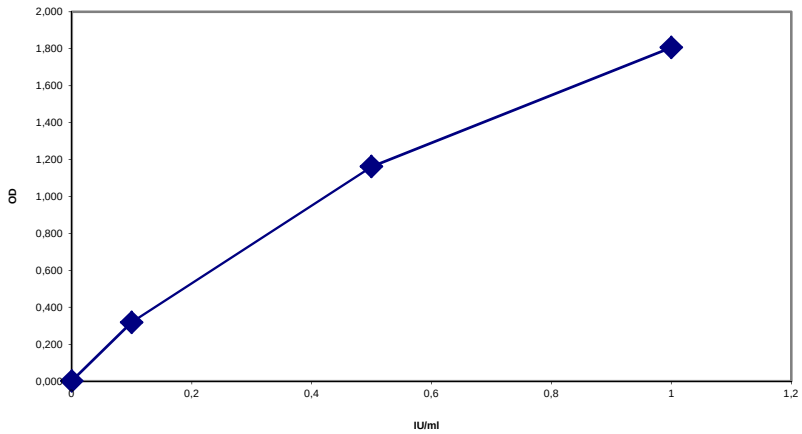
9.2. Calculation of Results

In order to obtain **quantitative results in IU/ml** blot the (mean) absorbance values of the 4 Standards A - D on (linear/linear) graph paper in a system of coordinates against their corresponding concentrations (0.0 / 0.1 / 0.5 and 1.0 IU/ml) and draw a standard curve (absorbance values on the y-axis, concentrations on the x-axis).

Read results from this standard curve employing the (mean) absorbance values of each patient sample.

For the calculation of the standard-curve mathematical Point to Point function should be used.

9.3. Typical standard Curve



9.4. Interpretation of Results and Recommendations [IU/ml]

< 0.1 IU/ml	No protective antibody level or no reliable protection! Immediate full course of basic immunization or booster injection and control of antibody concentration 4 to 6 weeks later is recommended.
0.11 - 0.5 IU/ml	Reliable protection! Booster injection and control of antibody concentration 4 to 6 weeks later is recommended.
0.51 - 1.0 IU/ml	Reliable protection; control of antibody concentration after about 2 years is recommended. Booster injection is not required. Note: In cases of antibody concentrations greater than 0.5 IU/ml vaccination can cause side effects!
1.1 - 5.0 IU/ml	Range of long term protection: Control after 5 to 10 years
> 5.0 IU/ml	Range of long term protection: Control after 10 years immunisation and to record the data on the certificate of vaccination.

Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as serological data.
In immunocompromised patients and newborns serological data only have restricted value.

10. SPECIFIC PERFORMANCE CHARACTERISTICS

The results refer to the groups of samples investigated; these are not guaranteed specifications.

For further information about the specific performance characteristics please contact NovaTec Immundiagnostica GmbH.

10.1. Precision

Intraassay	n	Mean (E)	Cv (%)
#1	24	1.306	3.60
#2	24	1.805	3.46
#3	24	1.591	5.34
Interassay	**n**	**Mean (IU/ml)**	**Cv (%)**
#1	12	0.060	9.62
#2	12	0.084	11.33
#3	12	0.658	13.99

10.2. Diagnostic Specificity

The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte.
It is 100.0% (95% confidence interval: 76.84% - 100.0%).

10.3. Diagnostic Sensitivity

The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte.
It is 99.22% (95% confidence interval: 95.76% - 99.98%).

10.4. Analytical Sensitivity

The analytical sensitivity (according to CLSI EP17-A) is defined as the apparent concentration of the analyte that can be distinguished from the zero calibrator. It is 0.01 IU/ml.

10.5. Interferences

Interferences with hemolytic, lipemic or icteric samples are not observed up to a concentration of 10 mg/ml hemoglobin, 5 mg/ml triglycerides and 0.5 mg/ml bilirubin.

10.6. Cross Reactivity

Investigation of a sample panel with antibody activities to potentially cross-reacting parameters did not reveal evidence of false-positive results due to cross-reactions.

10.7. Measurement range

The measurement range is 0.01 IU/ml – 1 IU/ml.

11. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or repeated freeze-thaw cycles of the sample may affect the absorbance values.

12. PRECAUTIONS AND WARNINGS

- In compliance with article 1 paragraph 2b European directive 98/79/EC the use of the in vitro diagnostic medical devices is intended by the manufacturer to secure suitability, performances and safety of the product. Therefore the test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the testkits with analyzers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.
- Only for in-vitro diagnostic use.
- All materials of human or animal origin should be regarded and handled as potentially infectious.
- All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive.
- Do not interchange reagents or strips of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- Use only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and standard/control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense reagents without splashing accurately into the wells.
- The ELISA is only designed for qualified personnel who are familiar with good laboratory practice.

12.1. Disposal Considerations

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

13. ORDERING INFORMATION

Prod. No.: TETG0430 Clostridium tetani toxin IgG ELISA (96 Determinations)

NovaTec Immundiagnostica GmbH

NovaLisa®

Coxiella burnetii (Q-Fever) Phase 1 IgG

ELISA

CE

Only for in-vitro diagnostic use

Product Number: COX1G0600 (96 Determinations)

ENGLISH

1. INTRODUCTION

Q-Fever is a disease that results from infection with small, polymorph and gram-negative bacteria called Coxiella burnetii. After an outbreak in Brisbane, Australia, the responsible organism was isolated and named Coxiella burnetii in honour of Dr. Herald Rae Cox and Sir Frank Burnet. New molecular research demonstrated a close relationship to Legionella. The zoonosis Q-Fever is found everywhere except New Zealand (no data available). There is an extensive reservoir (mainly ticks) of C. burnetii. Ticks are an important vector of the pathogen in the transmission between domestic and wildlife animals. But the ticks are unimportant in the direct infection of humans. Cattles, sheep and goats are usually the source of transmission of this microorganism to humans. However cats, dogs and rabbits are also important in this regard. In most instances humans become infected with Coxiella burnetii following inhalation of contaminated aerosols (respiratory tract). The incubation period for Q-Fever in humans is about 2 weeks. The resulting illness can be divided into acute and chronic varieties. During the acute phase of illness antibodies to the phase 2-antigen are formed. Anti phase-1 antibodies in high titers are typical for a chronic disease.
In areas where Q-Fever is endemic, 12% or more of the population have antibodies to C. burnetii. Most of the infections are subclinical or undiagnosed.
The acute infection shows symptoms of high fever, shivers, muscle pain and headache. Later on more severe diseases such as pneumonia or hepatitis can occur. Infections during pregnancy can lead to an abort or premature birth. Approximately 1% of all infections become chronic. The most frequent organ manifestation in Q-Fever is endocarditis.

Species	Disease	Symptoms (e.g.)	Transmission route
Coxiella burnetii	Q-Fever	Pneumonia white fever, headache, muscle pain; Hepatitis; Myocarditis and Endocarditis.	Inhalation of contaminated aerosols (respiratory tract). Transmission by ingestion of contaminated products such as milk or meat is likely.

Infection or presence of pathogen may be identified by:

- Cell culture
- PCR
- Serologa: e.g. ELISA, IFT

2. INTENDED USE

The Coxiella burnetii (Q-Fever) Phase 1 IgG ELISA is intended for the qualitative determination of IgG class antibodies against Coxiella burnetii (Q-Fever) Phase 1 in human serum or plasma (citrate, heparin).

3. PRINCIPLE OF THE ASSAY

The qualitative immunoenzymatic determination of specific antibodies is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique.
Microtiterplates are coated with specific antigens to bind corresponding antibodies of the sample. After washing the wells to remove all unbound sample material a horseradish peroxidase (HRP) labelled conjugate is added. This conjugate binds to the captured antibodies. In a second washing step unbound conjugate is removed. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product.
The intensity of this product is proportional to the amount of specific antibodies in the sample. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450/620 nm is read using an ELISA Microtiterplate reader.

4. MATERIALS

4.1. Reagents supplied

- **Microtiterplate:** 12 break-apart 8-well snap-off strips coated with Coxiella burnetii (Q-Fever) Phase 1 antigens; in resealable aluminium foil.
- **IgG Sample Dilution Buffer:** 1 bottle containing 100 mL of phosphate buffer (10 mM) for sample dilution; pH 7.2 ± 0.2; coloured yellow; ready to use; white cap; ≤ 0.0015% (v/v) CMIT/ MIT (3:1).
- **Stop Solution:** 1 bottle containing 15 mL sulphuric acid, 0.2 mol/L; ready to use; red cap.
- **Washing Buffer (20x conc.):** 1 bottle containing 50 mL of a 20-fold concentrated phosphate buffer (0.2 M), pH 7.2 ± 0.2, for washing the wells; white cap.
- **Conjugate:** 1 bottle containing 20 mL of peroxidase labelled antibody to human IgG in phosphate buffer (10 mM); coloured blue; ready to use; black cap.
- **TMB Substrate Solution:** 1 bottle containing 15 mL 3,3',5,5'-tetramethylbenzidine (TMB), < 0.1 %; ready to use; yellow cap.
- **Positive Control:** 1 vial containing 2 mL control; coloured yellow; ready to use; red cap; ≤ 0.02% (v/v) MIT.
- **Cut-off Control:** 1 vial containing 3 mL control; coloured yellow; ready to use; green cap; ≤ 0.02% (v/v) MIT.
- **Negative Control:** 1 vial containing 2 mL control; coloured yellow; ready to use; blue cap; ≤ 0.0015% (v/v) CMIT/ MIT (3:1).

For hazard and precautionary statements see 12.1
For potential hazardous substances please check the safety data sheet.

4.2. Materials supplied

- 1 Cover foil
- 1 Instruction for use (IFU)
- 1 Plate layout

4.3. Materials and Equipment needed

- ELISA Microtiterplate reader, equipped for the measurement of absorbance at 450/620 nm
- Incubator 37°C
- Manual or automatic equipment for rinsing Microtiterplates
- Pipettes to deliver volumes between 10 and 1000 µL
- Vortex tube mixer
- Distilled water
- Disposable tubes

5. STABILITY AND STORAGE

Store the kit at 2...8 °C. The opened reagents are stable up to the expiry date stated on the label when stored at 2...8 °C.

6. REAGENT PREPARATION

It is very important to bring all reagents and samples to room temperature (20…25 °C) and mix them before starting the test run!

6.1. Microtiterplate

The break-apart snap-off strips are coated with Coxiella burnetii (Q-Fever) Phase 1 antigens. Immediately after removal of the strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2...8 °C.

6.2. Washing Buffer (20x conc.)

Dilute Washing Buffer 1 + 19; e. g. 10 mL Washing Buffer + 190 mL distilled water. The diluted buffer is stable for 5 days at room temperature (20…25 °C). In case crystals appear in the concentrate, warm up the solution to 37°C e.g. in a water bath. Mix well before dilution.

6.3. TMB Substrate Solution

The reagent is ready to use and has to be stored at 2...8 °C, away from the light. The solution should be colourless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away.

7. SAMPLE COLLECTION AND PREPARATION

Use human serum or plasma (citrate, heparin) samples with this assay. If the assay is performed within 5 days after sample collection, the samples should be kept at 2...8 °C; otherwise they should be aliquoted and stored deep-frozen (-70…-20 °C). If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing.
Heat inactivation of samples is not recommended.

7.1. Sample Dilution

Before assaying, all samples should be diluted 1+100 with IgG Sample Dilution Buffer. Dispense 10 µL sample and 1 mL IgG Sample Dilution Buffer into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

8. ASSAY PROCEDURE

Please read the instruction for use carefully **before** performing the assay. Result reliability depends on strict adherence to the instruction for use as described. The following test procedure is only validated for manual procedure. If performing the test on ELISA automatic systems we recommend increasing the washing steps from three up to five and the volume of Washing Buffer from 300 µL to 350 µL to avoid washing effects. Pay attention to chapter 12. Prior to commencing the assay, the distribution and identification plan for all samples and standards/controls (duplicates recommended) should be carefully established on the plate layout supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder.

Perform all assay steps in the order given and without any delays.

A clean, disposable tip should be used for dispensing each standard/control and sample.

Adjust the incubator to 37 ± 1 °C.

1. Dispense 100 µL standards/controls and diluted samples into their respective wells. Leave well A1 for the Substrate Blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 1 hour ± 5 min at 37 ± 1 °C.**
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µL of Washing Buffer. Avoid overflows from the reaction wells. The interval between washing and aspiration should be > 5 sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!

   Note: Washing is important! Insufficient washing results in poor precision and false results.
5. Dispense 100 µL Conjugate into all wells except for the Substrate Blank well A1.
6. **Incubate for 30 min at room temperature (20...25 °C).** Do not expose to direct sunlight.
7. Repeat step 4.
8. Dispense 100 µL TMB Substrate Solution into all wells.
9. **Incubate for exactly 15 min at room temperature (20...25 °C) in the dark.** A blue colour occurs due to an enzymatic reaction.
10. Dispense 100 µL Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution, thereby a colour change from blue to yellow occurs.
11. Measure the absorbance at 450/620 nm within 30 min after addition of the Stop Solution.

8.1. Measurement

Adjust the ELISA Microtiterplate reader **to zero** using the **Substrate Blank**.

If - due to technical reasons - the ELISA Microtiterplate reader cannot be adjusted to zero using the Substrate Blank, subtract its absorbance value from all other absorbance values measured in order to obtain reliable results!

Measure the absorbance of all wells at **450 nm** and record the absorbance values for each standard/control and sample in the plate layout.

Bichromatic measurement using a reference wavelength of 620 nm is recommended.

Where applicable calculate the mean absorbance values of all duplicates.

9. RESULTS

9.1. Run Validation Criteria

In order for an assay run to be considered valid, these Instructions for Use have to be strictly followed and the following criteria must be met:

- **Substrate Blank**: Absorbance value **< 0.100**
- **Negative Control**: Absorbance value **< 0.200 and < Cut-off**
- **Cut-off Control**: Absorbance value **0.150 – 1.300**
- **Positive Control**: Absorbance value **> Cut-off**

If these criteria are not met, the test is not valid and must be repeated.

9.2. Calculation of Results

The Cut-off is the mean absorbance value of the Cut-off Control determinations.

Example: Absorbance value Cut-off Control 0.44 + absorbance value Cut-off control 0.42 = 0.86 / 2 = 0.43
Cut-off = 0.43

9.2.1. Results in Units [NTU]

$$\frac{\text{Sample (mean) absorbance value} \times 10}{\text{Cut-off}} = \text{[NovaTec Units = NTU]}$$

Example: $\frac{1.591 \times 10}{0.43} = 37$ NTU (Units)

9.3. Interpretation of Results

Cut-off	10 NTU	-
Positive	> 11 NTU	Antibodies against the pathogen are present. There has been a contact with the antigen (pathogen resp. vaccine).
Equivocal	9 – 11 NTU	Antibodies against the pathogen could not be detected clearly. It is recommended to repeat the test with a fresh sample in 2 to 4 weeks. If the result is equivocal again the sample is judged as **negative**.
Negative	< 9 NTU	The sample contains no antibodies against the pathogen. A previous contact with the antigen (pathogen resp. vaccine) is unlikely.
Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as serological data. In immunocompromised patients and newborns serological data only have restricted value.		

9.3.1 Antibody Isotypes and State of Infection

Serology	Significance
Phase II IgM	Characteristic of the primary antibody response May persist for several months
Phase II IgG	Characteristic of the primary antibody response May persist for several years
Phase I IgG	Characteristic of the chronic infection Occasionally during convalescent phase

10. SPECIFIC PERFORMANCE CHARACTERISTICS

The results refer to the groups of samples investigated; these are not guaranteed specifications.

For further information about the specific performance characteristics please contact NovaTec Immundiagnostica GmbH.

10.1. Precision

Intraassay	**n**	**Mean (E)**	**CV (%)**
#1	24	0.303	6.75
#2	24	0.601	7.03
#3	24	1.380	11.19
Interassay	**n**	**Mean (NTU)**	**CV (%)**
#1	12	21.41	4.84
#2	12	47.55	6.23
#3	12	2.13	8.84

10.2. Diagnostic Specificity

The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte.
It is 100% (95% confidence interval: 86.77% - 100%).

10.3. Diagnostic Sensitivity

The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte.
It is 100% (95% confidence interval: 59.04% - 100%).

10.4. Interferences

Interferences with hemolytic, lipemic or icteric samples are not observed up to a concentration of 10 mg/mL hemoglobin, 5 mg/mL triglycerides and 0.5 mg/mL bilirubin.

10.5. Cross Reactivity

Investigation of a sample panel with antibody activities to potentially cross-reacting parameters did not reveal evidence of false-positive results due to cross-reactions.

11. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or repeated freeze-thaw cycles of the sample may affect the absorbance values.

12. PRECAUTIONS AND WARNINGS

- The test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the testkits with analyzers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.
- Only for in-vitro diagnostic use.
- All materials of human or animal origin should be regarded and handled as potentially infectious.
- All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive.
- Do not interchange reagents or Microtiterplates of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- Use only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and standard/control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense reagents without splashing accurately into the wells.
- The ELISA is only designed for qualified personnel following the standards of good laboratory practice (GLP).
- For further internal quality control each laboratory should additionally use known samples.

12.1. Safety note for reagents containing hazardous substances

Reagents may contain CMIT/MIT (3:1) or MIT (refer to 4.1)
Therefore, the following hazard and precautionary statements apply.

Warning

H317	May cause an allergic skin reaction.
P261	Avoid breathing spray
P280	Wear protective gloves/ protective clothing.
P302+P352	IF ON SKIN: Wash with plenty of soap and water.
P333+P313	If skin irritation or rash occurs: Get medical advice/ attention.
P362+P364	Take off contaminated and Wash it before reuse.

Further information can be found in the safety data sheet.

12.2. Disposal Considerations

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

13. ORDERING INFORMATION

Prod. No.: COX1G0600 Coxiella burnetii (Q-Fever) Phase 1 IgG ELISA (96 Determinations)

NovaTec Immundiagnostica GmbH

NovaLisa®

Coxiella burnetii (Q-Fever) Phase 2 IgG

ELISA

CE

Only for in-vitro diagnostic use

Product Number: COX2G0600 (96 Determinations)

ENGLISH

1. INTRODUCTION

Q-Fever is a disease that results from infection with small, polymorph and gram-negative bacteria called Coxiella burnetii. After an outbreak in Brisbane, Australia, the responsible organism was isolated and named Coxiella burnetii in honour of Dr. Herald Rae Cox and Sir Frank Burnet. New molecular research demonstrated a close relationship to Legionella. The zoonosis Q-Fever is found everywhere except New Zealand (no data available). There is an extensive reservoir (mainly ticks) of C. burnetii. Ticks are an important vector of the pathogen in the transmission between domestic and wildlife animals. But the ticks are unimportant in the direct infection of humans. Cattles, sheep and goats are usually the source of transmission of this microorganism to humans. However cats, dogs and rabbits are also important in this regard. In most instances humans become infected with Coxiella burnetii following inhalation of contaminated aerosols (respiratory tract). The incubation period for Q-Fever in humans is about 2 weeks. The resulting illness can be divided into acute and chronic varieties. During the acute phase of illness antibodies to the phase 2-antigen are formed. Anti phase-1 antibodies in high titers are typical for a chronic disease.
In areas where Q-Fever is endemic, 12% or more of the population have antibodies to C. burnetii. Most of the infections are subclinical or undiagnosed.
The acute infection shows symptoms of high fever, shivers, muscle pain and headache. Later on more severe diseases such as pneumonia or hepatitis can occur. Infections during pregnancy can lead to an abort or premature birth. Approximately 1% of all infections become chronic. The most frequent organ manifestation in Q-Fever is endocarditis.

Species	Disease	Symptoms (e.g.)	Transmission route
Coxiella burnetii	Q-Fever	Pneumonia white fever, headache, muscle pain; Hepatitis; Myocarditis and Endocarditis.	Inhalation of contaminated aerosols (respiratory tract). Transmission by ingestion of contaminated products such as milk or meat is likely.

Infection or presence of pathogen may be identified:

- Cell culture
- PCR
- Serologa: e.g. ELISA, IFT

2. INTENDED USE

The Coxiella burnetii (Q-Fever) Phase 2 IgG ELISA is intended for the qualitative determination of IgG class antibodies against Coxiella burnetii (Q-Fever) Phase 2 in human serum or plasma (citrate, heparin).

3. PRINCIPLE OF THE ASSAY

The qualitative immunoenzymatic determination of specific antibodies is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique.
Microtiterplates are coated with specific antigens to bind corresponding antibodies of the sample. After washing the wells to remove all unbound sample material a horseradish peroxidase (HRP) labelled conjugate is added. This conjugate binds to the captured antibodies. In a second washing step unbound conjugate is removed. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product.
The intensity of this product is proportional to the amount of specific antibodies in the sample. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450/620 nm is read using an ELISA Microtiterplate reader.

4. MATERIALS

4.1. Reagents supplied

- **Microtiterplate:** 12 break-apart 8-well snap-off strips coated with Coxiella burnetii (Q-Fever) Phase 2 antigens; in resealable aluminium foil.
- **IgG Sample Dilution Buffer:** 1 bottle containing 100 mL of phosphate buffer (10 mM) for sample dilution; pH 7.2 ± 0.2; coloured yellow; ready to use; white cap; ≤ 0.0015% (v/v) CMIT/ MIT (3:1).
- **Stop Solution:** 1 bottle containing 15 mL sulphuric acid, 0.2 mol/L; ready to use; red cap.
- **Washing Buffer (20x conc.):** 1 bottle containing 50 mL of a 20-fold concentrated phosphate buffer (0.2 M), pH 7.2 ± 0.2, for washing the wells; white cap.
- **Conjugate:** 1 bottle containing 20 mL of peroxidase labelled antibody to human IgG in phosphate buffer (10 mM); coloured blue; ready to use; black cap.
- **TMB Substrate Solution:** 1 bottle containing 15 mL 3,3',5,5'-tetramethylbenzidine (TMB), < 0.1 %; ready to use; yellow cap.
- **Positive Control:** 1 vial containing 2 mL control; coloured yellow; ready to use; red cap; ≤ 0.02% (v/v) MIT.
- **Cut-off Control:** 1 vial containing 3 mL control; coloured yellow; ready to use; green cap; ≤ 0.02% (v/v) MIT.
- **Negative Control:** 1 vial containing 2 mL control; coloured yellow; ready to use; blue cap; ≤ 0.0015% (v/v) CMIT/ MIT (3:1).

For hazard and precautionary statements see 12.1
For potential hazardous substances please check the safety data sheet.

4.2. Materials supplied

- 1 Cover foil
- 1 Instruction for use (IFU)
- 1 Plate layout

4.3. Materials and Equipment needed

- ELISA Microtiterplate reader, equipped for the measurement of absorbance at 450/620 nm
- Incubator 37°C
- Manual or automatic equipment for rinsing Microtiterplates
- Pipettes to deliver volumes between 10 and 1000 µL
- Vortex tube mixer
- Distilled water
- Disposable tubes

5. STABILITY AND STORAGE

Store the kit at 2...8 °C. The opened reagents are stable up to the expiry date stated on the label when stored at 2...8 °C.

6. REAGENT PREPARATION

It is very important to bring all reagents and samples to room temperature (20…25 °C) and mix them before starting the test run!

6.1. Microtiterplate

The break-apart snap-off strips are coated with Coxiella burnetii (Q-Fever) Phase 2 antigens. Immediately after removal of the strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2...8 °C.

6.2. Washing Buffer (20x conc.)

Dilute Washing Buffer 1 + 19; e. g. 10 mL Washing Buffer + 190 mL distilled water. The diluted buffer is stable for 5 days at room temperature (20…25 °C). In case crystals appear in the concentrate, warm up the solution to 37 °C e.g. in a water bath. Mix well before dilution.

6.3. TMB Substrate Solution

The reagent is ready to use and has to be stored at 2...8 °C, away from the light. The solution should be colourless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away.

7. SAMPLE COLLECTION AND PREPARATION

Use human serum or plasma (citrate, heparin) samples with this assay. If the assay is performed within 5 days after sample collection, the samples should be kept at 2...8 °C; otherwise they should be aliquoted and stored deep-frozen (-70…-20 °C). If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing.
Heat inactivation of samples is not recommended.

7.1. Sample Dilution

Before assaying, all samples should be diluted 1+100 with IgG Sample Dilution Buffer. Dispense 10 µL sample and 1 mL IgG Sample Dilution Buffer into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

8. ASSAY PROCEDURE

Please read the instruction for use carefully **before** performing the assay. Result reliability depends on strict adherence to the instruction for use as described. The following test procedure is only validated for manual procedure. If performing the test on ELISA automatic systems we recommend increasing the washing steps from three up to five and the volume of Washing Buffer from 300 µL to 350 µL to avoid washing effects. Pay attention to chapter 12. Prior to commencing the assay, the distribution and identification plan for all samples and standards/controls (duplicates recommended) should be carefully established on the plate layout supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder.

Perform all assay steps in the order given and without any delays.

A clean, disposable tip should be used for dispensing each standard/control and sample.

Adjust the incubator to 37 ± 1 °C.

1. Dispense 100 µL standards/controls and diluted samples into their respective wells. Leave well A1 for the Substrate Blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 1 hour ± 5 min at 37 ± 1 °C.**
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µL of Washing Buffer. Avoid overflows from the reaction wells. The interval between washing and aspiration should be > 5 sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!

   Note: Washing is important! Insufficient washing results in poor precision and false results.
5. Dispense 100 µL Conjugate into all wells except for the Substrate Blank well A1.
6. **Incubate for 30 min at room temperature (20...25 °C).** Do not expose to direct sunlight.
7. Repeat step 4.
8. Dispense 100 µL TMB Substrate Solution into all wells.
9. **Incubate for exactly 15 min at room temperature (20...25 °C) in the dark.** A blue colour occurs due to an enzymatic reaction.
10. Dispense 100 µL Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution, thereby a colour change from blue to yellow occurs.
11. Measure the absorbance at 450/620 nm within 30 min after addition of the Stop Solution.

8.1. Measurement

Adjust the ELISA Microtiterplate reader **to zero** using the **Substrate Blank**.

If - due to technical reasons - the ELISA Microtiterplate reader cannot be adjusted to zero using the Substrate Blank, subtract its absorbance value from all other absorbance values measured in order to obtain reliable results!

Measure the absorbance of all wells at **450 nm** and record the absorbance values for each standard/control and sample in the plate layout.

Bichromatic measurement using a reference wavelength of 620 nm is recommended.

Where applicable calculate the mean absorbance values of all duplicates.

9. RESULTS

9.1. Run Validation Criteria

In order for an assay run to be considered valid, these Instructions for Use have to be strictly followed and the following criteria must be met:

- **Substrate Blank**: Absorbance value **< 0.100**
- **Negative Control**: Absorbance value **< 0.200 and < Cut-off**
- **Cut-off Control**: Absorbance value **0.150 – 1.300**
- **Positive Control**: Absorbance value **> Cut-off**

If these criteria are not met, the test is not valid and must be repeated.

9.2. Calculation of Results

The Cut-off is the mean absorbance value of the Cut-off Control determinations.

Example: Absorbance value Cut-off Control 0.44 + absorbance value Cut-off control 0.42 = 0.86 / 2 = 0.43
Cut-off = 0.43

9.2.1. Results in Units [NTU]

$$\frac{\text{Sample (mean) absorbance value} \times 10}{\text{Cut-off}} = \text{[NovaTec Units = NTU]}$$

Example: $\frac{1.591 \times 10}{0.43} = 37$ NTU (Units)

9.3. Interpretation of Results

Cut-off	10 NTU	-
Positive	> 11 NTU	Antibodies against the pathogen are present. There has been a contact with the antigen (pathogen resp. vaccine).
Equivocal	9 – 11 NTU	Antibodies against the pathogen could not be detected clearly. It is recommended to repeat the test with a fresh sample in 2 to 4 weeks. If the result is equivocal again the sample is judged as **negative**.
Negative	< 9 NTU	The sample contains no antibodies against the pathogen. A previous contact with the antigen (pathogen resp. vaccine) is unlikely.
Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as serological data. In immunocompromised patients and newborns serological data only have restricted value.		

9.3.1. Antibody Isotypes and State of Infection

Serology	Significance
Phase II IgM	Characteristic of the primary antibody response May persist for several months
Phase II IgG	Characteristic of the primary antibody response May persist for several years
Phase I IgG	Characteristic of the chronic infection Occasionally during convalescent phase

10. SPECIFIC PERFORMANCE CHARACTERISTICS

The results refer to the groups of samples investigated; these are not guaranteed specifications.

For further information about the specific performance characteristics please contact NovaTec Immundiagnostica GmbH.

10.1. Precision

Intraassay	**n**	**Mean (E)**	**CV (%)**
#1	24	0.428	9.69
#2	24	0.794	8.51
#3	24	0.645	6.27
Interassay	**n**	**Mean (NTU)**	**CV (%)**
#1	12	17.91	8.48
#2	12	14.30	12.09
#3	12	1.93	7.38

10.2. Diagnostic Specificity

The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte.
It is 100% (95% confidence interval: 91.4% - 100%).

10.3. Diagnostic Sensitivity

The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte.
It is 100% (95% confidence interval: 39.76% - 100%).

10.4. Interferences

Interferences with hemolytic, lipemic or icteric samples are not observed up to a concentration of 10 mg/mL hemoglobin, 5 mg/mL triglycerides and 0.5 mg/mL bilirubin.

10.5. Cross Reactivity

Investigation of a sample panel with antibody activities to potentially cross-reacting parameters did not reveal evidence of false-positive results due to cross-reactions.

11. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or repeated freeze-thaw cycles of the sample may affect the absorbance values.

12. PRECAUTIONS AND WARNINGS

- The test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the testkits with analyzers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.
- Only for in-vitro diagnostic use.
- All materials of human or animal origin should be regarded and handled as potentially infectious.
- All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive.
- Do not interchange reagents or Microtiterplates of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- Use only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and standard/control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense reagents without splashing accurately into the wells.
- The ELISA is only designed for qualified personnel following the standards of good laboratory practice (GLP).
- For further internal quality control each laboratory should additionally use known samples.

12.1. Safety note for reagents containing hazardous substances

Reagents may contain CMIT/MIT (3:1) or MIT (refer to 4.1)
Therefore, the following hazard and precautionary statements apply.

Warning

H317	May cause an allergic skin reaction.
P261	Avoid breathing spray
P280	Wear protective gloves/ protective clothing.
P302+P352	IF ON SKIN: Wash with plenty of soap and water.
P333+P313	If skin irritation or rash occurs: Get medical advice/ attention.
P362+P364	Take off contaminated and Wash it before reuse.

Further information can be found in the safety data sheet.

12.2. Disposal Considerations

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

13. ORDERING INFORMATION

Prod. No.: COX2G0600 Coxiella burnetii (Q-Fever) Phase 2 IgG ELISA (96 Determinations)

NovaTec Immundiagnostica GmbH

NovaLisa®

Coxiella burnetii (Q-Fever) Phase 2 IgM

ELISA

CE

Only for in-vitro diagnostic use

Product Number: COX2M0600 (96 Determinations)

ENGLISH

1. INTRODUCTION

Q-Fever is a disease that results from infection with small, polymorph and gram-negative bacteria called Coxiella burnetii. After an outbreak in Brisbane, Australia, the responsible organism was isolated and named Coxiella burnetii in honour of Dr. Herald Rae Cox and Sir Frank Burnet. New molecular research demonstrated a close relationship to Legionella. The zoonosis Q-Fever is found everywhere except New Zealand (no data available). There is an extensive reservoir (mainly ticks) of C. burnetii. Ticks are an important vector of the pathogen in the transmission between domestic and wildlife animals. But the ticks are unimportant in the direct infection of humans. Cattles, sheep and goats are usually the source of transmission of this microorganism to humans. However cats, dogs and rabbits are also important in this regard. In most instances humans become infected with Coxiella burnetii following inhalation of contaminated aerosols (respiratory tract). The incubation period for Q-Fever in humans is about 2 weeks. The resulting illness can be divided into acute and chronic varieties. During the acute phase of illness antibodies to the phase II-antigen are formed. Anti phase-I antibodies in high titers are typical for a chronic disease.
In areas where Q-Fever is endemic, 12% or more of the population have antibodies to C. burnetii. Most of the infections are subclinical or undiagnosed.
The acute infection shows symptoms of high fever, shivers, muscle pain and headache. Later on more severe diseases such as pneumonia or hepatitis can occur. Infections during pregnancy can lead to an abort or premature birth. Approximately 1% of all infections become chronic. The most frequent organ manifestation in Q-Fever is endocarditis.

Species	Disease	Symptoms (e.g.)	Transmission route
Coxiella burnetii	Q-Fever	Pneumonia white fever, headache, muscle pain; Hepatitis; Myocarditis and Endocarditis.	Inhalation of contaminated aerosols (respiratory tract). Transmission by ingestion of contaminated products such as milk or meat is likely.

Infection or presence of pathogen may be identified by:

- Cell culture
- PCR
- Serologa: e.g. ELISA, IFT

2. INTENDED USE

The Coxiella burnetii (Q-Fever) Phase 2 IgM ELISA is intended for the qualitative determination of IgM class antibodies against Coxiella burnetii (Q-Fever) Phase 2 in the early stages of infection in human serum or plasma (citrate, heparin).

3. PRINCIPLE OF THE ASSAY

The qualitative immunoenzymatic determination of specific antibodies is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique.
Microtiterplates are coated with specific antigens to bind corresponding antibodies of the sample. After washing the wells to remove all unbound sample material a horseradish peroxidase (HRP) labelled conjugate is added. This conjugate binds to the captured antibodies. In a second washing step unbound conjugate is removed. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product.
The intensity of this product is proportional to the amount of specific antibodies in the sample. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450/620 nm is read using an ELISA Microtiterplate reader.

4. MATERIALS

4.1. Reagents supplied

- **Microtiterplate:** 12 break-apart 8-well snap-off strips coated with Coxiella burnetii (Q-Fever) Phase 2 antigens; in resealable aluminium foil.
- **IgM Sample Dilution Buffer:** 1 bottle containing 100 mL of phosphate buffer (10 mM) for sample dilution; pH 7.2 ± 0.2; anti-human IgG (RF Absorbent); coloured green; ready to use; white cap; ≤ 0.0015% (v/v) CMIT/ MIT (3:1).
- **Stop Solution:** 1 bottle containing 15 mL sulphuric acid, 0.2 mol/L; ready to use; red cap.
- **Washing Buffer (20x conc.):** 1 bottle containing 50 mL of a 20-fold concentrated phosphate buffer (0.2 M), pH 7.2 ± 0.2, for washing the wells; white cap.
- **Conjugate:** 1 bottle containing 20 mL of peroxidase labelled antibody to human IgM in phosphate buffer (10 mM); coloured red; ready to use; black cap.
- **TMB Substrate Solution:** 1 bottle containing 15 mL 3,3',5,5'-tetramethylbenzidine (TMB), < 0.1 %; ready to use; yellow cap.
- **Positive Control:** 1 vial containing 2 mL control; coloured yellow; ready to use; red cap; ≤ 0.02% (v/v) MIT.
- **Cut-off Control:** 1 vial containing 3 mL control; coloured yellow; ready to use; green cap; ≤ 0.02% (v/v) MIT.
- **Negative Control:** 1 vial containing 2 mL control; coloured yellow; ready to use; blue cap; ≤ 0.0015% (v/v) CMIT/ MIT (3:1).

For hazard and precautionary statements see 12.1.
For potential hazardous substances please check the safety data sheet.

4.2. Materials supplied

- 1 Cover foil
- 1 Instruction for use (IFU)
- 1 Plate layout

4.3. Materials and Equipment needed

- ELISA Microtiterplate reader, equipped for the measurement of absorbance at 450/620 nm
- Incubator 37 °C
- Manual or automatic equipment for rinsing Microtiterplates
- Pipettes to deliver volumes between 10 and 1000 µL
- Vortex tube mixer
- Distilled water
- Disposable tubes

5. STABILITY AND STORAGE

Store the kit at 2...8 °C. The opened reagents are stable up to the expiry date stated on the label when stored at 2...8 °C.

6. REAGENT PREPARATION

It is very important to bring all reagents and samples to room temperature (20…25 °C) and mix them before starting the test run!

6.1. Microtiterplate

The break-apart snap-off strips are coated with Coxiella burnetii (Q-Fever) Phase 2 antigens. Immediately after removal of the strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2...8 °C.

6.2. Washing Buffer (20x conc.)

Dilute Washing Buffer 1 + 19; e. g. 10 mL Washing Buffer + 190 mL distilled water. The diluted buffer is stable for 5 days at room temperature (20…25 °C). In case crystals appear in the concentrate, warm up the solution to 37°C e.g. in a water bath. Mix well before dilution.

6.3. TMB Substrate Solution

The reagent is ready to use and has to be stored at 2...8 °C, away from the light. The solution should be colourless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away.

7. SAMPLE COLLECTION AND PREPARATION

Use human serum or plasma (citrate, heparin) samples with this assay. If the assay is performed within 5 days after sample collection, the samples should be kept at 2...8 °C; otherwise they should be aliquoted and stored deep-frozen (-70…-20 °C). If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing.
Heat inactivation of samples is not recommended.

7.1. Sample Dilution

Before assaying, all samples should be diluted 1+100 with IgM Sample Dilution Buffer. Dispense 10 µL sample and 1 mL IgM Sample Dilution Buffer into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

8. ASSAY PROCEDURE

Please read the instruction for use carefully **before** performing the assay. Result reliability depends on strict adherence to the instruction for use as described. The following test procedure is only validated for manual procedure. If performing the test on ELISA automatic systems we recommend increasing the washing steps from three up to five and the volume of Washing Buffer from 300 µL to 350 µL to avoid washing effects. Pay attention to chapter 12. Prior to commencing the assay, the distribution and identification plan for all samples and standards/controls (duplicates recommended) should be carefully established on the plate layout supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder.

Perform all assay steps in the order given and without any delays.

A clean, disposable tip should be used for dispensing each standard/control and sample.

Adjust the incubator to 37 ± 1 °C.

1. Dispense 100 µL standards/controls and diluted samples into their respective wells. Leave well A1 for the Substrate Blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 1 hour ± 5 min at 37 ± 1 °C.**
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µL of Washing Buffer. Avoid overflows from the reaction wells. The interval between washing and aspiration should be > 5 sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!

   Note: Washing is important! Insufficient washing results in poor precision and false results.
5. Dispense 100 µL Conjugate into all wells except for the Substrate Blank well A1.
6. **Incubate for 30 min at room temperature (20...25 °C).** Do not expose to direct sunlight.
7. Repeat step 4.
8. Dispense 100 µL TMB Substrate Solution into all wells.
9. **Incubate for exactly 15 min at room temperature (20...25 °C) in the dark.** A blue colour occurs due to an enzymatic reaction.
10. Dispense 100 µL Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution, thereby a colour change from blue to yellow occurs.
11. Measure the absorbance at 450/620 nm within 30 min after addition of the Stop Solution.

8.1. Measurement

Adjust the ELISA Microtiterplate reader **to zero** using the **Substrate Blank**.

If - due to technical reasons - the ELISA Microtiterplate reader cannot be adjusted to zero using the Substrate Blank, subtract its absorbance value from all other absorbance values measured in order to obtain reliable results!

Measure the absorbance of all wells at **450 nm** and record the absorbance values for each standard/control and sample in the plate layout.

Bichromatic measurement using a reference wavelength of 620 nm is recommended.

Where applicable calculate the mean absorbance values of all duplicates.

9. RESULTS

9.1. Run Validation Criteria

In order for an assay run to be considered valid, these Instructions for Use have to be strictly followed and the following criteria must be met:

- **Substrate Blank**: Absorbance value **< 0.100**
- **Negative Control**: Absorbance value **< 0.200 and < Cut-off**
- **Cut-off Control**: Absorbance value **0.150 – 1.300**
- **Positive Control**: Absorbance value **> Cut-off**

If these criteria are not met, the test is not valid and must be repeated.

9.2. Calculation of Results

The Cut-off is the mean absorbance value of the Cut-off Control determinations.

Example: Absorbance value Cut-off Control 0.44 + absorbance value Cut-off control 0.42 = 0.86 / 2 = 0.43
Cut-off = 0.43

9.2.1. Results in Units [NTU]

$$\frac{\text{Sample (mean) absorbance value} \times 10}{\text{Cut-off}} = \text{[NovaTec Units = NTU]}$$

Example: $\frac{1.591 \times 10}{0.43} = 37$ NTU (Units)

9.3. Interpretation of Results

Cut-off	10 NTU	-
Positive	> 11 NTU	Antibodies against the pathogen are present. There has been a contact with the antigen (pathogen resp. vaccine).
Equivocal	9 – 11 NTU	Antibodies against the pathogen could not be detected clearly. It is recommended to repeat the test with a fresh sample in 2 to 4 weeks. If the result is equivocal again the sample is judged as **negative**.
Negative	< 9 NTU	The sample contains no antibodies against the pathogen. A previous contact with the antigen (pathogen resp. vaccine) is unlikely.
Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as serological data. In immunocompromised patients and newborns serological data only have restricted value.		

9.3.1. Antibody Isotypes and State of Infection

Serology	Significance
Phase II IgM	Characteristic of the primary antibody response May persist for several months
Phase II IgG	Characteristic of the primary antibody response May persist for several years
Phase I IgG	Characteristic of the chronic infection Occasionally during convalescent phase

10. SPECIFIC PERFORMANCE CHARACTERISTICS

The results refer to the groups of samples investigated; these are not guaranteed specifications.

For further information about the specific performance characteristics please contact NovaTec Immundiagnostica GmbH.

10.1. Precision

Intraassay	n	Mean (E)	CV (%)
#1	24	0.485	3.11
#2	24	0.824	3.86
#3	24	0.794	3.23
Interassay	**n**	**Mean (NTU)**	**CV (%)**
#1	12	20.76	3.16
#2	12	14.60	8.05
#3	12	1.02	6.76

10.2. Diagnostic Specificity

The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte.
It is 95.95 % (95 % confidence interval: 88.61 % - 99.16 %).

10.3. Diagnostic Sensitivity

The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte.
It is 95.74 % (95 % confidence interval: 85.46 % - 99.48 %).

10.4. Interferences

Interferences with hemolytic, lipemic or icteric samples are not observed up to a concentration of 10 mg/mL hemoglobin, 5 mg/mL triglycerides and 0.5 mg/mL bilirubin.

10.5. Cross Reactivity

Cross reactivity with antibodies against CMV, Dengue virus, EBV and Mycoplasma cannot be excluded.

11. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or repeated freeze-thaw cycles of the sample may affect the absorbance values.

12. PRECAUTIONS AND WARNINGS

- The test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the testkits with analyzers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.
- Only for in-vitro diagnostic use.
- All materials of human or animal origin should be regarded and handled as potentially infectious.
- All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive.
- Do not interchange reagents or Microtiterplates of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- Use only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and standard/control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense reagents without splashing accurately into the wells.
- The ELISA is only designed for qualified personnel following the standards of good laboratory practice (GLP).
- For further internal quality control each laboratory should additionally use known samples.

12.1. Safety note for reagents containing hazardous substances

Reagents may contain CMIT/MIT (3:1) or MIT (refer to 4.1)
Therefore, the following hazard and precautionary statements apply.

Warning

H317	May cause an allergic skin reaction.
P261	Avoid breathing spray.
P280	Wear protective gloves/ protective clothing.
P302+P352	IF ON SKIN: Wash with plenty of soap and water.
P333+P313	If skin irritation or rash occurs: Get medical advice/ attention.
P362+P364	Take off contaminated and Wash it before reuse.

Further information can be found in the safety data sheet

12.2. Disposal Considerations

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

13. ORDERING INFORMATION

Prod. No.: COX2M0600 Coxiella burnetii (Q-Fever) Phase 2 IgM ELISA (96 Determinations)

Instructions For Use

Serazym® Adenovirus

Enzyme immunoassay for detection of *Adenovirus* in faecal samples

REF E-017 Σ 96 REF E-017-A2 Σ 2x 96 IVD *In-vitro*-diagnostic medical device CE

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Introduction

Adenovirus is the causative agent of respiratory tract infections, conjunctivitis or enteritis. *Adenovirus* infections are spread via faecal-oral transmission or aerosols (1). Most infections are mild to moderate and do not last longer than one week. *Adenovirus* is responsible for 2 - 8% of respiratory tract infections and 7 - 17% of diarrheal diseases in children (2). Thirty percent of viral diarrheas in immunocompromised patients are caused by *Adenovirus* infections (1). The diagnosis of *Adenovirus* infections is preferably done by direct detection in faecal specimens or swabs. Due to the difficulty of *Adenovirus* culture on tissue or cell culture virus detection so far has been performed by electron microscopy. Meanwhile immunological methods like enzyme immunoassay (ELISA) have been developed for antigen detection (3). ELISA techniques are based on poly- and/or monoclonal antibodies to the protein hexon representing the major part of the virus capsid.

References:

1. Mentel, R. und Döhner, L. (1996): „Humane Adenoviren.“ Diagnostische Bibliothek Band 1 Virusdiagnostik, Hrsg. Tomas Porstmann, Blackwell Wissenschafts-Verlag Berlin, Wien 1996, S. 103-114
2. Schoenemann W. (1988): “Bedeutung von Adenovirusinfektionen im Säuglings- und Kleinkindesalter” Monatsschrift Kinderheilkunde 136: 680-685
3. August, M. J. and Warford, A. L. (1987): „Evaluation of a Commercial Monoclonal Antibody for Detection of Adenovirus Antigen.” Journal Of Clinical Microbiology, Vol. 25, No. 11: 2233-2235

Intended Use

***Serazym*® Adenovirus is an *in-vitro*-diagnostic medical device for direct detection of *Adenovirus* in faecal samples.**

Principle Of The Test

Serazym® Adenovirus is a one-step enzyme immunoassay on the basis of monoclonal antibodies against an epitope of the capsid protein hexon, common to all human pathogenic *Adenovirus* serotypes. Diluted stool specimens and horseradish peroxidase (HRP) labelled monoclonal anti-Adenovirus-antibodies are dispensed simultaneously into the wells of a microtitration plate coated with monoclonal anti-Adenovirus-antibodies. After an incubation time of 60 min at room temperature (RT) unbound components are removed by a washing step. HRP converts the subsequently added colourless substrate solution of 3,3',5,5'-Tetramethylbenzidine (TMB) within a 10 min reaction time at room temperature protected from light into a blue product. The enzyme reaction is terminated by sulphuric acid dispensed into the wells turning the solution from blue to yellow. The optical density (OD) of the solution read at 450 / ≥ 620 nm is directly proportional to the specifically bound amount of *Adenovirus*. Considering the cut-off value results are interpreted as positive or negative.

Test Components

			For 96 Wells	For 2x 96 Wells
1	WELLS	**Microtitration plate** coated with monoclonal anti-Adenovirus-antibodies (mouse)	12 single breakable 8-well strips colour coding violet vacuum-sealed with desiccant	2x 12 single breakable 8-well strips colour coding violet vacuum-sealed with desiccant
2	WASHBUF CONC 10x	**Wash buffer** 10-fold	100 ml concentrate for 1000 ml solution white cap	2x 100 ml concentrate for 2x 1000 ml solution white cap
3	DIL	**Sample diluent**	100 ml · ready to use coloured yellow black cap	2x 100 ml · ready to use coloured yellow black cap
4	CONTROL +	**Positive control** *Adenovirus* antigen Ad 41 (inactivated)	1.5 ml · ready to use coloured blue red cap	3.0 ml · ready to use coloured blue red cap
5	CONTROL –	**Negative control** *Adenovirus* negative sample	1.5 ml · ready to use coloured blue green cap	3.0 ml · ready to use coloured blue green cap
6	CONJ HRP	**HRP-conjugate** HRP-labelled, monoclonal anti-Adenovirus-antibodies (mouse)	12 ml · ready to use coloured green brown cap	24 ml · ready to use coloured green brown cap
7	SUBSTR TMB	**Substrate** 3,3',5,5'-Tetramethylbenzidine and hydrogen peroxide	15 ml · ready to use blue cap	28 ml · ready to use blue cap
8	STOP	**Stop solution** 0.25 M sulphuric acid	15 ml · ready to use yellow cap	28 ml · ready to use yellow cap

Preparation And Storage Of Samples

Collection and storage
Stool samples should be stored at 2…8°C immediately after collection and processed within 72 hours. Longer storage is possible at -20°C. Repeated freezing and thawing of samples should be avoided. Stool samples already diluted with the *Serazym*® sample diluent can be stored for up to 72 h at 2…8°C before testing in the ELISA.

Preparation
Quickly thaw frozen samples. Warm samples to room temperature and mix well.
The *Serazym*® Adenovirus can be performed with 1 : 6 or 1 : 11 diluted specimens. In case of additional testing of the same sample in the *Serazym*® Norovirus, the *Serazym*® Campylobacter or the *Serazym*® Clostridium difficile Toxin A+B the 1 : 6 dilution is recommended.

Preparation of a 1 : 11 sample dilution:
Pipette 1000 µl of sample diluent into a clean tube. Using a disposable stirring rod transfer about 100 mg (diameter about 2 - 3 mm) of faeces if solid or pipette 100 µl if liquid into the tube and suspend thoroughly. If necessary, sediment floating particles by a centrifugation step with a micro centrifuge for one min at maximum speed.

Preparation of a 1 : 6 sample dilution:
Pipette 1000 µl of sample diluent into a clean tube. Using a disposable stirring rod transfer about 200 mg (diameter about 4 - 6 mm) of faeces if solid or pipette 200 µl if liquid into the tube and suspend thoroughly. If necessary, sediment floating particles by a centrifugation step with a micro centrifuge for one min at maximum speed.

Materials Required But Not Provided

Micropipettes · multi-channel pipette or multi-pipette · reagent container for multi-channel pipette · 8-channel wash comb with vacuum pump and waste bottle or microplate washer · microplate reader with optical filters of 450 nm for measurement and ≥ 620 nm for reference · distilled or deionized water · glassware · tubes (2 ml) for sample preparation

Preparation And Storage Of Reagents

Kit size and expiry
One kit is designed for 1x 96 or 2x 96 determinations. The expiry date of each component is reported on its respective label; that of the complete kit on the outer box label. Upon receipt, all test components have to be kept at 2…8°C, preferably in the original kit box. After opening all kit components are stable for at least 2 months, provided proper storage. The ready to use wash solution can be used for at least 30 days when stored at 2…8°C.

Reagent preparation
Allow all components to reach room temperature prior to use in the assay. The microtitration plate is vacuum-sealed in a foil with desiccant. The plate consists of a frame and strips with breakable wells. Allow the sealed plate to reach room temperature before opening. Unused wells should be stored refrigerated and protected from moisture in the original cover carefully resealed.
Prepare a sufficient amount of wash solution by diluting the 10-fold concentrated wash buffer 1 + 9 with distilled or deionized water.
For Example: 10 ml wash buffer concentrate *(2)* + 90 ml distilled or deionized water.

Assay Procedure

Dilute samples with sample diluent *(3)* 1 : 11 or 1 : 6, e.g. 100 mg or 100 µl stool + 1.0 ml (1 : 11) sample diluent *(3)* or 200 mg or 200 µl stool + 1.0 ml (1 : 6) sample diluent *(3)*.
Avoid any time shift during dispensing of reagents and samples.
Make sure the soak time of the wash buffer in the wells is at least 5 seconds per wash cycle and that the remaining fluid is completely drained in every single wash cycle!
Avoid light exposure of the TMB substrate solution!

Working steps

1. Warm all reagents to room temperature (RT) before use. Mix gently without causing foam.
2. Dispense 2 drops (or 75 µl) **CONJ HRP** HRP-conjugate *(6)* per well and
3. Pipette: 75 µl **CONTROL +** positive control *(4)*
   75 µl **CONTROL –** negative control *(5)*
   50 µl **diluted sample**, mix gently.
4. Cover plate and incubate for 60 min at RT.
5. Decant, then wash each well 5x with 300 µl wash solution (diluted from *(2)*) and tap dry onto absorbent paper.
6. Dispense 2 drops (or 75 µl) **SUBSTR TMB** substrate *(7)* per well.
7. Incubate for 10 min at RT protected from light.
8. Dispense 2 drops (or 75 µl) **STOP** stop solution *(8)* per well, mix gently.
9. Read OD at 450 nm / ≥ 620 nm with a microplate reader within 30 min after reaction Stop.

Result Interpretation

Qualitative evaluation
Cut-off determination: OD negative control + 0.20
Samples with OD values equal with or higher than the cut-off are considered positive, samples with OD values below the cut-off are considered negative for *Adenovirus* antigen.

Reference Values

Serazym® Adenovirus	
Positive	≥ Cut-off
Negative	< Cut-off

It is recommended that each laboratory establishes its own normal and pathological reference ranges as usually done for other diagnostic parameters, too. Therefore, the mentioned reference values provide a guide only to values which might be expected.

Test validity
The test run is valid if:

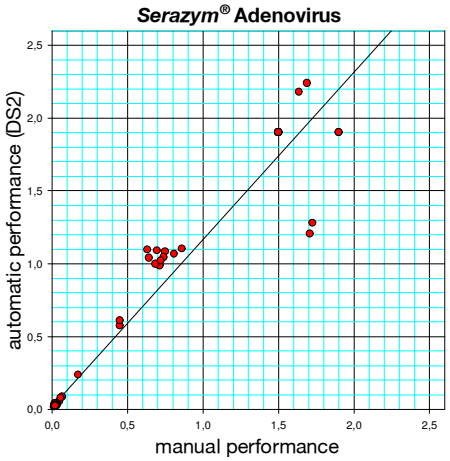
- the mean OD of the negative control is ≤ 0.20 (manual performance)
  ≤ 0.30 (automatic performance)
- the mean OD of the positive control is ≥ 1.20

If the above mentioned quality criteria are not met, repeat the test and make sure that the test procedure is followed correctly (incubation times and temperatures, sample and wash buffer dilution, wash steps etc.). In case of repeated failure of the quality criteria contact your supplier.

Limitations of the procedure
There is no correlation between measured absorbance and seriousness of the infection. It is also not allowed to correlate absorbances of the samples with that of the positive control. Cross contamination of reagents and samples can produce false positive results. Incorrect dilutions, not sufficiently homogenized samples or solid particles after centrifugation of the suspension can cause false negative as well as false positive results. A negative test result not necessarily excludes an *Adenovirus* infection. Inhomogeneous virus distribution in the sample can cause false negative results. The investigation of samples that were taken beyond the acute phase of the disease can cause false negative results, because the number of virus particles has decreased under the detection limit of the test. It is therefore recommended to take samples within the acute phase of the disease where a maximum number of excreted virus particles are to be expected. A final interpretation of the test results should consider clinical findings as well.

Automatic Processing
Performing the *Serazym*® Adenovirus on fully automated microplate processors (e.g. DS2, DSX) may cause elevated absorbances in comparison to the manual procedure due to individual differences concerning wash procedures and general technical specifications of the equipment. In these cases a maximum value of 0.3 absorbance units is permissible for the negative control. It is recommended to use a wash procedure including 10 seconds soak time per strip and wash step followed by a wash step with distilled or deionized water with 10 seconds of soak time after the final wash step of each wash cycle. If necessary the number of washing steps can be enhanced from 5x to 7x - 8x.

Correlation: manual – automatic processing
A panel of 110 stool specimens was investigated in parallel by manual and automatic processing method (DS2, Dynex Technologies) resp. The correlation was calculated with r = 0.974.



Performance Characteristics

Precision

Intra-assay coefficient of variation (CV) in the *Serazym*® Adenovirus from 8-fold determinations of samples:

sample	mean OD	standard deviation	CV (%)
1	2.792	0.160	5.7
2	2.059	0.167	8.1
3	1.368	0.094	6.9
4	0.718	0.068	9.4

Inter-assay coefficient of variation (CV) in the *Serazym*® Adenovirus in 6 different test runs from 8-fold determinations of samples:

sample	mean OD	standard deviation	CV (%)
1	1.850	0.107	5.8
2	1.057	0.069	6.5
3	0.574	0.042	7.3
4	0.312	0.030	9.6

Lower detection limit

The lower detection limit of *Adenovirus* antigen in the *Serazym*® Adenovirus was determined by titration of purified *Adenovirus* antigen (hexon). Lower detection limit: 6 ng / ml

Specificity and sensitivity

A total of 330 stool samples were investigated in parallel in the *Serazym*® Adenovirus and in another commercially available ELISA.

	comparative ELISA positive	comparative ELISA negative
***Serazym*® ELISA positive**	55	1
***Serazym*® ELISA negative**	2	272

Specifity: 99.6% · Sensitivity: 96.5%

Cross reactivity

Stool samples positive for one of the subsequent pathogens have been tested with the *Serazym*® Adenovirus and showed no cross reactivity:
Rotavirus (n = 10), *Astrovirus* (n = 8), *Norovirus* (n = 31), *Clostridium difficile* (n = 11), *Campylobacter jejuni* (n = 7), *Campylobacter coli* (n = 1), *Salmonella enteritidis* (n = 18), *Giardia lamblia* (n = 1).

Negative stool specimens have been spiked with $\geq 10^8$ colony forming units of the following microorganisms and tested negative with the *Serazym*® ELISA (OD 450 / 620 nm < Cut-Off):

Aeromonas hydrophila	(ATCC 7966)	*Klebsiella pneumoniae*	(ATCC 13883)
Bacillus cereus	(ATCC 11778)	*Peptostreptococcus anaerobius*	(ATCC 27337)
Bacillus subtilis	(ATCC 6633)	*Proteus vulgaris*	(ATCC 8427)
Bacteroides fragilis	(ATCC 25285)	*Pseudomonas aeruginosa*	(ATCC 10145)
Candida albicans	(ATCC 10231)	*Salmonella enterica Serovar enteritidis*	(ATCC 13076)
Campylobacter coli	(ATCC 33559)	*Salmonella enterica Serovar typhimurium*	(ATCC 14028)
Campylobacter jejuni	(ATCC 33291)	*Shigella flexneri*	(ATCC 12022)
Citrobacter freundii	(ATCC 8090)	*Shigella sonnei*	(ATCC 25931)
Clostridium sordellii	(ATCC 9714)	*Staphylococcus aureus*	(ATCC 25923)
Enterobacter aerogenes	(ATCC 13048)	*Staphylococcus epidermidis*	(ATCC 12228)
Enterobacter cloacae	(ATCC 13047)	*Vibrio parahaemolyticus*	(ATCC 17802)
Enterococcus faecalis	(ATCC 29212)	*Vibrio cholerae*	clinical isolate
Escherichia coli	(ATCC 25922)	*Yersinia enterocolitica Serotyp 03, 09*	clinical isolates

Interference
None of the following substances added to positive and negative stool samples showed a significant impact on the test result:
Barium sulfate (5%), Buscopan® (2 mg/ml), Cyclamate (5%), Diclofenac (2 mg/ml), Hemoglobine human (5 mg/ml), Blood human (1,25%), Hylak® N (5%), Iberogast® (5%), Immodium® akut duo (0.2/12.5 mg/ml), Loperamide (0.2 mg/ml), Metronidazole (2 mg/ml), Mucin (5 mg/ml), Nexium® (2 mg/ml), Palmitic acid (20%), Pentofuryl® (2 mg/ml), Pepto-Bismol (1 mg/ml), Perenterol (2.5 mg/ml), Rennie® (8 mg/ml), Simagel® (2 mg/ml), Stearic acid (20%), Vancomycin (2 mg/ml).

Common Advices and Precautions

This kit is for *in-vitro* use only. Follow the working instructions carefully. The kit should be performed by trained technical staff only. Do not use reagents from damaged packages or bottles. The expiration dates stated on the respective labels are to be observed. **Do not use or mix reagents from different lots except for sample diluent, wash buffer, TMB/substrate solution and stop solution.**
The sample diluent, wash buffer, TMB/substrate solution and stop solution is universally applicable for the *Serazym*® stool ELISA Adenovirus (E-017), Rotavirus (E-020), Astrovirus (E-045), Norovirus (E-061), Clostridium difficile Toxin A+B (E-040), Clostridium difficile GDH (E-107), Campylobacter (E-093), H. pylori 2nd Gen. (E-114), Entamoeba histolytica (E-018), Cryptosporidium parvum (E-039), Giardia lamblia (E-038) and Giardia (E-106).
Do not use reagents from other manufacturers. Avoid time shift during dispensing of reagents. All reagents should be kept at 2…8°C before use. Some of the reagents may contain biocides as preservative. Further information can be found in the safety data sheet. They must not be swallowed or allowed to come into contact with skin or mucous membranes. Handle all components and all patient samples as if potentially hazardous. Since the kit contains potentially hazardous materials, the following precautions should generally be observed:

Do not smoke, eat or drink while handling kit material!
Always use protective gloves!
Never pipette material by mouth!
Note safety precautions of the single test components!

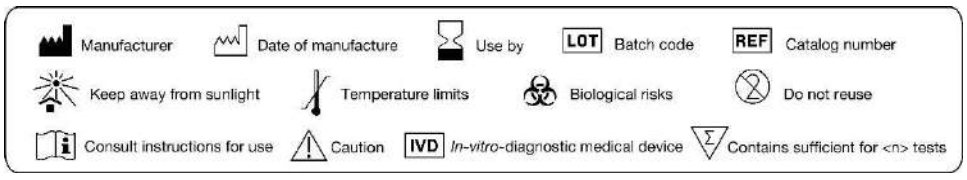
History of Changes

Version	Section	Modifications
2020-11-04	Common Advices and Precautions	Update
	History of Changes	Adding section “History of Changes “
	Test Components	Update

Incubation Scheme *Serazym*® Adenovirus (E-017)

1.	2 drops (or 75 µl)	CONJ HRP *(6)*
	+	
	pipette	
	75 µl	CONTROL + *(4)*
	75 µl	CONTROL – *(5)*
	50 µl	**diluted stool sample,** mix gently
	60 min	incubation (room temperature)
	5 x wash	with wash solution
2.	2 drops (or 75 µl)	SUBSTR TMB *(7)*
	10 min	incubation (room temperature) protected from light
3.	2 drops (or 75 µl)	STOP *(8)*

Read OD at 450 / ≥ 620 nm



Instructions For Use

Serazym® Astrovirus

Enzyme immunoassay for detection of *Astrovirus* in faecal samples

REF E-045 Σ 96 REF E-045-A2 Σ 2x 96 IVD *In-vitro*- diagnostic medical device CE

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Introduction

Astrovirus was firstly described in 1975 and named according to its star-shaped structure visible under the electron microscope. *Astrovirus* belongs to the family *Astroviridae*. Human *Astroviruses* are subdivided into 7 serotypes (1). Together with Rotavirus and Adenovirus *Astrovirus* is one of the most common causes of non-bacterial gastroenteritis in children under 5 years of age all over the world. Thus 80% of children between 5 and 10 years of age are anti-Astrovirus-antibody positive. *Astrovirus* caused gastroenteritis in adults and nosocomial infections are observed as well (2). The course of the disease is usually self-limiting and of short duration. After the incubation time of 1 - 2 days a 1 - 4 days lasting gastroenteritis develops accompanied by vomiting, diarrhea, fever and abdominal pain finally causing dehydration. Although occurring all over the year *Astrovirus* infections are mainly observed during the winter months (3, 4). *Astrovirus* infections are spread via faecal-oral transmission from person to person or via contaminated things or food. Infected persons excrete high amounts of *Astrovirus* particles with their faeces (1, 2).The detection of *Astrovirus* may be performed by electron microscopy or by molecular biology techniques such as polymerase chain reaction (PCR). Meanwhile immunological methods like enzyme immunoassay have established as preferential methods for routine laboratory diagnosis since these methods are fast, safe and automation is possible (1).

References:

1. Rohwedder, A. (2000): “Virale Gastroenteritiden, Erreger und Diagnostik”, Mikrobiologe, 10. Jg. p.121-126.
2. Palombo, E. A. and Bishop, R. F. (1996): “Annual Incidence, Serotype Distribution and Genetic Diversity of Human Astrovirus Isolates from Hospitalized Children in Melbourne, Australia”; Journal of Clinical Microbiology, Vol. 34, No. 7, p. 1750-1753.
3. Cukor, G. and Blacklow, N. R. (1984): “Human Viral Gastroenteritis”, Microbiological Reviews, June, Vol. 48 No. 2, p. 157-179.

4. Gaggero, A.; O´Ryan, M. et al. (1998): "Prevalence of Astrovirus Infection among Chilean Children with Acute Gastroenteritis", Journal of Clinical Microbiology, Vol. 36 No. 12, p. 3691-3693.

Intended use

***Serazym*® Astrovirus is an *in-vitro*-diagnostic medical device for direct detection of *Astrovirus* in faecal samples.**

Principle of the test

Serazym® Astrovirus is a one-step enzyme immunoassay on the basis of polyclonal and monoclonal antibodies against *Astrovirus* antigens. Diluted stool specimens and horseradish peroxidase (HRP) labelled monoclonal anti-Astrovirus-antibodies are dispensed simultaneously into the wells of a microtitration plate coated with polyclonal anti-Astrovirus-antibodies. After an incubation time of 60 min at room temperature (RT) unbound components are removed by a washing step. HRP converts the subsequently added colourless substrate solution of 3,3',5,5'-Tetramethylbenzidine (TMB) within a 10 min reaction time at room temperature protected from light into a blue product. The enzyme reaction is terminated by sulphuric acid dispensed into the wells turning the solution from blue to yellow. The optical density (OD) of the solution read at 450 / ≥ 620 nm is directly proportional to the specifically bound amount of *Astrovirus*.

Test components

			For 96 Wells	For 2x 96 Wells
1	WELLS	**Microtitration plate** coated with polyclonal anti-Astrovirus-antibodies (sheep)	12 single breakable 8-well strips colour coding light blue vacuum-sealed with desiccant	2x 12 single breakable 8-well strips colour coding light blue vacuum-sealed with desiccant
2	WASHBUF CONC 10x	**Wash buffer, 10-fold**	100 ml concentrate for 1000 ml solution white cap	2x 100 ml concentrate for 2x 1000 ml solution white cap
3	DIL	**Sample diluent**	100 ml · ready to use coloured yellow black cap	2x 100 ml · ready to use coloured yellow black cap
4	CONTROL +	**Positive control** *Astrovirus* reactive sample	1.5 ml · ready to use coloured blue red cap	3.0 ml · ready to use coloured blue red cap
5	CONTROL –	**Negative control** *Astrovirus* negative sample	1.5 ml · ready to use coloured blue green cap	3.0 ml · ready to use coloured blue green cap
6	CONJ HRP	**HRP-conjugate** HRP-labelled, monoclonal anti-Astrovirus-antibodies	12 ml · ready to use coloured green brown cap	24 ml · ready to use coloured green brown cap
7	SUBSTR TMB	**Substrate** 3,3',5,5'-Tetramethylbenzidine and hydrogen peroxide	15 ml · ready to use blue cap	28 ml · ready to use blue cap
8	STOP	**Stop solution** 0.25 M sulphuric acid	15 ml · ready to use yellow cap	28 ml · ready to use yellow cap

Preparation and storage of samples

Collection and storage
Stool samples should be stored at 2…8°C immediately after collection and processed within 72 hours. Longer storage is possible at -20°C. Repeated freezing and thawing of samples should be avoided. Stool samples already diluted with the *Serazym*® sample diluent can be stored for up to 48 h at 2…8°C before testing in the ELISA.

Preparation
Quickly thaw frozen samples. Warm samples to room temperature and mix well.
The *Serazym*® Astrovirus can be performed with 1 : 6 or 1 : 11 diluted specimens. In case of additional testing of the same sample in the *Serazym*® Norovirus, the *Serazym*® Campylobacter or the *Serazym*® Clostridium difficile Toxin A+B the 1 : 6 dilution is recommended.

Preparation of a 1 : 11 sample dilution: Pipette 1000 µl of sample diluent into a clean tube. Using a disposable stirring rod transfer about 100 mg (diameter about 2 - 3 mm) of faeces if solid or pipette 100 µl if liquid into the tube and suspend thoroughly. If necessary, sediment floating particles by a centrifugation step with a micro centrifuge for one min at maximum speed.

Preparation of a 1 : 6 sample dilution: Pipette 1000 µl of sample diluent into a clean tube. Using a disposable stirring rod transfer about 200 mg (diameter about 4 - 6 mm) of faeces if solid or pipette 200 µl if liquid into the tube and suspend thoroughly. If necessary, sediment floating particles by a centrifugation step with a micro centrifuge for one min at maximum speed.

Materials required but not provided

Micropipettes · multi-channel pipette or multi-pipette · Reagent container for multi-channel pipette · 8-channel wash comb with vacuum pump and waste bottle or microplate washer · microplate reader with optical filters of 450 nm for measurement and ≥ 620 nm for reference · distilled or deionized water · glassware · tubes (2 ml) for sample preparation

Preparation and storage of reagents

Kit size and expiry
One kit is designed for 1x 96 or 2x 96 determinations. The expiry date of each component is reported on its respective label, that of the complete kit on the outer box label. Upon receipt, all test components have to be kept at 2…8°C, preferably in the original kit box. After opening all kit components are stable for at least 2 months, provided proper storage. The ready to use wash solution can be used for at least 30 days when stored at 2…8°C.

Reagent preparation
Allow all components to reach room temperature prior to use in the assay. The microtitration plate is vacuum-sealed in a foil with desiccant. The plate consists of a frame and strips with breakable wells. Allow the sealed plate to reach room temperature before opening. Unused wells should be stored refrigerated and protected from moisture in the original cover carefully resealed. Prepare a sufficient amount of wash solution by diluting the 10-fold concentrated wash buffer 1 + 9 with distilled or deionized water.
For Example: 10 ml wash buffer concentrate *(2)* + 90 ml distilled or deionized water.

Assay procedure

Dilute samples with sample diluent *(3)* 1 : 11 or 1 : 6, e.g. 100 mg or 100 µl stool + 1.0 ml (1 : 11) sample diluent *(3)* or 200 mg or 200 µl stool + 1.0 ml (1 : 6) sample diluent *(3)*.
Avoid any time shift during dispensing of reagents and samples.
Make sure the soak time of the wash buffer in the wells is at least 5 seconds per wash cycle and that the remaining fluid is completely drained in every single wash cycle!
Avoid light exposure of the TMB substrate solution!

Working steps

1. Warm all reagents to room temperature (RT) before use.
   Mix gently without causing foam.
2. Dispense 2 drops (or 75 µl) **CONJ HRP** HRP-conjugate *(6)* per well and
3. Pipette: 75 µl **CONTROL +** positive control *(4)*
   75 µl **CONTROL –** negative control *(5)*
   50 µl **diluted sample**, mix gently.
4. Cover plate and incubate for 60 min at RT.
5. Decant, then wash each well 5x with 300 µl wash solution (diluted from *(2)*) and tap dry onto absorbent paper if necessary.
6. Dispense 2 drops (or 75 µl) **SUBSTR TMB** substrate *(7)* per well.
7. Incubate for 10 min at RT protected from light.
8. Dispense 2 drops (or 75 µl) **STOP** stop solution *(8)* per well, mix gently.
9. Read OD at 450 nm / ≥ 620 nm with a microplate reader within 30 min after reaction stop.

Result interpretation

Qualitative evaluation
Cut-off determination: OD negative control + 0.10
Samples with OD values equal with or higher than the cut-off are considered positive, samples with OD values below the cut-off are considered negative for *Astrovirus* antigen.

Reference values

It is recommended that each laboratory establishes its own normal and pathological reference ranges as usually done for other diagnostic parameters, too. Therefore, the mentioned reference values provide a guide only to values which might be expected.

Test validity

The test run is valid if:

- the mean OD of the negative control is ≤ 0.15 (manual test performance)
  ≤ 0.30 (automatic test performance)
- the mean OD of the positive control is ≥ 1.00

If the above-mentioned quality criteria are not met, repeat the test and make sure that the test procedure is followed correctly (incubation times and temperatures, sample and wash buffer dilution, wash steps etc.). In case of repeated failure of the quality criteria contact your supplier.

Limitations of the procedure

There is no correlation between measured absorbance and seriousness of the infection. It is also not allowed to correlate absorbances of the samples with that of the positive control. Cross contamination of reagents and samples can produce false positive results. Incorrect dilutions, not sufficiently homogenized samples or solid particles after centrifugation of the suspension can cause false negative as well as false positive results. A negative test result not necessarily excludes an *Astrovirus* infection. Inhomogeneous virus distribution in the sample can cause false negative results. The investigation of samples that were taken beyond the acute phase of the disease can cause false negative results, because the number of virus particles has decreased under the detection limit of the test. It is therefore recommended to take samples within the acute phase of the disease where a maximum number of excreted virus particles are to be expected. A final interpretation of the test results should consider clinical findings as well.

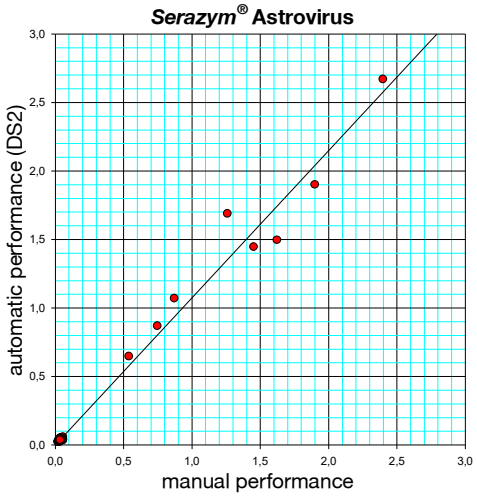
Automatic Processing

Performing the *Serazym*® Astrovirus on fully automated microplate processors (e.g. DS2, DSX) may cause elevated absorbances in comparison to the manual procedure due to individual differences concerning wash procedures and general technical specifications of the equipment. In these cases a maximum value of 0.3 absorbance units is permissible for the negative control. It is recommended to use a wash procedure including 10 seconds soak time per strip and wash step followed by a wash step with distilled or deionized water with 10 seconds of soak time after the final wash step of each wash cycle. If necessary, the number of washing steps can be enhanced from 5x to 7x - 8x.

Serazym® Astrovirus	
Positive	≥ Cut-off
Negative	< Cut-off

Correlation: manual – automatic processing

A panel of 96 stool specimens was investigated in parallel by manual and automatic processing method (DS2, Dynex Technologies) resp. The correlation was calculated with r = 0.993.



Performance characteristics

Precision

Intra-assay coefficient of variation (CV) in the *Serazym*® Astrovirus from 8-fold determinations of samples:

sample	mean absorbance	standard deviation	CV (%)
1	1.667	0.148	8.9
2	0.994	0.063	6.4
3	0.443	0.027	6.1
4	0.185	0.018	9.8

Inter-assay coefficient of variation (CV) in the *Serazym*® Astrovirus in 6 different test runs from 8-fold determinations of samples:

sample	mean absorbance	standard deviation	CV (%)
1	1.853	0.071	3.8
2	1.019	0.059	5.8
3	0.583	0.069	11.9
4	0.350	0.034	9.7

Lower detection limit

The lower detection limit of *Astrovirus* antigen in the *Serazym*® Astrovirus was determined by titration of purified *Astrovirus*-antigen. Lower detection limit: 6 ng / ml.

Specificity and sensitivity

A total of 98 stool samples were investigated in parallel in the *Serazym*® Astrovirus and in another commercially available ELISA.

	comparative ELISA positive	comparative ELISA negative
Serazym® ELISA positive	49	0
Serazym® ELISA negative	2	47

Specificity: 100% Sensitivity: 96%

Cross reactivity
Stool samples positive for one of the subsequent pathogens have been tested with the *Serazym*® Astrovirus and showed no cross reactivity:
Rotavirus (n = 10), *Adenovirus* (n = 20), *Norovirus* (n = 31), *Clostridium difficile* (n = 11), *Campylobacter jejuni* (n = 7), *Campylobacter coli* (n = 1), *Salmonella enteritidis* (n = 18), *Giardia lamblia* (n = 1).

Negative stool specimens have been spiked with $\geq 10^8$ colony forming units of the following microorganisms and tested negative with the *Serazym*® ELISA (OD 450 / 620 nm < Cut-off):

Aeromonas hydrophila	(ATCC 7966)	*Klebsiella pneumoniae*	(ATCC 13883)
Bacillus cereus	(ATCC 11778)	*Peptostreptococcus anaerobius*	(ATCC 27337)
Bacillus subtilis	(ATCC 6633)	*Proteus vulgaris*	(ATCC 8427)
Bacteroides fragilis	(ATCC 25285)	*Pseudomonas aeruginosa*	(ATCC 10145)
Candida albicans	(ATCC 10231)	*Salmonella enterica Serovar enteritidis*	(ATCC 13076)
Campylobacter coli	(ATCC 33559)	*Salmonella enterica Serovar typhimurium*	(ATCC 14028)
Campylobacter jejuni	(ATCC 33291)	*Shigella flexneri*	(ATCC 12022)
Citrobacter freundii	(ATCC 8090)	*Shigella sonnei*	(ATCC 25931)
Clostridium sordellii	(ATCC 9714)	*Staphylococcus aureus*	(ATCC 25923)
Enterobacter aerogenes	(ATCC 13048)	*Staphylococcus epidermidis*	(ATCC 12228)
Enterobacter cloacae	(ATCC 13047)	*Vibrio parahaemolyticus*	(ATCC 17802)
Enterococcus faecalis	(ATCC 29212)	*Vibrio cholerae*	Clinical isolate
Escherichia coli	(ATCC 25922)	*Yersinia enterocolitica Serotyp 03, 09*	Clinical isolates

Interference
None of the following substances added to positive and negative stool samples showed a significant impact on the test result:
barium sulphate (5%), Buscopan® (2 mg/ml), cyclamate (5%), Diclofenac (2 mg/ml), haemoglobine (5 mg/ml), Hylak® N (5%), Immodium® akut duo (0.2/12.5 mg/ml), Iberogast® (5%), loperamide (0.2 mg/ml), metronidazole (2 mg/ml), mucin (5 mg/ml), Nexium® (2 mg/ml), palmitic acid (20%), Pentofuryl® (2 mg/ml), Pepto-Bismol (1 mg/ml), Perenterol (2.5 mg/ml), Rennie® (8 mg/ml), Simagel® (2 mg/ml), stearic acid (20%).

* The addition of 5% (v/v) Hylak® N (lactic acid containing preparation against digestive complaints) to Astrovirus positive stool suspensions may decrease OD values.

Common advices and precautions

This kit is for *in-vitro* use only. Follow the working instructions carefully. The kit should be performed by trained technical staff only. Do not use reagents from damaged packages or bottles. The expiration dates stated on the respective labels are to be observed. **Do not use or mix reagents from different lots except for sample diluent, wash buffer, TMB/substrate solution and stop solution.**
The sample diluent, wash buffer, TMB/substrate solution and stop solution are universally applicable for the *Serazym*® stool ELISA Adenovirus (E-017), Rotavirus (E-020), Astrovirus (E-045), Norovirus (E-061), Clostridium difficile Toxin A+B (E-040), Clostridium difficile GDH (E-107), Campylobacter (E-093), H. pylori 2nd Gen. (E-114), Entamoeba histolytica (E-018), Cryptosporidium parvum (E-039), Giardia lamblia (E-038) and Giardia (E-106).
Do not use reagents from other manufacturers. Avoid time shift during dispensing of reagents. All reagents should be kept at 2…8°C before use Some of the reagents may contain biocides as preservative. Further information can be found in the safety data sheet. They must not be swallowed or allowed to come into contact with skin or mucous membranes. Handle all components and all patient samples as if potentially hazardous. Since the kit contains potentially hazardous materials, the following precautions should generally be observed:

Do not smoke, eat or drink while handling kit material!
Always use protective gloves!
Never pipette material by mouth!
Note safety precautions of the single test components!

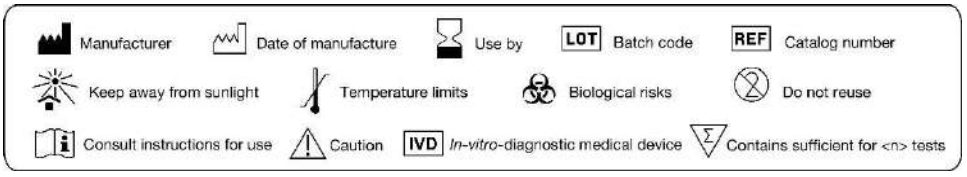
History of Changes

Version	Section	Modifications
2020-07-21	Intended use	Correction
	Test Components	Correction
	Assay Procedure	Update
	Common Advices and Precautions	Update
	History of Changes	New section “History of Changes“

Incubation scheme *Serazym*® Astrovirus (E-045)

1.		2 drops (or 75 µl)	CONJ HRP *(6)*
		+	
		pipette	
		75 µl	CONTROL + *(4)*
		75 µl	CONTROL – *(5)*
		50 µl	**stool sample,** mix gently
		60 min	incubation (room temperature)
		5 x wash	with wash solution
2.		2 drops (or 75 µl)	SUBSTR TMB *(7)*
		10 min	incubation (room temperature) protected from light
3.		2 drops (or 75 µl)	STOP *(8)*

Read OD at 450 / ≥ 620 nm



Instructions For Use

Serazym® Norovirus

Enzyme immunoassay for detection of *Norovirus* in stool specimens

REF E-061 Σ 96 REF E-061-A2 Σ 2x 96 IVD *In-vitro*-diagnostic medical device CE

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Introduction

Noroviruses belong to the family *Caliciviridae*, single stranded RNA viruses of 30 - 40 nm in size characterized by a typical cup-shaped capsid. Within the genus *Norovirus* the two human pathogenic genogroups GGI and GGII have been identified. GGI and GGII strains can be further subclassified into at least 15 and 18 genotypes resp. The genetic heterogeneity of Noroviruses causes distinct capsid protein divergences between different genogroups (about 60%) as well as between different genotypes within one genogroup (about 20 - 30%). Since 1994 genotype GGII.4 is predominantly circulating. Noroviruses are very resistant to environmental conditions and highly contagious. The infection is transmitted by direct contact to already infected people either by faecal-oral transmission or by ingestion of aerosols from vomit or by contaminated food, drinking water or objects. After a short, 10 - 50 hours lasting incubation time fulminant diarrhea and often vomiting develop as the characteristic symptoms. The infection is usually self-limiting and symptoms disappear after 2 - 3 days. Norovirus infections are characterized by seasonal fluctuations with a climax during the winter months. They are considered as the most common cause of non-bacterial gastroenteritis outbreaks worldwide, but may also be responsible for single cases of viral gastroenteritis. The high sequence variability of the capsid proteins circumvents the production of protective antibodies and hampers diagnostic detection. Methods like PCR (usually as “Real time Reverse Transcriptase PCR – Rt RT-PCR) and enzyme immunoassay are commonly used for laboratory diagnosis.

References:

1. Venkataram, B.V. et al. (1999): "X-ray Crystallographic Structure of the Norwalk Virus Capsid" Science Vol 286: 287-290.
2. Künkel, U. und Schreier, E. (2002): "Caliciviren-Virale Auslöser akuter Gastroenteritiden" Bundesgesundheitsbl-Gesundheitsforsch-Gesundheitsschutz 45: 534-542.
3. Hansman, G.S. et al. (2006): "Genetic and antigenic diversity among noroviruses" Journal of General Virology 87:909-919.
4. Marshall, J.A. and Bruggink, L.D. (2006): "Laboratory Diagnosis of Norovirus" Clin. Lab. 52: 571-581
5. Tomoyuki Shiota et al. (2007): "Characterization of a Broadly Reactive Monoclonal Antibody against Norovirus Genogroups I and II: Recognition of a Novel Conformational Epitope" Journal of Virology, Vol. 81, No. 22, p.12298-12306.
6. Lindesmith, L.C. et al. 2008: „Mechanisms of GII.4 Norovirus Persistence in Human Populations" PLoS Medicine Vol 5 (2): 269-289

Intended Use

The *Serazym*® Norovirus is an *in-vitro*-diagnostic medical device for direct detection of *Norovirus* specific antigens in stool specimens.

Principle Of The Test

Norovirus antigens from stool specimens and the positive control react with polyclonal anti-Norovirus antibodies coated on the solid phase of the microplate. After incubation non-bound material is removed by a washing step. Subsequently bound antigens specifically react with horseradish peroxidase (HRP) labeled polyclonal anti-Norovirus antibodies during a second incubation period. Unbound conjugate is removed by a washing step. HRP converts the subsequently added colourless chromogenic substrate solution (TMB / H_2O_2) into a blue product. The enzyme reaction is terminated by sulphuric acid dispensed into the wells turning the solution from blue to yellow. The optical density (OD) of the solution read at 450 / $\geq$ 620 nm is directly proportional to the specifically bound amount of *Norovirus* antigens. By means of the calculated cut-off value, results are interpreted either as positive or negative.

Test Components

			For 96 Wells	For 2x 96 Wells
1	WELLS	**Microtitration plate** coated with polyclonal anti-Norovirus antibodies (sheep)	12 single breakable 8-well strips colour coding silver vacuum-sealed with desiccant	2x 12 single breakable 8-well strips colour coding silver vacuum-sealed with desiccant
2	WASHBUF CONC 10x	**Wash buffer** 10-fold	100 ml concentrate for 1000 ml solution white cap	2x 100 ml concentrate for 2x 1000 ml solution white cap
3	DIL	**Sample diluent**	100 ml · ready to use coloured yellow black cap	2x 100 ml · ready to use coloured yellow black cap
4	CONTROL +	**Positive control** Recombinant Norovirus capsid proteins	2.0 ml · ready to use coloured blue red cap	4.0 ml · ready to use coloured blue red cap
5	CONTROL –	**Negative control** Norovirus negative sample	2.0 ml · ready to use coloured blue green cap	4.0 ml · ready to use coloured blue green cap
6	CONJ HRP	**HRP-conjugate** HRP-labelled polyclonal anti-Norovirus antibodies (sheep)	15 ml · ready to use coloured green brown cap	25 ml · ready to use coloured green brown cap
7	SUBSTR TMB	**Substrate** 3,3',5,5'-Tetramethylbenzidine and hydrogen peroxide	15 ml · ready to use blue cap	28 ml · ready to use blue cap
8	STOP	**Stop solution** 0.25 M sulphuric acid	15 ml · ready to use yellow cap	28 ml · ready to use yellow cap

Preparation And Storage Of Samples

Collection and storage

Stool samples should be stored at 2…8°C immediately after collection and processed within 72 hours. Longer storage is possible at -20°C. Repeated freezing and thawing of samples should be avoided. Stool samples already diluted with the *Serazym*® sample diluent can be stored for up to 72 h at 2…8°C before testing in the ELISA.

Preparation

Warm samples to room temperature and mix thoroughly. Pipette 1000 µl of sample diluent into a clean tube. Using a disposable stirring rod transfer about 200 mg (diameter about 4 - 6 mm) of faeces if solid or pipette 200 µl if liquid into the tube and suspend thoroughly. If necessary spin down floating particles in a micro centrifuge for one min at maximum speed.

Materials Required But Not Provided

Micropipettes · multi-channel pipette or multi-pipette · Reagent container for multi-channel pipette · 8-channel wash comb with vacuum pump and waste bottle or microplate washer · microplate reader with 450 nm filter for measurement and ≥ 620 nm for reference · distilled or deionized water · glassware · tubes (2 ml) for sample preparation

Preparation And Storage Of Reagents

Kit size and expiry
One kit is designed for 1x 96 or 2x 96 determinations. The expiry date of each component is reported on its respective label, that of the complete kit on the outer box label. Upon receipt, all test components have to be kept at 2…8°C, preferably in the original kit box. After opening all kit components are stable for at least 2 months, provided proper storage. The ready to use wash solution can be used for at least one month when stored at 2…8°C.

Reagent preparation
Allow all components to reach room temperature prior to use in the assay. The microtitration plate is vacuum-sealed in a foil with desiccant. The plate consists of a frame and strips with breakable wells. Allow the sealed plate to reach room temperature before opening. Unused wells should be stored refrigerated and protected from moisture in the original cover carefully resealed. Prepare a sufficient amount of wash solution by diluting the 10-fold concentrated wash buffer 1 + 9 with distilled or deionized water.
For Example: 10 ml wash buffer concentrate *(2)* + 90 ml distilled or deionized water.

Assay Procedure

Dilute samples with sample diluent *(3)* 1 : 6, e.g. 200 mg or 200 µl stool + 1.0 ml sample diluent *(3)* and mix thoroughly on a vortex.
Avoid any time shift during dispensing of reagents and samples.
Make sure the soak time of the wash buffer in the wells is at least 5 seconds per wash cycle and that the remaining fluid is completely drained in every single wash cycle!
Avoid direct light exposure of the TMB substrate solution!

Working steps

1. Warm all reagents to room temperature (RT) before use. Mix gently without causing foam.
2. Pipette: 100 µl CONTROL + positive control *(4)*
   100 µl CONTROL – negative control *(5)*
   100 µl **diluted stool specimen.**
3. Cover plate and incubate for 60 min at RT.
4. Decant, then wash each well 5x with 300 µl wash solution (diluted from *(2)*) and tap dry onto absorbent paper if necessary.
5. Dispense 3 drops (or 100 µl) CONJ HRP HRP-conjugate *(6)* per well.
6. Cover plate and incubate for 30 min at RT.
7. Decant, then wash each well 5x with 300 µl wash solution (diluted from *(2)*) and tap dry onto absorbent paper if necessary.
8. Dispense 3 drops (or 100 µl) SUBSTR TMB substrate *(7)* per well.
9. Incubate for 10 min at RT protected from light.
10. Dispense 3 drops (or 100 µl) STOP stop solution *(8)* per well, mix gently.
11. Read OD at 450 nm / ≥ 620 nm with a microplate reader within 30 min after reaction stop.

Result Interpretation

Qualitative evaluation
Cut-off determination: OD negative control + 0.10
Samples with OD values higher than or equal to the cut-off value are considered positive, samples with OD values below the cut-off value are considered negative for *Norovirus* antigen.

Reference Values

Serazym® Norovirus	
Positive	≥ Cut-off
Negative	< Cut-off

It is recommended that each laboratory establishes its own normal and pathological reference ranges as usually done for other diagnostic parameters too. Therefore, the mentioned reference values provide a guide only to values which might be expected.

Test validity
The test run is valid if:

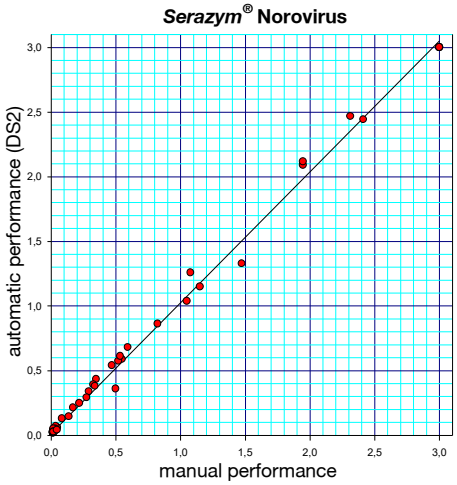
- the mean OD of the negative control is ≤ 0.20 (manual test performance)
  ≤ 0.30 (automatic test performance)
- the mean OD of the positive control is ≥ 1.20

If the above mentioned quality criteria are not met, repeat the test and make sure that the test procedure is followed correctly (incubation times and temperatures, sample and wash buffer dilution, wash steps etc.). In case of repeated failure of the quality criteria contact your supplier.

Limitations of the procedure
There is no correlation between measured absorbance and seriousness of the infection. It is also not allowed to correlate absorbances of the samples with that of the positive control. Cross contamination of reagents and samples can produce false positive results. Incorrect dilutions, not sufficiently homogenized samples or solid particles after centrifugation of the suspension can cause false negative as well as false positive results. A negative test result not necessarily excludes a *Norovirus* infection. Inhomogeneous virus distribution in the sample can cause false negative results. The investigation of samples that were taken beyond the acute phase of the disease can cause false negative results, because the number of virus particles has decreased under the detection limit of the test. It is therefore recommended to take samples within the acute phase of the disease where a maximum number of excreted virus particles are to be expected. Genetic recombination between different *Norovirus* strains may cause antigenic shift finally leading to the occurrence of virus variants that are not detected by ELISA. The overall interpretation of the ELISA results should always consider clinical findings.

Automatic Processing
Performing the *Serazym*® Norovirus on fully automated microplate processors (e.g. DS2 or DSX) may cause elevated absorbances in comparison to the manual procedure due to individual differences concerning wash procedures and general technical specifications of the equipment. In these cases a maximum value of 0.3 absorbance units is permissible for the negative control.
It is recommended to use a wash procedure including 10 seconds soak time per strip and wash step followed by a wash step with distilled or deionized water with 10 seconds of soak time after the final wash step of each wash cycle. If necessary the number of washing steps can be enhanced from 5x to 7x-8x.

Correlation: manual – automatic processing
A panel of 90 stool specimens was investigated in parallel by manual and automatic processing method (DS2, Dynex Technologies) resp. The correlation was calculated with r = 0.999.



Performance Characteristics

Precision
Intra-assay coefficient of variation (CV) in the *Serazym*® Norovirus calculated from 8-fold determinations of samples:

sample	mean absorbance	standard deviation	CV (%)
1	2.132	0.043	2.00
2	0.902	0.035	3.93
3	0.534	0.017	3.28
4	0.217	0.013	5.92

Inter-assay coefficient of variation (CV) in the *Serazym*® Norovirus in 8 different test runs on 2 different days from 8-fold determinations of samples:

sample	mean absorbance	standard deviation	CV (%)
1	1.924	0.146	7.59
2	0.813	0.026	3.17
3	0.562	0.021	3.74
4	0.247	0.007	2.76

Lower detection limit
The lower detection limit of the *Serazym*® Norovirus was determined < 10 ng/ml capsid protein for genogroup I and II.

Specificity and sensitivity
One retrospective study with 159 stool specimens was performed to compare the *Serazym*® Norovirus with another commercially available ELISA.

	comparative ELISA positive	comparative ELISA negative
Serazym® ELISA positive	111	3
Serazym® ELISA negative	6	39

Specificity: 92.9% Sensitivity: 94.9%

Cross reactivity
Faecal samples positive for one of the following pathogens did not show any cross reaction in the *Serazym*® Norovirus:
Adenovirus (n = 6), *Astrovirus* (n = 8), *Rotavirus* (n = 6), *Clostridium difficile* (n = 8), *Campylobacter jejuni* (n = 6), *Helicobacter pylori* (n = 5), *Giardia lamblia* (n = 8), *Cryptosporidium parvum* (n = 7), *Entamoeba histolytica/dispar* (n = 6).

Negative stool specimens have been spiked with ≥ 10^8 colony forming units and virus particles per ml stool suspension resp. All microorganisms were tested negative with the *Serazym*® ELISA (OD 450 / 620 nm < Cut-Off):

Adenovirus	typ e41
Aeromonas hydrophila	ATCC 7966
Astrovirus	serotype 4
Bacillus cereus	ATCC 117788
Bacillus subtilis	ATCC 6633
Bacteroides fragilis	ATCC 25285
Campylobacter jejuni	ATCC 33291
Candida albicans	clinical isolate
Citrobacter freundii	ATCC 8090
Clostridium sordelli	ATCC 9714
Enterobacter aerogenes	ATCC 13048
Enterobacter cloacae	ATCC 13047
Enterococcus faecalis	ATCC 29212
Escherichia coli	ATCC 25922
Klebsiella pneumonia	ATCC 13883

Peptostreptococcus anaerobius	ATCC 27337
Proteus vulgaris	ATCC 8427
Pseudomonas aeroginosa	ATCC 10145
Rotavirus	strain SA11
Salmonella enterica Serovar thyphimurium	ATCC 14028
Salmonella enterica ssp. galolyticus	ATCC 13076
Shigella flexneri	ATCC 12022
Shigella sonnei	ATCC 25931
Staphylococus aureus	ATCC 25923
Staphylococcus epidermidis	ATCC 12228
Vibrio cholerae	RV 2011/ST5
Vibrio parahaemolyticus	ATCC 17802
Yersinia enterocolitica O:9	clinical isolate
Yersinia enterocolitica O:3	clinical isolate

Interference
None of the following substances added to positive and negative stool samples showed a significant impact on the test result:
Barium sulfate (5%), Buscopan® (2 mg/ml), Cyclamate (5%), Diclofenac (2 mg/ml), Hemoglobine human (5 mg/ml), Blood human (5%), Hylak® N (5%), Iberogast® (5%), Immodium® akut duo (0.2/12.5 mg/ml), Loperamide (0.2 mg/ml), Metronidazole (2 mg/ml), Mucin (5 mg/ml), Nexium® (2 mg/ml), Palmitic acid (20%), Pentofuryl® (2 mg/ml), Pepto-Bismol (1 mg/ml), Perenterol (2.5 mg/ml), Rennie® (8 mg/ml), Simagel® (2 mg/ml), Stearic acid (20%), Vancomycin (2 mg/ml).

Common Advices and Precautions

This kit is for *in-vitro* use only. Follow the working instructions carefully. The kit should be performed by trained technical staff only. Do not use reagents from damaged packages or bottles. The expiration dates stated on the respective labels are to be observed. **Do not use or mix reagents from different lots except for sample diluent, wash buffer, TMB/substrate solution and stop solution.**
The sample diluent, wash buffer, TMB/substrate solution and stop solution is universally applicable for the *Serazym*® stool ELISA Adenovirus (E-017), Rotavirus (E-020), Astrovirus (E-045), Norovirus (E-061), Clostridium difficile Toxin A+B (E-040), Clostridium difficile GDH (E-107), Campylobacter (E-093), H. pylori 2nd Gen. (E-114), Entamoeba histolytica (E-018), Cryptosporidium parvum (E-039), Giardia lamblia (E-038) and Giardia (E-106).
Do not use reagents from other manufacturers. Avoid time shift during dispensing of reagents. All reagents should be kept at 2…8°C before use. Some of the reagents may contain biocides as preservative. Further information can be found in the safety data sheet. They must not be swallowed or allowed to come into contact with skin or mucous membranes. Handle all components and all patient samples as if potentially hazardous. Since the kit contains potentially hazardous materials, the following precautions should generally be observed:

Do not smoke, eat or drink while handling kit material!
Always use protective gloves!
Never pipette material by mouth!
Note safety precautions of the single test components!

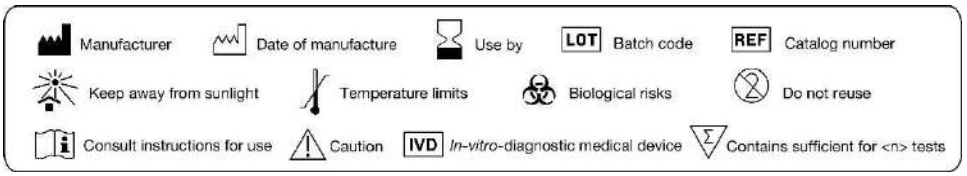
History of Changes

Version	Section	Modifications
2021-02-02	Common Advices and Precautions	Update
	History of Changes	Adding section “History of Changes“
	Test Components	Update
	Assay Procedure	Update

Incubation Scheme *Serazym*® Norovirus (E-061)

1.		pipette	
		100 µl	CONTROL + *(4)*
		100 µl	CONTROL – *(5)*
		100 µl	**diluted stool sample**
		60 min	incubation (room temperature)
		5 x wash	with wash solution
2.		3 drops (or 100 µl)	CONJ HRP *(6)*
		30 min	incubation (room temperature)
		5 x wash	with wash solution
3.		3 drops (or 100 µl)	SUBSTR TMB *(7)*
		10 min	incubation (room temperature) protected from light
4.		3 drops (or 100 µl)	STOP *(8)*

Read OD at 450 / ≥ 620 nm



NovaTec Immundiagnostica GmbH

NovaLisa®

Brucella IgG

ELISA

CE

Only for in-vitro diagnostic use

Product Number: BRUG0050 (96 Determinations)

ENGLISH

1. INTRODUCTION

Brucella is a small Gram-negative bacterium (0.4-0.8 µm in diameter and 0.4-3.0 µm in length) which is non-flagellated, and non-spore-forming. They are named after the military doctor David Bruce, who on Malta in 1887 isolated the pathogens from the spleen of a soldier who died of undulating fever. Four species are pathogenic to human: Brucella abortus, Brucella melitensis, Brucella suis and Brucella canis. All four species are exciters of Brucellosis, a disease characterized by undulating fever. Depending on exciter the disease is also called Morbus Bang (B. abortus) or Malta fever (B. melitensis).

The pathogens are transmitted from animals, which are mainly affected. The infection is caused by contact with ill animals or their excrements as well as by non-pasteurized milk and milk products like fresh cheese from sheep or goat. Main entrances are skin wounds, conjunctives and digestive tract. The intact pathogens are transported by granulocytes into local lymph nodes, from where they spread haematogenous. All kind of organs can be infected. Symptoms depend up on the infected organ, where an inflammation takes place. Until now the pathogenic mechanism is not completely understood.

Brucellosis appears worldwide. In non-pasteurized milk and milk products Brucella is viably and infectious for weeks. Bovine brucellosis caused by Brucella abortus is still the most widespread form, although reported incidence and prevalence of the disease vary widely from country to country (from <0.01 to >200 per 100,000 population). Brucella melitensis is endemic in areas where keeping of sheeps and goats is frequent. It causes serious human infections. Working with these bacteria in laboratories necessitates highest carefulness because of high contagiosity. Risk groups include abattoir workers, meat inspectors, animal handlers, veterinarians, and laboratorians. Brucellosis is a nationally notifiable disease and reportable to the local health authority.

Species	Disease	Symptoms (e.g.)	Transmission route
B. abortus (cattle) B. melitensis (sheep, goats) B. suis (pigs) B. canis (dogs)	Brucella	Fever, chills (undulating fever), malaise, arthritis, hepatitis, endocarditis, hepatomegalie, osteomyelitis (OM)	Oral (non-pasteurized milk and milk products) Percutan (contact with ill animals or their excrements) In general no transmission from human to human

The presence of pathogen or infection may be identified by

- Histology
- Serology: e.g. ELISA

2. INTENDED USE

The Brucella IgG ELISA is intended for the qualitative determination of IgG class antibodies against Brucella in human serum or plasma (citrate, heparin).

3. PRINCIPLE OF THE ASSAY

The qualitative immunoenzymatic determination of specific antibodies is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique.
Microplates are coated with specific antigens to bind corresponding antibodies of the sample. After washing the wells to remove all unbound sample material a horseradish peroxidase (HRP) labelled conjugate is added. This conjugate binds to the captured antibodies. In a second washing step unbound conjugate is removed. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product.
The intensity of this product is proportional to the amount of specific antibodies in the sample. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450/620 nm is read using an ELISA microwell plate reader.

4. MATERIALS

4.1. Reagents supplied

- **Brucella Coated Microplate (IgG):** 12 break-apart 8-well snap-off strips coated with Brucella antigens; in resealable aluminium foil.
- **IgG Sample Diluent:** 1 bottle containing 100 ml of phosphate buffer (10 mM) for sample dilution; pH 7.2 ± 0.2; coloured yellow; ready to use; white cap.
- **Stop Solution:** 1 bottle containing 15 ml sulphuric acid, 0.2 mol/l; ready to use; red cap.
- **Washing Buffer (20x conc.):** 1 bottle containing 50 ml of a 20-fold concentrated phosphate buffer (0.2 M), pH 7.2 ± 0.2, for washing the wells; white cap.
- **Brucella anti-IgG Conjugate:** 1 bottle containing 20 ml of peroxidase labelled antibody to human IgG in phosphate buffer (10 mM); coloured blue; ready to use; black cap.
- **TMB Substrate Solution:** 1 bottle containing 15 ml 3,3',5,5'-tetramethylbenzidine (TMB), < 0.1 %; ready to use; yellow cap; < 5 % NMP.
- **Brucella IgG Positive Control:** 1 vial containing 2 ml control (human serum or plasma); coloured yellow; ready to use; red cap.
- **Brucella IgG Cut-off Control:** 1 vial containing 3 ml control (human serum or plasma); coloured yellow; ready to use; green cap.
- **Brucella IgG Negative Control:** 1 vial containing 2 ml control (human serum or plasma); coloured yellow; ready to use; blue cap.

For potential hazardous substances please check the safety data sheet.

4.2. Materials supplied

- 1 Cover foil
- 1 Instruction for use (IFU)
- 1 Plate layout

4.3. Materials and Equipment needed

- ELISA microwell plate reader, equipped for the measurement of absorbance at 450/620 nm
- Incubator 37 °C
- Manual or automatic equipment for rinsing wells
- Pipettes to deliver volumes between 10 and 1000 µl
- Vortex tube mixer
- Distilled water
- Disposable tubes

5. STABILITY AND STORAGE

Store the kit at 2...8 °C. The opened reagents are stable up to the expiry date stated on the label when stored at 2...8 °C.

6. REAGENT PREPARATION

It is very important to bring all reagents and samples to room temperature (20…25 °C) and mix them before starting the test run!

6.1. Coated Microplate

The break-apart snap-off strips are coated with Brucella antigens. Immediately after removal of the strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2...8 °C.

6.2. Washing Buffer (20x conc.)

Dilute Washing Buffer 1 + 19; e. g. 10 ml Washing Buffer + 190 ml distilled water. The diluted buffer is stable for 5 days at room temperature (20…25 °C). In case crystals appear in the concentrate, warm up the solution to 37°C e.g. in a water bath. Mix well before dilution.

6.3. TMB Substrate Solution

The reagent is ready to use and has to be stored at 2...8 °C, away from the light. The solution should be colourless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away.

7. SAMPLE COLLECTION AND PREPARATION

Use human serum or plasma (citrate, heparin) samples with this assay. For CSF please use the instruction for use ABVL0001. If the assay is performed within 5 days after sample collection, the samples should be kept at 2...8 °C; otherwise they should be aliquoted and stored deep-frozen (-70…-20 °C). If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing.
Heat inactivation of samples is not recommended.

7.1. Sample Dilution

Before assaying, all samples should be diluted 1+100 with IgG Sample Diluent. Dispense 10 µl sample and 1 ml IgG Sample Diluent into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

8. ASSAY PROCEDURE

8.1. Test Preparation

Please read the instruction for use carefully before performing the assay. Result reliability depends on strict adherence to the instruction for use as described. The following test procedure is only validated for manual procedure. If performing the test on ELISA automatic systems we recommend increasing the washing steps from three to five and the volume of Washing Buffer from 300 µl to 350 µl to avoid washing effects. Pay attention to chapter 12. Prior to commencing the assay, the distribution and identification plan for all samples and standards/controls (duplicates recommended) should be carefully established on the plate layout supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder.

Perform all assay steps in the order given and without any delays.

A clean, disposable tip should be used for dispensing each standard/control and sample.

Adjust the incubator to 37 ± 1 °C.

1. Dispense 100 µl standards/controls and diluted samples into their respective wells. Leave well A1 for the Substrate Blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 1 hour ± 5 min at 37 ± 1 °C.**
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µl of Washing Buffer. Avoid overflows from the reaction wells. The interval between washing and aspiration should be > 5 sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!

   Note: Washing is important! Insufficient washing results in poor precision and false results.
5. Dispense 100 µl Conjugate into all wells except for the Substrate Blank well A1.
6. **Incubate for 30 min at room temperature (20...25 °C).** Do not expose to direct sunlight.
7. Repeat step 4.
8. Dispense 100 µl TMB Substrate Solution into all wells.
9. **Incubate for exactly 15 min at room temperature (20...25 °C) in the dark.** A blue colour occurs due to an enzymatic reaction.
10. Dispense 100 µl Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution, thereby a colour change from blue to yellow occurs.
11. Measure the absorbance at 450/620 nm within 30 min after addition of the Stop Solution.

8.2. Measurement

Adjust the ELISA microwell plate reader **to zero** using the **Substrate Blank**.

If - due to technical reasons - the ELISA microwell plate reader cannot be adjusted to zero using the Substrate Blank, subtract its absorbance value from all other absorbance values measured in order to obtain reliable results!

Measure the absorbance of all wells at **450 nm** and record the absorbance values for each standard/control and sample in the plate layout.

Bichromatic measurement using a reference wavelength of 620 nm is recommended.

Where applicable calculate the **mean absorbance values** of all duplicates.

9. RESULTS

9.1. Run Validation Criteria

In order for an assay to be considered valid, the following criteria must be met:

- **Substrate Blank**: Absorbance value **< 0.100**
- **Negative Control**: Absorbance value **< 0.200 and < Cut-off**
- **Cut-off Control**: Absorbance value **0.150 – 1.300**
- **Positive Control**: Absorbance value **> Cut-off**

If these criteria are not met, the test is not valid and must be repeated.

9.2. Calculation of Results

The Cut-off is the mean absorbance value of the Cut-off Control determinations.

Example: Absorbance value Cut-off Control 0.44 + absorbance value Cut-off control 0.42 = 0.86 / 2 = 0.43
Cut-off = 0.43

9.2.1. Results in Units [NTU]

$$\frac{\text{Sample (mean) absorbance value} \times 10}{\text{Cut-off}} = \text{[NovaTec Units = NTU]}$$

Example: $\frac{1.591 \times 10}{0.43} = 37$ NTU (Units)

9.3. Interpretation of Results

Cut-off	10 NTU	-
Positive	> 11 NTU	Antibodies against the pathogen are present. There has been a contact with the antigen (pathogen resp. vaccine).
Equivocal	9 – 11 NTU	Antibodies against the pathogen could not be detected clearly. It is recommended to repeat the test with a fresh sample in 2 to 4 weeks. If the result is equivocal again the sample is judged as **negative**.
Negative	< 9 NTU	The sample contains no antibodies against the pathogen. A previous contact with the antigen (pathogen resp. vaccine) is unlikely.
Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as serological data. In immunocompromised patients and newborns serological data only have restricted value.		

9.3.1. Antibody Isotypes and State of Infection

Serology	Significance
IgM	Characteristic of the primary antibody response High IgM titer with low IgG titer: → suggests a current or very recent infection Rare: → persisting IgM
IgG	Characteristic of the secondary antibody response May persist for several years High IgG titer with low IgM titer: → may indicate a past infection

10. SPECIFIC PERFORMANCE CHARACTERISTICS

The results refer to the groups of samples investigated; these are not guaranteed specifications.

For further information about the specific performance characteristics please contact NovaTec Immundiagnostica GmbH.

10.1. Precision

Intraassay	**n**	**Mean (OD)**	**CV (%)**
#1	24	0.577	4.14
#2	24	1.276	3.34
#3	24	1.200	2.75
Interassay	**n**	**Mean (NTU)**	**CV (%)**
#1	12	23.22	4.97
#2	12	20.13	6.05
#3	12	5.10	8.55

10.2. Diagnostic Specificity

The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte.
It is 98.78% (95% confidence interval: 93.39% - 99.97%)

10.3. Diagnostic Sensitivity

The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte.
It is 100.0% (95% confidence interval: 66.37% - 100.0%).

10.4. Interferences

Interferences with hemolytic, lipemic or icteric samples are not observed up to a concentration of 10 mg/ml hemoglobin, 5 mg/ml triglycerides and 0.5 mg/ml bilirubin.

10.5. Cross Reactivity

Investigation of a sample panel with antibody activities to potentially cross-reacting parameters did not reveal evidence of false-positive results due to cross-reactions.

11. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or repeated freeze-thaw cycles of the sample may affect the absorbance values.

12. PRECAUTIONS AND WARNINGS

- In compliance with article 1 paragraph 2b European directive 98/79/EC the use of the in vitro diagnostic medical devices is intended by the manufacturer to secure suitability, performances and safety of the product. Therefore the test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the testkits with analyzers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.
- Only for in-vitro diagnostic use.
- All materials of human or animal origin should be regarded and handled as potentially infectious.
- All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive.
- Do not interchange reagents or strips of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- Use only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and standard/control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense reagents without splashing accurately into the wells.
- The ELISA is only designed for qualified personnel who are familiar with good laboratory practice.

12.1. Disposal Considerations

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

13. ORDERING INFORMATION

Prod. No.: BRUG0050 Brucella IgG ELISA (96 Determinations)

DEUTSCH

1. EINLEITUNG

Brucellen sind sehr kleine, gramnegative, unbewegliche Stäbchenbakterien (0.4-0.8 µm Durchmeser und 0.4-3.0 µm lang). Sie wurden benannt nach dem englischen Militärarzt David Bruce, der die Erreger 1887 auf Malta aus der Milz eines an undulierendem Fieber verstorbenen Soldaten isolierte. Von humanpathogener Bedeutung sind vier Arten: Brucella abortus, Brucella melitensis, Brucella suis und Brucella canis. Alle vier Spezies sind Erreger der Brucellose, einem Krankheitsbild, das als undulierendes Fieber bezeichnet wird. Je nach Erregernachweis wird die Erkrankung auch als Morbus Bang (B. abortus) oder Maltafieber (B. melitensis) bezeichnet.

Betroffen sind in erster Linie Tiere, von denen der Erreger auf den Menschen übertragen werden kann. Infektionen erfolgen durch Kontakt mit kranken Tieren oder deren Ausscheidungen, sowie durch unpasteurisierte Milch und deren Produkte (frischer Schaf-, Ziegenkäse). Haupteintrittspforten sind Hautverletzungen, die Konjunktiven und der Gastrointestinaltrakt. Die Erreger werden von Granulozyten, in denen sie unbeschadet überleben, in die lokalen Lymphknoten geschleppt, von wo aus sie hämatogen streuen. Praktisch alle Organe können befallen werden. Die Manifestationsorte bestimmen das Krankheitsbild. In den befallenen Organen entsteht eine granulomatöse Entzündung. Die genauen Mechanismen der Pathogenese der Brucellose sind noch nicht vollständig aufgeklärt.

Brucellosen sind weltweit verbreitet. In unpasteurisierter Milch und deren Produkten sind Brucellen wochenlang lebensfähig und infektiös. Die bovine Brucellose, verursacht durch B. abortus bleibt die am weitesten verbreitete Form dieser Erkrankungen, auch wenn Inzidenz und Prävalenz regional sehr stark variieren (von <0.01 bis >200 pro 100.000). In Gebieten mit Schaf- und Ziegenhaltung tritt besonders B. melitensis endemisch auf und führt zu schweren humanen Infektionen. Der Umgang mit diesen Bakterien im Labor erfordert aufgrund ihrer Kontagiosität größte Sorgfalt. Risikopersonen sind vor allem Schäfer, Landwirte, Tierpfleger, Tierzüchter, Tierärzte, Melker, Molkerei- und Laborpersonal.

Spezies	**Erkrankung**	**Symptome (z.B.)**	**Infektionsweg**
B. abortus (Rind) B. melitensis (Ziegen, Schafe) B. suis (Schweine) B. canis (Hunde)	Brucella	Fieber, Schüttelfrost (Febris undulans), Unwohlsein, Arthritis, Hepatitis, Hepatomegalie, Endokarditis, Osteomyelitis	oral (unpasteurisierte Milch und Milchprodukte) perkutan (durch Kontakt mit kranken Tieren oder deren Ausscheidungen) i. d R. keine Übertragung von Mensch zu Mensch

Nachweis des Erregers bzw. der Infektion durch:

- Histologie
- Serologie: z.B. ELISA

2. VERWENDUNGSZWECK

Der Brucella IgG ELISA ist für den qualitativen Nachweis spezifischer IgG-Antikörper gegen Brucella in humanem Serum oder Plasma (Citrat, Heparin) bestimmt.

3. TESTPRINZIP

Die qualitative immunenzymatische Bestimmung von spezifischen Antikörpern beruht auf der ELISA (Enzyme-linked Immunosorbent Assay) Technik.
Die Mikrotiterplatten sind mit spezifischen Antigenen beschichtet, an welche die korrespondierenden Antikörper aus der Probe binden. Ungebundenes Probenmaterial wird durch Waschen entfernt. Anschließend erfolgt die Zugabe eines Meerettich-Peroxidase (HRP) Konjugates. Dieses Konjugat bindet an die an der Mikrotiterplatte gebundenen spezifischen Antikörper. In einem zweiten Waschschritt wird ungebundenes Konjugat entfernt. Die Immunkomplexe, die durch die Bindung des Konjugates entstanden sind, werden durch die Zugabe von Tetramethylbenzidin (TMB)-Substratlösung und eine resultierende Blaufärbung nachgewiesen.
Die Intensität des Reaktionsproduktes ist proportional zur Menge der spezifischen Antikörper in der Probe. Die Reaktion wird mit Schwefelsäure gestoppt, wodurch ein Farbumschlag von blau nach gelb erfolgt. Die Absorption wird bei 450/620 nm mit einem Mikrotiterplatten-Photometer gemessen.

NovaTec Immundiagnostica GmbH

NovaLisa®

Brucella IgM

ELISA

CE

Only for in-vitro diagnostic use

Product Number: BRUM0050 (96 Determinations)

ENGLISH

1. INTRODUCTION

Brucella is a small Gram-negative bacterium (0.4-0.8 µm in diameter and 0.4-3.0 µm in length) which is non-flagellated, and non-spore-forming. They are named after the military doctor David Bruce, who on Malta in 1887 isolated the pathogens from the spleen of a soldier who died of undulating fever. Four species are pathogenic to human: Brucella abortus, Brucella melitensis, Brucella suis and Brucella canis. All four species are exciters of Brucellosis, a disease characterized by undulating fever. Depending on exciter the disease is also called Morbus Bang (B. abortus) or Malta fever (B. melitensis).

The pathogens are transmitted from animals, which are mainly affected. The infection is caused by contact with ill animals or their excrements as well as by non-pasteurized milk and milk products like fresh cheese from sheep or goat. Main entrances are skin wounds, conjunctives and digestive tract. The intact pathogens are transported by granulocytes into local lymph nodes, from where they spread haematogenous. All kind of organs can be infected. Symptoms depend up on the infected organ, where an inflammation takes place. Until now the pathogenic mechanism is not completely understood.

Brucellosis appears worldwide. In non-pasteurized milk and milk products Brucella is viably and infectious for weeks. Bovine brucellosis caused by Brucella abortus is still the most widespread form, although reported incidence and prevalence of the disease vary widely from country to country (from <0.01 to >200 per 100,000 population). Brucella melitensis is endemic in areas where keeping of sheeps and goats is frequent. It causes serious human infections. Working with these bacteria in laboratories necessitates highest carefulness because of high contagiosity. Risk groups include abattoir workers, meat inspectors, animal handlers, veterinarians, and laboratorians. Brucellosis is a nationally notifiable disease and reportable to the local health authority.

Species	Disease	Symptoms (e.g.)	Transmission route
B. abortus (cattle) B. melitensis (sheep, goats) B. suis (pigs) B. canis (dogs)	Brucella	Fever, chills (undulating fever), malaise, arthritis, hepatitis, endocarditis, hepatomegalie, osteomyelitis (OM)	Oral (non-pasteurized milk and milk products) Percutan (contact with ill animals or their excrements) In general no transmission from human to human

The presence of pathogen or infection may be identified by

- Histology
- Serology: e.g. ELISA

2. INTENDED USE

The Brucella IgM ELISA is intended for the qualitative determination of IgM class antibodies against Brucella in human serum or plasma (citrate, heparin).

3. PRINCIPLE OF THE ASSAY

The qualitative immunoenzymatic determination of specific antibodies is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique.
Microplates are coated with specific antigens to bind corresponding antibodies of the sample. After washing the wells to remove all unbound sample material a horseradish peroxidase (HRP) labelled conjugate is added. This conjugate binds to the captured antibodies. In a second washing step unbound conjugate is removed. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product.
The intensity of this product is proportional to the amount of specific antibodies in the sample. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450/620 nm is read using an ELISA microwell plate reader.

4. MATERIALS

4.1. Reagents supplied

- **Brucella Coated Microplate (IgM):** 12 break-apart 8-well snap-off strips coated with Brucella antigens; in resealable aluminium foil.
- **IgM Sample Diluent:** 1 bottle containing 100 ml of phosphate buffer (10 mM) for sample dilution; pH 7.2 ± 0.2; anti-human IgG (RF Absorbent); coloured green; ready to use; white cap.
- **Stop Solution:** 1 bottle containing 15 ml sulphuric acid, 0.2 mol/l; ready to use; red cap.
- **Washing Buffer (20x conc.):** 1 bottle containing 50 ml of a 20-fold concentrated phosphate buffer (0.2 M), pH 7.2 ± 0.2, for washing the wells; white cap.
- **Brucella anti-IgM Conjugate:** 1 bottle containing 20 ml of peroxidase labelled antibody to human IgM in phosphate buffer (10 mM); coloured red; ready to use; black cap.
- **TMB Substrate Solution:** 1 bottle containing 15 ml 3,3',5,5'-tetramethylbenzidine (TMB), < 0.1 %; ready to use; yellow cap; < 5 % NMP.
- **Brucella IgM Positive Control:** 1 vial containing 2 ml control (human serum or plasma); coloured yellow; ready to use; red cap.
- **Brucella IgM Cut-off Control:** 1 vial containing 3 ml control (human serum or plasma); coloured yellow; ready to use; green cap.
- **Brucella IgM Negative Control:** 1 vial containing 2 ml control (human serum or plasma); coloured yellow; ready to use; blue cap.

For potential hazardous substances please check the safety data sheet.

4.2. Materials supplied

- 1 Cover foil
- 1 Instruction for use (IFU)
- 1 Plate layout

4.3. Materials and Equipment needed

- ELISA microwell plate reader, equipped for the measurement of absorbance at 450/620 nm
- Incubator 37 °C
- Manual or automatic equipment for rinsing wells
- Pipettes to deliver volumes between 10 and 1000 µl
- Vortex tube mixer
- Distilled water
- Disposable tubes

5. STABILITY AND STORAGE

Store the kit at 2...8 °C. The opened reagents are stable up to the expiry date stated on the label when stored at 2...8 °C.

6. REAGENT PREPARATION

It is very important to bring all reagents and samples to room temperature (20…25 °C) and mix them before starting the test run!

6.1. Coated Microplate

The break-apart snap-off strips are coated with Brucella antigens. Immediately after removal of the strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2...8 °C.

6.2. Washing Buffer (20x conc.)

Dilute Washing Buffer 1 + 19; e. g. 10 ml Washing Buffer + 190 ml distilled water. The diluted buffer is stable for 5 days at room temperature (20…25 °C). In case crystals appear in the concentrate, warm up the solution to 37°C e.g. in a water bath. Mix well before dilution.

6.3. TMB Substrate Solution

The reagent is ready to use and has to be stored at 2...8 °C, away from the light. The solution should be colourless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away.

7. SAMPLE COLLECTION AND PREPARATION

Use human serum or plasma (citrate, heparin) samples with this assay. For CSF please use the instruction for use ABVL0001. If the assay is performed within 5 days after sample collection, the samples should be kept at 2...8 °C; otherwise they should be aliquoted and stored deep-frozen (-70…-20 °C). If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing.
Heat inactivation of samples is not recommended.

7.1. Sample Dilution

Before assaying, all samples should be diluted 1+100 with IgM Sample Diluent. Dispense 10 µl sample and 1 ml IgM Sample Diluent into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

8. ASSAY PROCEDURE

8.1. Test Preparation

Please read the instruction for use carefully before performing the assay. Result reliability depends on strict adherence to the instruction for use as described. The following test procedure is only validated for manual procedure. If performing the test on ELISA automatic systems we recommend increasing the washing steps from three to five and the volume of Washing Buffer from 300 µl to 350 µl to avoid washing effects. Pay attention to chapter 12. Prior to commencing the assay, the distribution and identification plan for all samples and standards/controls (duplicates recommended) should be carefully established on the plate layout supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder.

Perform all assay steps in the order given and without any delays.

A clean, disposable tip should be used for dispensing each standard/control and sample.

Adjust the incubator to 37 ± 1 °C.

1. Dispense 100 µl standards/controls and diluted samples into their respective wells. Leave well A1 for the Substrate Blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 1 hour ± 5 min at 37 ± 1 °C.**
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µl of Washing Buffer. Avoid overflows from the reaction wells. The interval between washing and aspiration should be > 5 sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!

   Note: Washing is important! Insufficient washing results in poor precision and false results.
5. Dispense 100 µl Conjugate into all wells except for the Substrate Blank well A1.
6. **Incubate for 30 min at room temperature (20...25 °C).** Do not expose to direct sunlight.
7. Repeat step 4.
8. Dispense 100 µl TMB Substrate Solution into all wells.
9. **Incubate for exactly 15 min at room temperature (20...25 °C) in the dark.** A blue colour occurs due to an enzymatic reaction.
10. Dispense 100 µl Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution, thereby a colour change from blue to yellow occurs.
11. Measure the absorbance at 450/620 nm within 30 min after addition of the Stop Solution.

8.2. Measurement

Adjust the ELISA microwell plate reader **to zero** using the **Substrate Blank**.

If - due to technical reasons - the ELISA microwell plate reader cannot be adjusted to zero using the Substrate Blank, subtract its absorbance value from all other absorbance values measured in order to obtain reliable results!

Measure the absorbance of all wells at **450 nm** and record the absorbance values for each standard/control and sample in the plate layout.

Bichromatic measurement using a reference wavelength of 620 nm is recommended.

Where applicable calculate the **mean absorbance values** of all duplicates.

9. RESULTS

9.1. Run Validation Criteria

In order for an assay to be considered valid, the following criteria must be met:

- **Substrate Blank**: Absorbance value **< 0.100**
- **Negative Control**: Absorbance value **< 0.200 and < Cut-off**
- **Cut-off Control**: Absorbance value **0.150 – 1.300**
- **Positive Control**: Absorbance value **> Cut-off**

If these criteria are not met, the test is not valid and must be repeated.

9.2. Calculation of Results

The Cut-off is the mean absorbance value of the Cut-off Control determinations.

Example: Absorbance value Cut-off Control 0.44 + absorbance value Cut-off control 0.42 = 0.86 / 2 = 0.43
Cut-off = 0.43

9.2.1. Results in Units [NTU]

$$\frac{\text{Sample (mean) absorbance value} \times 10}{\text{Cut-off}} = \text{[NovaTec Units = NTU]}$$

Example: $\frac{1.591 \times 10}{0.43} = 37$ NTU (Units)

9.3. Interpretation of Results

Cut-off	10 NTU	-
Positive	> 11 NTU	Antibodies against the pathogen are present. There has been a contact with the antigen (pathogen resp. vaccine).
Equivocal	9 – 11 NTU	Antibodies against the pathogen could not be detected clearly. It is recommended to repeat the test with a fresh sample in 2 to 4 weeks. If the result is equivocal again the sample is judged as **negative**.
Negative	< 9 NTU	The sample contains no antibodies against the pathogen. A previous contact with the antigen (pathogen resp. vaccine) is unlikely.
Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as serological data. In immunocompromised patients and newborns serological data only have restricted value.		

9.3.1. Antibody Isotypes and State of Infection

Serology	Significance
IgM	Characteristic of the primary antibody response High IgM titer with low IgG titer: → suggests a current or very recent infection Rare: → persisting IgM
IgG	Characteristic of the secondary antibody response May persist for several years High IgG titer with low IgM titer: → may indicate a past infection

10. SPECIFIC PERFORMANCE CHARACTERISTICS

The results refer to the groups of samples investigated; these are not guaranteed specifications.

For further information about the specific performance characteristics please contact NovaTec Immundiagnostica GmbH.

10.1. Precision

Intraassay	**n**	**Mean (OD)**	**CV (%)**
#1	24	0.550	5.82
#2	24	1.048	4.27
#3	24	0.998	4.18
Interassay	**n**	**Mean (NTU)**	**CV (%)**
#1	12	19.82	8.53
#2	12	14.08	13.38
#3	12	2.28	7.43

10.2. Diagnostic Specificity

The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte.
It is 100.0% (95% confidence interval: 97.49% - 100.0%).

10.3. Diagnostic Sensitivity

The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte.
It is 100.0% (95% confidence interval: 81.47% - 100.0%).

10.4. Interferences

Interferences with hemolytic, lipemic or icteric samples are not observed up to a concentration of 10 mg/ml hemoglobin, 5 mg/ml triglycerides and 0.5 mg/ml bilirubin.

10.5. Cross Reactivity

Investigation of a sample panel with antibody activities to potentially cross-reacting parameters did not reveal evidence of false-positive results due to cross-reactions.

11. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or repeated freeze-thaw cycles of the sample may affect the absorbance values.

12. PRECAUTIONS AND WARNINGS

- In compliance with article 1 paragraph 2b European directive 98/79/EC the use of the in vitro diagnostic medical devices is intended by the manufacturer to secure suitability, performances and safety of the product. Therefore the test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the testkits with analyzers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.
- Only for in-vitro diagnostic use.
- All materials of human or animal origin should be regarded and handled as potentially infectious.
- All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive.
- Do not interchange reagents or strips of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- Use only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and standard/control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense reagents without splashing accurately into the wells.
- The ELISA is only designed for qualified personnel who are familiar with good laboratory practice.

12.1. Disposal Considerations

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

13. ORDERING INFORMATION

Prod. No.: BRUM0050 Brucella IgM ELISA (96 Determinations)

NovaTec Immundiagnostica GmbH

NovaLisa®

Hantavirus IgG

ELISA

CE

Only for in-vitro diagnostic use

Product Number: HANG0670 (96 Determinations)

ENGLISH

1. INTRODUCTION

Hantaviruses are negative sense RNA viruses in the Bunyaviridae family. Humans may be infected with Hantaviruses through urine, saliva or contact with rodent waste products. Some Hantaviruses may lead to serious diseases in humans, such as hemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS).

Human infections of Hantaviruses have almost entirely been linked to human contact with rodent excrement, but recent human-to-human transmission has been reported with the Andes virus in South America.

Hantavirus has an incubation time of two to four weeks in humans before symptoms of infection occur. The symptoms of HFRS can be split into five phases:

- Febrile phase: Symptoms include fever, chills, sweaty palms, diarrhea, malaise, headaches, nausea, abdominal and back pain, respiratory problems such as the ones common in influenza virus infection, as well as gastro-intestinal problems. These symptoms normally occur for three to seven days and arise about two to three weeks after exposure.
- Hypotensive phase: This occurs when the blood platelet levels drop and symptoms can lead to tachycardia and hypoxemia. This phase can last for 2 days.
- Oliguric phase: This phase lasts for three to seven days and is characterized by the onset of renal failure and proteinuria occurs.
- Diuretic phase: This is characterized by diuresis of three to six liters per day, which can last for a couple of days up to weeks.
- Convalescent phase: This is normally when recovery occurs and symptoms begin to improve.

Regions especially affected by HFRS include China, the Korean Peninsula, Russia (Hantaan, Puumala and Seoul viruses), and northern and western Europe (Puumala and Dobrava virus).

Species	Disease	Symptoms (e.g.)	Transmission route
Puumala virus Dobrava virus Hantaan virus Seoul virus	Hemorrhagic fever with renal syndrome (HFRS)	Initial: suddenly occurring symptoms like intense headache, back and abdominal pain, fever, chills, nausea, and blurred vison. Late: low blood pressure, acute shock, vascular leakage, and acute kidney failure	After exposure to aerosolized urine, droppings, or saliva of infected rodents or their nests (airborne transmission). Also by direct contact with these materials to broken skin or onto mucous membranes. Bites by infected rodents. Human to human transmission can not be excluded (for New World strains).
Andes virus Sin-Nombre-virus (New world strains)	Hantavirus pulmonary syndrome (HPS)	Initial: universal symptoms include fatigue, fever and muscle aches, especially in the large muscle groups - thighs, hips, back, and sometimes shoulders. There may also be headache, dizziness, chills, and abdominal problems, such as nausea, vomiting, diarrhea, and abdominal pain. Late: coughing and shortness of breath, lungs fill with fluid.	

The presence of pathogen or infection may be identified by:

- PCR
- Serology (e. g. ELISA)

2. INTENDED USE

The Hantavirus IgG ELISA is intended for the qualitative determination of IgG antibodies against Hantavirus in human serum or plasma (citrate or heparin).

3. PRINCIPLE OF THE ASSAY

The qualitative immunoenzymatic determination of specific antibodies is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique.
Microplates are coated with specific antigens to bind corresponding antibodies of the sample. After washing the wells to remove all unbound sample material a horseradish peroxidase (HRP) labelled conjugate is added. This conjugate binds to the captured antibodies. In a second washing step unbound conjugate is removed. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product.
The intensity of this product is proportional to the amount of specific antibodies in the sample. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450/620 nm is read using an ELISA microwell plate reader.

4. MATERIALS

4.1. Reagents supplied

- **Hantavirus Coated Microplate (IgG):** 12 breakapart 8-well snap-off strips coated with recombinant Hantavirus antigens in resealable aluminium foil.
- **IgG Sample Diluent**: 1 bottle containing 100 ml of phosphate buffer (10 mM) for sample dilution; pH 7.2 ± 0.2; coloured yellow; ready to use; white cap.
- **Stop Solution**: 1 bottle containing 15 ml sulphuric acid, 0.2 mol/l; ready to use; red cap.
- **Washing Buffer (20x conc.):** 1 bottle containing 50 ml of a 20-fold concentrated phosphate buffer (0.2 M); pH 7.2 ± 0.2; for washing the wells; white cap.
- **Hantavirus anti-IgG Conjugate:** 1 bottle containing 20 ml of peroxidase labelled antibody to human IgG; in phosphate buffer (10 mM); coloured blue, ready to use; black cap.
- **TMB Substrate Solution:** 1 bottle containing 15 ml 3,3',5,5'-tetramethylbenzidine (TMB), < 0.1%; ready to use; yellow cap; < 5% NMP.
- **Hantavirus IgG Positive Control:** 1 bottle containing 2 ml control (human serum or plasma); coloured yellow; ready to use; red cap.
- **Hantavirus IgG Cut-off Control:** 1 bottle containing 3 ml control (human serum or plasma); coloured yellow; ready to use; green cap.
- **Hantavirus IgG Negative Control:** 1 bottle containing 2 ml control (human serum or plasma); coloured yellow; ready to use; blue cap.

For potential hazardous substances please check the safety data sheet.

4.2. Materials supplied

- 1 Cover foil
- 1 Instruction for use (IFU)
- 1 Plate layout

4.3. Materials and Equipment needed

- ELISA microwell plate reader, equipped for the measurement of absorbance at 450/620 nm
- Incubator 37°C
- Manual or automatic equipment for rinsing wells
- Pipettes to deliver volumes between 10 and 1000 µl
- Vortex tube mixer
- Distilled water
- Disposable tubes

5. STABILITY AND STORAGE

Store the kit at 2...8 °C. The opened reagents are stable up to the expiry date stated on the label when stored at 2...8 °C.

6. REAGENT PREPARATION

It is very important to bring all reagents and samples to room temperature (20…25 °C) and mix them before starting the test run!

6.1. Coated Microplate

The break-apart snap-off strips are coated with recombinant Hantavirus antigens. Immediately after removal of the strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2...8 °C.

6.2. Washing Buffer (20x conc.)

Dilute Washing Buffer 1 + 19; e. g. 10 ml Washing Buffer + 190 ml distilled water. The diluted buffer is stable for 5 days at room temperature (20…25 °C). In case crystals appear in the concentrate, warm up the solution to 37°C e.g. in a water bath. Mix well before dilution.

6.3. TMB Substrate Solution

The reagent is ready to use and has to be stored at 2...8 °C, away from the light. The solution should be colourless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away.

7. SAMPLE COLLECTION AND PREPARATION

Use human serum or plasma (citrate or heparin) samples with this assay. For CSF please use the instruction for use ABVL0001. If the assay is performed within 5 days after sample collection, the samples should be kept at 2...8 °C; otherwise they should be aliquoted and stored deep-frozen (-70…-20 °C). If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing.
Heat inactivation of samples is not recommended.

7.1. Sample Dilution

Before assaying, all samples should be diluted 1+100 with IgG Sample Diluent. Dispense 10 µl sample and 1 ml IgG Sample Diluent into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

8. ASSAY PROCEDURE

8.1. Test Preparation

Please read the instruction for use carefully **before** performing the assay. Result reliability depends on strict adherence to the instruction for use as described. The following test procedure is only validated for manual procedure. If performing the test on ELISA automatic systems we recommend increasing the washing steps from three to five and the volume of Washing Buffer from 300 µl to 350 µl to avoid washing effects. Pay attention to chapter 12. Prior to commencing the assay, the distribution and identification plan for all samples and standards/controls (duplicates recommended) should be carefully established on the plate layout supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder.

Perform all assay steps in the order given and without any delays.

A clean, disposable tip should be used for dispensing each standard/control and sample.

Adjust the incubator to 37 ± 1 °C.

1. Dispense 100 µl standards/controls and diluted samples into their respective wells. Leave well A1 for the Substrate Blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 1 hour ± 5 min at 37 ± 1 °C.**
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µl of Washing Buffer. Avoid overflows from the reaction wells. The interval between washing and aspiration should be > 5 sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!

   Note: Washing is important! Insufficient washing results in poor precision and false results.
5. Dispense 100 µl Conjugate into all wells except for the Substrate Blank well A1.
6. **Incubate for 30 min at room temperature (20...25 °C).** Do not expose to direct sunlight.
7. Repeat step 4.
8. Dispense 100 µl TMB Substrate Solution into all wells.
9. **Incubate for exactly 15 min at room temperature (20...25 °C) in the dark.** A blue colour occurs due to an enzymatic reaction.
10. Dispense 100 µl Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution, thereby a colour change from blue to yellow occurs.
11. Measure the absorbance at 450/620 nm within 30 min after addition of the Stop Solution.

8.2. Measurement

Adjust the ELISA microwell plate reader **to zero** using the **Substrate Blank**.

If - due to technical reasons - the ELISA microwell plate reader cannot be adjusted to zero using the Substrate Blank, subtract its absorbance value from all other absorbance values measured in order to obtain reliable results!

Measure the absorbance of all wells at **450 nm** and record the absorbance values for each standard/control and sample in the plate layout.

Bichromatic measurement using a reference wavelength of 620 nm is recommended.

Where applicable calculate the **mean absorbance values** of all duplicates.

9. RESULTS

9.1. Run Validation Criteria

In order for an assay to be considered valid, the following criteria must be met:

- **Substrate Blank**: Absorbance value **< 0.100**
- **Negative Control**: Absorbance value **< 0.200 and < Cut-off**
- **Cut-off Control**: Absorbance value **0.150 – 1.300**
- **Positive Control**: Absorbance value **> Cut-off**

If these criteria are not met, the test is not valid and must be repeated.

9.2. Calculation of Results

The Cut-off is the mean absorbance value of the Cut-off Control determinations.

Example: Absorbance value Cut-off Control 0.44 + absorbance value Cut-off control 0.42 = 0.86 / 2 = 0.43

Cut-off = 0.43

9.2.1. Results in Units [NTU]

$$\frac{\text{Sample (mean) absorbance value} \times 10}{\text{Cut-off}} = \text{[NovaTec Units = NTU]}$$

Example: $\frac{1.591 \times 10}{0.43} = 37$ NTU (Units)

9.3. Interpretation of Results

Cut-off	10 NTU	-
Positive	> 11 NTU	Antibodies against the pathogen are present. There has been a contact with the antigen (pathogen resp. vaccine).
Equivocal	9 – 11 NTU	Antibodies against the pathogen could not be detected clearly. It is recommended to repeat the test with a fresh sample in 2 to 4 weeks. If the result is equivocal again the sample is judged as **negative**.
Negative	< 9 NTU	The sample contains no antibodies against the pathogen. A previous contact with the antigen (pathogen resp. vaccine) is unlikely.
Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as serological data. In immunocompromised patients and newborns serological data only have restricted value.		

9.3.1. Antibody Isotypes and State of Infection

Serology	Significance
IgM	Characteristic of the primary antibody response High IgM titer with low IgG titer: → suggests a current or very recent infection Rare: → persisting IgM
IgG	Characteristic of the secondary antibody response May persist for several years High IgG titer with low IgM titer: → may indicate a past infection

10. SPECIFIC PERFORMANCE CHARACTERISTICS

The results refer to the groups of samples investigated; these are not guaranteed specifications.

For further information about the specific performance characteristics please contact NovaTec Immundiagnostica GmbH.

10.1. Precision

Intraassay	n	Mean (E)	Cv (%)
#1	24	0.450	3.61
#2	24	1.333	6.41
#3	24	1.264	4.78

Interassay	n	Mean (NTU)	Cv (%)
#1	12	27.44	5.34
#2	12	25.44	8.15
#3	12	1.09	12.09

10.2. Diagnostic Specificity

The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte. It is 96.59% (95% confidence interval: 90.36% - 99.29%).

10.3. Diagnostic Sensitivity

The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte. It is 99.16% (95% confidence interval: 95.41% - 99.98%).

10.4. Interferences

Interferences with hemolytic, lipemic or icteric samples are not observed up to a concentration of 10 mg/ml hemoglobin, 5 mg/ml triglycerides and 0.5 mg/ml bilirubin.

10.5. Cross Reactivity

Investigation of a sample panel with antibody activities to potentially cross-reacting parameters did not reveal significant evidence of false-positive results due to cross-reactions.

11. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or repeated freeze-thaw cycles of the sample may affect the absorbance values.

12. PRECAUTIONS AND WARNINGS

- In compliance with article 1 paragraph 2b European directive 98/79/EC the use of the in vitro diagnostic medical devices is intended by the manufacturer to secure suitability, performances and safety of the product. Therefore the test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the testkits with analyzers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.
- Only for in-vitro diagnostic use.
- All materials of human or animal origin should be regarded and handled as potentially infectious.
- All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive.
- Do not interchange reagents or strips of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- Use only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and standard/control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense reagents without splashing accurately into the wells.
- The ELISA is only designed for qualified personnel who are familiar with good laboratory practice.

12.1. Disposal Considerations

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

13. ORDERING INFORMATION

Prod. No.: HANG0670 Hantavirus IgG ELISA (96 Determinations)

DEUTSCH

1. EINLEITUNG

Hantaviren sind Einzelstrang ss(-)RNA Viren der Bunyaviridae Familie. Die Infektion von Menschen findet durch Urin, Speichel oder Kontakt mit anderen Abfallprodukten von Nagetieren statt. Einige Hantaviren können ernsthafte Erkrankungen verursachen wie das Hämorrhagische Fieber mit Renalem Syndrom (HFRS) oder das Hantavirus-assoziierte pulmonale Syndrom, HPS.

Infektionen mit Hantaviren lassen sich fast vollständig auf den Kontakt mit Ausscheidungsprodukten von Nagetieren zurückführen, es wurden aber auch Mensch zu Mensch Übertragungen mit dem Andes Virus in Südamerika beschrieben.

Hantaviren haben eine Inkubationszeit von 2-4 Wochen bevor erste Symptome der Infektion auftreten. Die Symptome von HFRS können in 5 Phasen unterteilt werden:

- Febrile Phase: Die Symptome beinhalten Fieber, Schüttelfrost, Schweißausbrüche, Brechdurchfall, Unwohlsein, Kopfschmerzen, Übelkeit, abdominal und Rückenschmerzen, Atemprobleme wie sie auch durch gewöhnliche Influenzaviren hervorgerufen werden, als auch gastrointestinale Probleme. Die Symptome treten normalerweise 2-3 Wochen nach der Infektion für die Dauer von 3-7 Tagen auf.
- Hypotonische Phase: Diese tritt auf, wenn der Level an Thrombozyten fällt und Herzrasen sowie Hypoxämien auftreten. Diese Phase kann 2 Tage andauern.
- Oligurische Phase: Diese Phase dauert 3-7 Tage an und ist charakterisiert durch das Auftreten von Nierenversagen und Eiweißausscheidungen in Harn.
- Diuretische Phase: Diese Phase ist charakterisiert durch Harnmengen von 3-6 Litern pro Tag. Sie kann wenige Tage bis zu einigen Wochen andauern.
- Rekonvaleszente Phase: Hier findet die Erholung von der Krankheit statt und die Symptome gehen zurück.

Speziell von HFRS betroffene Regionen sind China, die koreanische Halbinsel, Russland (Hantaan, Puumala und Seoul Viren) sowie den Norden und Westen von Europa (Puumala und Dobrava Viren).

Spezies	**Erkrankung**	**Symptome (z.B.)**	**Infektionsweg**
Puumala Virus Dobrava Virus Hantaan Virus Seoul Virus	Hämorrhagisches Fieber mit renalem Syndrom (HFRS)	Früh: plötzlich auftretende Symptome wie Kopf- und Rückenschmerzen, abdominelle Schmerzen, Fieber, Schüttelfrost, Übelkeit und verschwommene Sicht. Spät: Niedriger Blutdruck, akuter Schock, Gefäßdurchlässigkeitsstörung und akutes Nierenversagen.	Durch Einatmen von Stäuben, die Urin, Kot, Speichel oder Nestmaterial infizierter Nager enthalten (luftübertragene Transmission). Ebenso durch direkten Kontakt mit diesen Materialien mit verletzter Haut oder Schleimhäuten. Durch Nagerbisse. Eine Übertragung von Mensch zu Mensch kann nicht ausgeschlossen werden (v.a. bei den Neue Welt Stämmen).
Andes Virus Sin-Nombre-virus (Neue Welt Stämme)	Hantavirus-induziertes-(kardio-) pulmonales Syndrom (HPS/ HCPS)	Früh: universell auftretende Symptome wie Schwindel, Fieber und Muskelschmerzen, v.a. der großen Muskulaturen von Oberschenkel, Hüfte, Rücken und manchmal Schultern. Ebenso können Kopfschmerzen, Benommenheit, Schüttelfrost, und abdominale Probleme wie Übelkeit, Erbrechen, Durchfall und Unterleibsschmerzen auftreten. Spät: Husten und Kurzatmigkeit, Flüssigkeitsansammlung in den Lungen.	

Nachweis des Erregers bzw. der Infektion durch:

- PCR
- Serologie (z.B. ELISA)

2. VERWENDUNGSZWECK

Der Hantavirus IgG ELISA ist für den qualitativen Nachweis spezifischer IgG Antikörper gegen Hantavirus in humanem Serum oder Plasma (Citrat oder Heparin) bestimmt.

NovaTec Immundiagnostica GmbH

NovaLisa®

Hantavirus IgM

ELISA

CE

Only for in-vitro diagnostic use

Product Number: HANM0670 (96 Determinations)

ENGLISH

1. INTRODUCTION

Hantaviruses are negative sense RNA viruses in the Bunyaviridae family. Humans may be infected with Hantaviruses through urine, saliva or contact with rodent waste products. Some Hantaviruses may cause serious diseases in humans, such as hemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS).

Human infections of Hantaviruses have almost entirely been linked to human contact with rodent excrement, but recent human-to-human transmission has been reported with the Andes virus in South America.

Hantavirus has an incubation time of two to four weeks in humans before symptoms of infection occur. The symptoms of HFRS can be split into five phases:

- Febrile phase: Symptoms include fever, chills, sweaty palms, diarrhea, malaise, headaches, nausea, abdominal and back pain, respiratory problems such as the ones common in influenza virus infection, as well as gastro-intestinal problems. These symptoms normally occur for three to seven days and arise about two to three weeks after exposure.
- Hypotensive phase: This occurs when the blood platelet levels drop and symptoms can lead to tachycardia and hypoxemia. This phase can last for 2 days.
- Oliguric phase: This phase lasts for three to seven days and is characterized by the onset of renal failure and proteinuria occurs.
- Diuretic phase: This is characterized by diuresis of three to six liters per day, which can last for a couple of days up to weeks.
- Convalescent phase: This is normally when recovery occurs and symptoms begin to improve.

Regions especially affected by HFRS include China, the Korean Peninsula, Russia (Hantaan, Puumala and Seoul viruses), and northern and western Europe (Puumala and Dobrava virus).

Species	Disease	Symptoms (e.g.)	Transmission route
Puumala virus Dobrava virus Hantaan virus Seoul virus	Hemorrhagic fever with renal syndrome (HFRS)	Initial: suddenly occurring symptoms like intense headache, back and abdominal pain, fever, chills, nausea, and blurred vison. Late: low blood pressure, acute shock, vascular leakage, and acute kidney failure	After exposure to aerosolized urine, droppings, or saliva of infected rodents or their nests (airborne transmission). Also by direct contact with these materials to broken skin or onto mucous membranes. Bites by infected rodents. Human to human transmission can not be excluded (for New World strains).
Andes virus Sin-Nombre-virus (New world strains)	Hantavirus pulmonary syndrome (HPS)	Early: universal symptoms include fatigue, fever and muscle aches, especially in the large muscle groups - thighs, hips, back, and sometimes shoulders. There may also be headaches, dizziness, chills, and abdominal problems, such as nausea, vomiting, diarrhea, and abdominal pain. Late: coughing and shortness of breath, lungs fill with fluid.	

The presence of pathogen or infection may be identified by:

- PCR
- Serology (e. g. ELISA)

2. INTENDED USE

The Hantavirus IgM ELISA is intended for the qualitative determination of IgM antibodies against Hantavirus in human serum or plasma (citrate or heparin).

3. PRINCIPLE OF THE ASSAY

The qualitative immunoenzymatic determination of specific antibodies is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique.
Microplates are coated with specific antigens to bind corresponding antibodies of the sample. After washing the wells to remove all unbound sample material a horseradish peroxidase (HRP) labelled conjugate is added. This conjugate binds to the captured antibodies. In a second washing step unbound conjugate is removed. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product.
The intensity of this product is proportional to the amount of specific antibodies in the sample. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450/620 nm is read using an ELISA microwell plate reader.

4. MATERIALS

4.1. Reagents supplied

- **Hantavirus Coated Microplate (IgM):** 12 breakapart 8-well snap-off strips coated with recombinant Hantavirus antigens in resealable aluminium foil.
- **IgM Sample Diluent**: 1 bottle containing 100 ml of phosphate buffer (10 mM) for sample dilution; pH 7.2 ± 0.2; anti-human IgG (RF Absorbent); coloured green; ready to use; white cap.
- **Stop Solution**: 1 bottle containing 15 ml sulphuric acid, 0.2 mol/l; ready to use; red cap.
- **Washing Buffer (20x conc.):** 1 bottle containing 50 ml of a 20-fold concentrated phosphate buffer (0.2 M); pH 7.2 ± 0.2; for washing the wells; white cap.
- **Hantavirus anti-IgM Conjugate:** 1 bottle containing 20 ml of peroxidase labelled antibody to human IgM; in phosphate buffer (10 mM); coloured red, ready to use; black cap.
- **TMB Substrate Solution:** 1 bottle containing 15 ml 3,3',5,5'-tetramethylbenzidine (TMB), < 0.1%; ready to use; yellow cap; < 5% NMP.
- **Hantavirus IgM Positive Control:** 1 bottle containing 2 ml control (human serum or plasma); coloured yellow; ready to use; red cap.
- **Hantavirus IgM Cut-off Control:** 1 bottle containing 3 ml control (human serum or plasma); coloured yellow; ready to use; green cap.
- **Hantavirus IgM Negative Control:** 1 bottle containing 2 ml control (human serum or plasma); coloured yellow; ready to use; blue cap.

For potential hazardous substances please check the safety data sheet.

4.2. Materials supplied

- 1 Cover foil
- 1 Instruction for use (IFU)
- 1 Plate layout

4.3. Materials and Equipment needed

- ELISA microwell plate reader, equipped for the measurement of absorbance at 450/620 nm
- Incubator 37°C
- Manual or automatic equipment for rinsing wells
- Pipettes to deliver volumes between 10 and 1000 µl
- Vortex tube mixer
- Distilled water
- Disposable tubes

5. STABILITY AND STORAGE

Store the kit at 2...8 °C. The opened reagents are stable up to the expiry date stated on the label when stored at 2...8 °C.

6. REAGENT PREPARATION

It is very important to bring all reagents and samples to room temperature (20…25 °C) and mix them before starting the test run!

6.1. Coated Microplate

The break-apart snap-off strips are coated with recombinant Hantavirus antigens. Immediately after removal of the strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2...8 °C.

6.2. Washing Buffer (20x conc.)

Dilute Washing Buffer 1 + 19; e. g. 10 ml Washing Buffer + 190 ml distilled water. The diluted buffer is stable for 5 days at room temperature (20…25 °C). In case crystals appear in the concentrate, warm up the solution to 37°C e.g. in a water bath. Mix well before dilution.

6.3. TMB Substrate Solution

The reagent is ready to use and has to be stored at 2...8 °C, away from the light. The solution should be colourless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away.

7. SAMPLE COLLECTION AND PREPARATION

Use human serum or plasma (citrate or heparin) samples with this assay. For CSF please use the instruction for use ABVL0001. If the assay is performed within 5 days after sample collection, the samples should be kept at 2...8 °C; otherwise they should be aliquoted and stored deep-frozen (-70…-20 °C). If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing.
Heat inactivation of samples is not recommended.

7.1. Sample Dilution

Before assaying, all samples should be diluted 1+100 with IgM Sample Diluent. Dispense 10 µl sample and 1 ml IgM Sample Diluent into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

8. ASSAY PROCEDURE

8.1. Test Preparation

Please read the instruction for use carefully **before** performing the assay. Result reliability depends on strict adherence to the instruction for use as described. The following test procedure is only validated for manual procedure. If performing the test on ELISA automatic systems we recommend increasing the washing steps from three to five and the volume of Washing Buffer from 300 µl to 350 µl to avoid washing effects. Pay attention to chapter 12. Prior to commencing the assay, the distribution and identification plan for all samples and standards/controls (duplicates recommended) should be carefully established on the plate layout supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder.

Perform all assay steps in the order given and without any delays.

A clean, disposable tip should be used for dispensing each standard/control and sample.

Adjust the incubator to 37 ± 1 °C.

1. Dispense 100 µl standards/controls and diluted samples into their respective wells. Leave well A1 for the Substrate Blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 1 hour ± 5 min at 37 ± 1 °C.**
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µl of Washing Buffer. Avoid overflows from the reaction wells. The interval between washing and aspiration should be > 5 sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!

   Note: Washing is important! Insufficient washing results in poor precision and false results.
5. Dispense 100 µl Conjugate into all wells except for the Substrate Blank well A1.
6. **Incubate for 30 min at room temperature (20...25 °C).** Do not expose to direct sunlight.
7. Repeat step 4.
8. Dispense 100 µl TMB Substrate Solution into all wells.
9. **Incubate for exactly 15 min at room temperature (20...25 °C) in the dark.** A blue colour occurs due to an enzymatic reaction.
10. Dispense 100 µl Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution, thereby a colour change from blue to yellow occurs.
11. Measure the absorbance at 450/620 nm within 30 min after addition of the Stop Solution.

8.2. Measurement

Adjust the ELISA microwell plate reader **to zero** using the **Substrate Blank**.

If - due to technical reasons - the ELISA microwell plate reader cannot be adjusted to zero using the Substrate Blank, subtract its absorbance value from all other absorbance values measured in order to obtain reliable results!

Measure the absorbance of all wells at **450 nm** and record the absorbance values for each standard/control and sample in the plate layout.

Bichromatic measurement using a reference wavelength of 620 nm is recommended.

Where applicable calculate the **mean absorbance values** of all duplicates.

9. RESULTS

9.1. Run Validation Criteria

In order for an assay to be considered valid, the following criteria must be met:

- **Substrate Blank**: Absorbance value **< 0.100**
- **Negative Control**: Absorbance value **< 0.200 and < Cut-off**
- **Cut-off Control**: Absorbance value **0.150 – 1.300**
- **Positive Control**: Absorbance value **> Cut-off**

If these criteria are not met, the test is not valid and must be repeated.

9.2. Calculation of Results

The Cut-off is the mean absorbance value of the Cut-off Control determinations.

Example: Absorbance value Cut-off Control 0.44 + absorbance value Cut-off control 0.42 = 0.86 / 2 = 0.43
Cut-off = 0.43

9.2.1. Results in Units [NTU]

$$\frac{\text{Sample (mean) absorbance value} \times 10}{\text{Cut-off}} = \text{[NovaTec Units = NTU]}$$

Example: $\frac{1.591 \times 10}{0.43} = 37$ NTU (Units)

9.3. Interpretation of Results

Cut-off	10 NTU	-
Positive	> 11 NTU	Antibodies against the pathogen are present. There has been a contact with the antigen (pathogen resp. vaccine).
Equivocal	9 – 11 NTU	Antibodies against the pathogen could not be detected clearly. It is recommended to repeat the test with a fresh sample in 2 to 4 weeks. If the result is equivocal again the sample is judged as **negative**.
Negative	< 9 NTU	The sample contains no antibodies against the pathogen. A previous contact with the antigen (pathogen resp. vaccine) is unlikely.
Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as serological data. In immunocompromised patients and newborns serological data only have restricted value.		

9.3.1. Antibody Isotypes and State of Infection

Serology	Significance
IgM	Characteristic of the primary antibody response High IgM titer with low IgG titer: → suggests a current or very recent infection Rare: → persisting IgM
IgG	Characteristic of the secondary antibody response May persist for several years High IgG titer with low IgM titer: → may indicate a past infection

10. SPECIFIC PERFORMANCE CHARACTERISTICS

The results refer to the groups of samples investigated; these are not guaranteed specifications.

For further information about the specific performance characteristics please contact NovaTec Immundiagnostica GmbH.

10.1. Precision

Intraassay	n	Mean (E)	Cv (%)
#1	24	0.649	4.11
#2	24	1.322	3.24
#3	24	1.064	4.29

Interassay	n	Mean (NTU)	Cv (%)
#1	12	23.49	12.94
#2	12	15.31	12.08
#3	12	0.73	12.86

10.2. Diagnostic Specificity

The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte. It is 99.03% (95% confidence interval: 94.71% - 99.98%).

10.3. Diagnostic Sensitivity

The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte. It is 96.3% (95% confidence interval: 89.56% - 99.23%).

10.4. Interferences

Interferences with hemolytic, lipemic or icteric samples are not observed up to a concentration of 10 mg/ml hemoglobin, 5 mg/ml triglycerides and 0.5 mg/ml bilirubin.

10.5. Cross Reactivity

Investigation of a sample panel with antibody activities to potentially cross-reacting parameters did not reveal significant evidence of false-positive results due to cross-reactions.

11. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or repeated freeze-thaw cycles of the sample may affect the absorbance values.

12. PRECAUTIONS AND WARNINGS

- In compliance with article 1 paragraph 2b European directive 98/79/EC the use of the in vitro diagnostic medical devices is intended by the manufacturer to secure suitability, performances and safety of the product. Therefore the test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the testkits with analyzers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.
- Only for in-vitro diagnostic use.
- All materials of human or animal origin should be regarded and handled as potentially infectious.
- All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive.
- Do not interchange reagents or strips of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- Use only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and standard/control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense reagents without splashing accurately into the wells.
- The ELISA is only designed for qualified personnel who are familiar with good laboratory practice.

12.1. Disposal Considerations

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

13. ORDERING INFORMATION

Prod. No.: HANM0670 Hantavirus IgM ELISA (96 Determinations)

Instructions For Use

Serazym® Rotavirus

Enzyme immunoassay for detection of *Rotavirus* in faecal samples

REF E-020 Σ 96 REF E-020-A2 Σ 2x 96 IVD *In-vitro*- diagnostic medical device CE

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Introduction

Group A Rotaviruses are the most common cause of non-bacterial gastroenteritis in children aged between 4 months and 3 years (1 - 5). Rotavirus is excreted into the intestine in large amounts (10^9 – 10^{11} virus particles per g faeces). Nosocomial infections therefore cause problems especially on baby wards and in childrens hospitals (3).
Rotavirus may also be responsible for travellers diarrhea in adults and have been detected in stool specimens of asymptomatic carriers as well (1). Rotavirus is spread by faecal-oral transmission from person to person or via contaminated staff. In temperate climates Rotavirus infections are mainly observed during the winter months (1).
Since virus culture on primary monkey kidney cells or permanent cell lines is time-consuming, these methods are of no diagnostic relevance. The golden standard is direct virus detection by electron microscopy (1, 2). Meanwhile antigen detection methods based on immunological techniques like agglutination tests or enzyme immunoassays using polyclonal or monoclonal antibodies against the group A specific antigen (VP-6) have been established (1 - 5).

References:

1. Böthig, B. und Diedrich, S. (1996):"Rotaviren" Diagnostische Bibliothek Band 1 Virusdiagnostik, Hrsg. Tomas Porstmann, Blackwell Wissenschafts-Verlag Berlin, Wien 1996, S. 441-451
2. Grauballe, B.F. et al. (1981):"Optimized Enzyme-Linked Immunosorbent Assay for Detection of Human and Bovine Rotavirus in Stools: Comparison with Electron-Microscopy, Immunoelectro-Osmophoresis and Fluorescent Antibody Techniques." Journal of Medical Virology 7: 29-40
3. Coulson, B.S. and I.H. Holmes (1984): "An Improved Enzyme-Linked Immunosorbent Assay For The Detection Of Rotavirus In Faeces Of Neonates." Journal of Virological Methods, 8: 165-179
4. Cukor G. et al. (1984): "Detection of Rotavirus in Human Stools by Using Monoclonal Antibody." Journal of Clinical Microbiology 19: 888-892
5. Cukor G. and N.R. Blacklow (1984): "Human Viral Gastroenteritis", Microbiological Reviews 48 No.2,p. 157-179.

Intended Use

The *Serazym*® Rotavirus is an *in-vitro*-diagnostic medical device for direct detection of Rotavirus in faecal samples.

Principle Of The Test

Serazym® Rotavirus is a one-step enzyme immunoassay on the basis of polyclonal antibodies to the group specific VP-6 antigen, the major protein of group A Rotaviruses. Diluted stool specimens and horseradish peroxidase (HRP) labelled polyclonal anti-Rotavirus-antibodies are dispensed simultaneously into the wells of a microtitration plate coated with polyclonal anti-Rotavirus antibodies. After an incubation time of 60 min at room temperature unbound components are removed by a washing step. HRP converts the subsequently added colourless substrate solution of 3,3',5,5'-Tetramethylbenzidine (TMB) within a 10 min reaction time into a blue product. The enzyme reaction is terminated by sulphuric acid dispensed into the wells turning the solution from blue to yellow. The optical density (OD) of the solution read at 450 / 620 nm is directly proportional to the specifically bound amount of Rotavirus. Considering the cut-off value results are interpreted as positive or negative.

Test Components

			For 96 Wells	For 2x 96 Wells
1	WELLS	**Microtitration plate** coated with polyclonal anti-Rotavirus-antibodies (sheep)	12 single breakable 8-well strips colour coding dark blue vacuum-sealed with desiccant	2x 12 single breakable 8-well strips colour coding dark blue vacuum-sealed with desiccant
2	WASHBUF CONC 10x	**Wash buffer** 10-fold	100 ml concentrate for 1000 ml solution white cap	2x 100 ml concentrate for 2x 1000 ml solution white cap
3	DIL	**Sample diluent**	100 ml · ready to use coloured yellow black cap	2x 100 ml · ready to use coloured yellow black cap
4	CONTROL +	**Positive control** Rotavirus reactive sample	1.5 ml · ready to use coloured blue red cap	3.0 ml · ready to use coloured blue red cap
5	CONTROL –	**Negative control** Rotavirus negative sample	1.5 ml · ready to use coloured blue green cap	3.0 ml · ready to use coloured blue green cap
6	CONJ HRP	**HRP-conjugate** HRP-labelled, polyclonal anti-Rotavirus-antibodies (rabbit)	12 ml · ready to use coloured green brown cap	24 ml · ready to use coloured green brown cap
7	SUBSTR TMB	**Substrate** 3,3',5,5'-Tetramethylbenzidine and hydrogen peroxide	15 ml · ready to use blue cap	28 ml · ready to use blue cap
8	STOP	**Stop solution** 0.25 M sulphuric acid	15 ml · ready to use yellow cap	28 ml · ready to use yellow cap

Preparation And Storage Of Samples

Collection and storage

Stool samples should be stored at 2…8°C immediately after collection and processed within 72 hours. Longer storage is possible at -20°C. Repeated freezing and thawing of samples should be avoided. Stool samples already diluted with the *Serazym*® sample diluent can be stored for up to 72 h at 2…8 °C before testing in the ELISA.

Preparation

Quickly thaw frozen samples. Warm samples to room temperature and mix well.
The *Serazym*® Rotavirus can be performed with 1 : 6 or 1 : 11 diluted specimens. In case of additional testing of the same sample in the *Serazym*® Norovirus, the *Serazym*® Campylobacter or the *Serazym*® Clostridium difficile Toxin A+B the 1 : 6 dilution is recommended.

Preparation of a 1 : 11 sample dilution:
Pipette 1000 µl of sample diluent into a clean tube. Using a disposable stirring rod transfer about 100 mg (diameter about 2 - 3 mm) of faeces if solid or pipette 100 µl if liquid into the tube and suspend thoroughly. If necessary, sediment floating particles by a centrifugation step with a micro centrifuge for one min at maximum speed.

Preparation of a 1 : 6 sample dilution:
Pipette 1000 µl of sample diluent into a clean tube. Using a disposable stirring rod transfer about 200 mg (diameter about 4 - 6 mm) of faeces if solid or pipette 200 µl if liquid into the tube and suspend thoroughly. If necessary, sediment floating particles by a centrifugation step with a micro centrifuge for one min at maximum speed.

Materials Required But Not Provided

Micropipettes · multi-channel pipette or multi-pipette · reagent container for multi-channel pipette · 8-channel wash comb with vacuum pump and waste bottle or microplate washer · microplate reader with optical filters of 450 nm for measurement and ≥ 620 nm for reference · distilled or deionized water · glassware · tubes (2 ml) for sample preparation

Preparation And Storage Of Reagents

Kit size and expiry
One kit is designed for 1x 96 or 2x 96 determinations. The expiry date of each component is reported on its respective label, that of the complete kit on the outer box label. Upon receipt, all test components have to be kept at 2…8°C, preferably in the original kit box. After opening all kit components are stable for at least 2 months, provided proper storage. The ready to use wash solution can be used for at least 1 month when stored at 2…8°C.

Reagent preparation
Allow all components to reach room temperature prior to use in the assay. The microtitration plate is vacuum-sealed in a foil with desiccant. The plate consists of a frame and strips with breakable wells. Allow the sealed plate to reach room temperature before opening. Unused wells should be stored refrigerated and protected from moisture in the original cover carefully resealed. Prepare a sufficient amount of wash solution by diluting the 10-fold concentrated wash buffer 1 + 9 with distilled or deionized water.
For Example: 10 ml wash buffer concentrate *(2)* + 90 ml distilled or deionized water.

Assay Procedure

Dilute samples with sample diluent *(3)* 1 : 11 or 1 : 6, e.g. 100 mg or 100 µl stool + 1.0 ml (1 : 11) sample diluent *(3)* or 200 mg or 200 µl stool + 1.0 ml (1 : 6) sample diluent *(3)*.
Avoid any time shift during dispensing of reagents and samples.
Make sure the soak time of the wash buffer in the wells is at least 5 seconds per wash cycle and that the remaining fluid is completely drained in every single wash cycle!
Avoid light exposure of the TMB substrate solution!

Working steps

1. Warm all reagents to room temperature (RT) before use. Mix gently without causing foam.
2. Dispense 2 drops (or 75 µl) **CONJ HRP** HRP-conjugate *(6)* per well and
3. Pipette: 75 µl **CONTROL +** positive control *(4)*
   75 µl **CONTROL –** negative control *(5)*
   50 µl **diluted sample**, mix gently.
4. Cover plate and incubate for 60 min at RT.
5. Decant, then wash each well 5x with 300 µl wash solution (diluted from *(2)*) and tap dry onto absorbent paper if necessary.
6. Dispense 2 drops (or 75 µl) **SUBSTR TMB** substrate *(7)* per well.
7. Incubate for 10 min at RT protected from light.
8. Dispense 2 drops (or 75 µl) **STOP** stop solution *(8)* per well, mix gently.
9. Read OD at 450 nm / ≥ 620 nm with a microplate reader within 30 min after reaction stop.

Result Interpretation

Qualitative evaluation
Cut-off determination: OD negative control + 0.20
Samples with OD values equal with or higher than the cut-off are considered positive, samples with OD values below the cut-off are considered negative for Rotavirus antigen.

Reference Values

Serazym® Rotavirus	
Positive	≥ Cut-off
Negative	< Cut-off

It is recommended that each laboratory establishes its own normal and pathological reference ranges as usually done for other diagnostic parameters, too. Therefore, the mentioned reference values provide a guide only to values which might be expected.

Test validity
The test run is valid if:

- the mean OD of the negative control is ≤ 0.20 (manual performance)
  ≤ 0.30 (automatic performance)
- the mean OD of the positive control is ≥ 1.20

If the above mentioned quality criteria are not met, repeat the test and make sure that the test procedure is followed correctly (incubation times and temperatures, sample and wash buffer dilution, wash steps etc.). In case of repeated failure of the quality criteria contact your supplier.

Limitations of the procedure

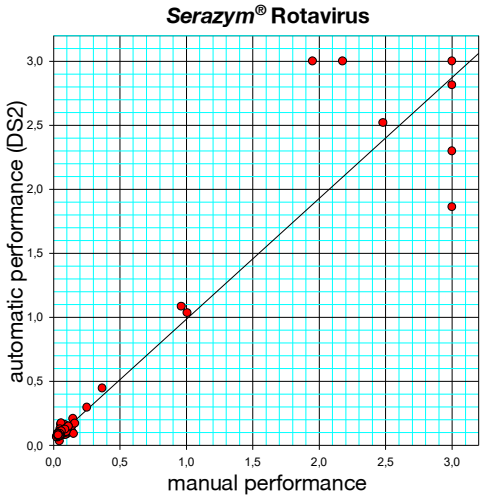
There is no correlation between measured absorbance and seriousness of the infection. It is also not allowed to correlate absorbances of the samples with that of the positive control. Cross contamination of reagents and samples can produce false positive results. Incorrect dilutions, not sufficiently homogenized samples or solid particles after centrifugation of the suspension can cause false negative as well as false positive results. A negative test result not necessarily excludes a *Rotavirus* infection. Inhomogeneous virus distribution in the sample can cause false negative results. The investigation of samples that were taken beyond the acute phase of the disease can cause false negative results, because the number of virus particles has decreased under the detection limit of the test. It is therefore recommended to take samples within the acute phase of the disease where a maximum number of excreted virus particles are to be expected. Faecal samples from vaccinated children may contain vaccine virus causing positive ELISA results. A final interpretation of the test results should consider clinical findings as well.

Automatic Processing

Performing the *Serazym*® Rotavirus on fully automated microplate processors (e.g. DS2, DSX) may cause elevated absorbances in comparison to the manual procedure due to individual differences concerning wash procedures and general technical specifications of the equipment. In these cases a maximum value of 0.3 absorbance units is permissible for the negative control. It is recommended to use a wash procedure including 10 seconds soak time per strip and wash step followed by a wash step with distilled or deionized water with 10 seconds of soak time after the final wash step of each wash cycle. If necessary, the number of washing steps can be enhanced from 5x to 7x - 8x.

Correlation: manual – automatic processing

A panel of 133 stool specimens was investigated in parallel by manual and automatic processing method (DS2, Dynex Technologies) resp. The correlation was calculated with r = 0.96.



Performance Characteristics

Precision

Intra-assay coefficient of variation (CV) in the *Serazym*® Rotavirus from 12-fold determinations of samples:

sample	mean OD	standard deviation	CV (%)
1	1.841	0.137	7.45
2	1.208	0.078	6.50
3	0.620	0.040	6.38
4	0.463	0.024	5.26

Inter-assay coefficient of variation (CV) in the *Serazym*® Rotavirus in 10 different test runs from 3-fold determinations of samples:

sample	mean OD	standard deviation	CV (%)
1	2.720	0.128	4.71
2	1.647	0.122	7.38
3	0.968	0.074	7.69
4	0.409	0.019	4.58

Lower detection limit

The lower detection limit of *Rotavirus* antigen in the *Serazym*® Rotavirus was determined by titration of purified *Rotavirus* antigen SA-11: < 10 ng / ml corresponding to 10^6 virus particles / g faeces.

Specificity and sensitivity

A total of 488 stool samples were investigated in parallel in the *Serazym*® Rotavirus and in another commercially available ELISA.

	comparative ELISA positive	comparative ELISA negative
***Serazym*® ELISA positive**	246	0
***Serazym*® ELISA negative**	4	238

Specifity: 100% · Sensitivity: 98.4%

Cross reactivity

Stool samples positive for one of the subsequent pathogens have been tested with the *Serazym*® Rotavirus and showed no cross reactivity:
Adenovirus (n = 20), Astrovirus (n = 8), Norovirus (n = 31), *Clostridium difficile* (n = 11), *Campylobacter jejuni* (n = 7), *Campylobacter coli* (n = 1), *Salmonella enteritidis* (n = 18), *Giardia lamblia* (n = 1) and stool samples (n = 93) with detectable levels (> 10 µg / g) of haemoglobin.

Negative stool specimens have been spiked with ≥ 10^8 colony forming units of the following microorganisms and tested negative with the *Serazym*® ELISA (OD 450 / 620 nm < Cut-Off):

Aeromonas hydrophila	(ATCC 7966)	*Klebsiella pneumoniae*	(ATCC 13883)
Bacillus cereus	(ATCC 11778)	*Peptostreptococcus anaerobius*	(ATCC 27337)
Bacillus subtilis	(ATCC 6633)	*Proteus vulgaris*	(ATCC 8427)
Bacteroides fragilis	(ATCC 25285)	*Pseudomonas aeruginosa*	(ATCC 10145)
Candida albicans	(ATCC 10231)	*Salmonella enterica Serovar enteritidis*	(ATCC 13076)
Campylobacter coli	(ATCC 33559)	*Salmonella enterica Serovar typhimurium*	(ATCC 14028)
Campylobacter jejuni	(ATCC 33291)	*Shigella flexneri*	(ATCC 12022)
Citrobacter freundii	(ATCC 8090)	*Shigella sonnei*	(ATCC 25931)
Clostridium sordellii	(ATCC 9714)	*Staphylococcus aureus*	(ATCC 25923)
Enterobacter aerogenes	(ATCC 13048)	*Staphylococcus epidermidis*	(ATCC 12228)
Enterobacter cloacae	(ATCC 13047)	*Vibrio parahaemolyticus*	(ATCC 17802)
Enterococcus faecalis	(ATCC 29212)	*Vibrio cholerae*	clinical isolate
Escherichia coli	(ATCC 25922)	*Yersinia enterocolitica Serotyp 03, 09*	clinical isolates

Interference

None of the following substances added to positive and negative stool samples showed a significant impact on the test result:
Barium sulfate (5%), Buscopan® (2 mg/ml), Cyclamate (5%), Diclofenac (2 mg/ml), Hemoglobin human (5 mg/ml), Blood human (5%), Hylak® N (5%), Iberogast® (5%), Immodium® akut duo (0.2/12.5 mg/ml), Loperamide (0.2 mg/ml), Metronidazole (2 mg/ml), Mucin (5 mg/ml), Nexium® (2 mg/ml), Palmitic acid (20%), Pentofuryl® (2 mg/ml), Pepto-Bismol (1 mg/ml), Perenterol (2.5 mg/ml), Rennie® (8 mg/ml), Simagel® (2 mg/ml), Stearic acid (20%), Vancomycin (2 mg/ml).

Common Advices and Precautions

This kit is for *in-vitro* use only. Follow the working instructions carefully. The kit should be performed by trained technical staff only. Do not use reagents from damaged packages or bottles. The expiration dates stated on the respective labels are to be observed. **Do not use or mix reagents from different lots except for sample diluent, wash buffer, TMB/substrate solution and stop solution.**
The sample diluent, wash buffer, TMB/substrate solution and stop solution is universally applicable for the *Serazym*® stool ELISA Adenovirus (E-017), Rotavirus (E-020), Astrovirus (E-045), Norovirus (E-061), Clostridium difficile Toxin A+B (E-040), Clostridium difficile GDH (E-107), Campylobacter (E-093), H. pylori 2nd Gen. (E-114), Entamoeba histolytica (E-018), Cryptosporidium parvum (E-039), Giardia lamblia (E-038) and Giardia (E-106).
Do not use reagents from other manufacturers. Avoid time shift during dispensing of reagents. All reagents should be kept at 2…8°C before use Some of the reagents may contain biocides as preservative. Further information can be found in the safety data sheet. They must not be swallowed or allowed to come into contact with skin or mucous membranes. Handle all components and all patient samples as if potentially hazardous. Since the kit contains potentially hazardous materials, the following precautions should generally be observed:

Do not smoke, eat or drink while handling kit material!
Always use protective gloves!
Never pipette material by mouth!
Note safety precautions of the single test components!

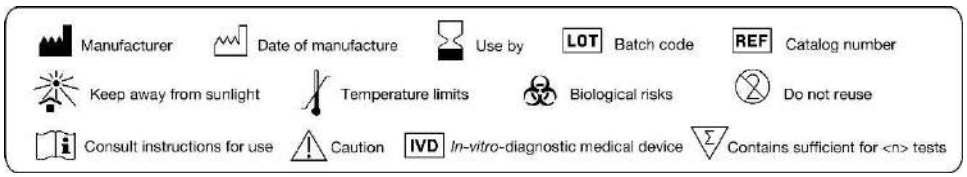
History of Changes

Version	Section	Modifications
2020-11-16	Common Advices and Precautions	Update
	History of Changes	Adding section “History of Changes “
	Test Components	Update

Incubation Scheme *Serazym*® Rotavirus (E-020)

1.	2 drops (or 75 µl)	CONJ HRP *(6)*
	+	
	pipette	
	75 µl	CONTROL + *(4)*
	75 µl	CONTROL – *(5)*
	50 µl	**diluted stool sample,** mix gently
	60 min	incubation (room temperature)
	5 x Wash	with wash solution
2.	2 drops (or 75 µl)	SUBSTR TMB *(7)*
	10 min	incubation (room temperature) protected from light
3.	2 drops (or 75 µl)	STOP *(8)*

Read OD at 450 / ≥ 620 nm



NovaTec Immundiagnostica GmbH

NovaLisa®

Legionella pneumophila IgM

ELISA

CE

Only for in-vitro diagnostic use

Product Number: LEGM0650 (96 Determinations)

ENGLISH

1. INTRODUCTION

Legionellae are aerobic gram-negative facultative intracellular parasites of certain protozoa. They are found in freshwater environments worldwide and can cause respiratory disease (legionellosis) in humans.

Legionella was first identified after an outbreak of pneumonia involving delegates of the 1976 American Legion Convention at a Philadelphia hotel.

The genus Legionella currently has at least 50 species comprising 70 distinct serogroups. One species of Legionella, L. pneumophila, is the aetiological agent of approximately 90 % of legionellosis cases, and serogroup 1 (Sg1) accounts for about 84 % of these cases.

L. pneumophila multiplies itself at temperatures between 25 and 42 °C, with an optimal growth temperature of 35 °C. Legionella thrives in warm, stagnant water in the environment and in artificial systems such as cooling towers, evaporative condensers, hot and cold water systems and spa pools that mimic the natural environment in which the organism thrives. These systems also provide the means by which aerosols/droplets are generated and the organism dispersed into the atmosphere.

Legionellosis can be acquired by the inhalation of aerosols containing Legionella bacteria or by micro-aspiration of ingested water contaminated with Legionella. Person-to-person transmission is not thought to be a risk.

The likelihood of contracting Legionnaires' disease depends on the level of contamination in the water source, the susceptibility of the person exposed, and the intensity of exposure. Legionnaires' disease is characterized as an "opportunistic" disease that attacks individuals who have an underlying illness or a weakened immune system. Predisposing risks include increasing age, being male, heavy smoking, alcohol abuse, chronic lung disease, immunosuppressive therapy, cancer chemotherapy, organ or bone marrow transplant, and corticosteroid therapy.

Legionellosis can appear in two distinct clinical presentations: Legionella pneumonia (Legionnaires' disease) with an incubation period of approx. 2-10 days (may extend up to 16-20 days) and Pontiac fever (incubation period: normally 12-48 hours).

Legionella pneumonia (Legionnaires' disease) is a serious form of pneumonia that carries with it a case-fatality ratio of 10-15 %. Legionnaires' disease patients initially present with cough, fever and nonspecific symptoms including malaise, myalgia and headache. Some patients develop shaking chills, chest pain, diarrhea, delirium or other neurologic symptoms. Extra pulmonary involvement is rare.

Pontiac fever is a milder form of the disease without manifestations of pneumonia and presents as an influenza-like illness. Symptoms may include headache, chills, muscle aches, a dry cough and fever. It is usually self-limiting and typically does not require treatment. The attack rate is much higher than for Legionnaires' disease (up to 95 % of those exposed).

Species	Disease	Symptoms (e.g.)	Transmission route
Legionella pneumophila	Legionella pneumonia (Legionnaires' disease)	Cough, fever and nonspecific symptoms (malaise, myalgia, headache). Some patients develop shaking chills, chest pain, diarrhea, delirium or other neurologic symptoms.	Inhalation of aerosols containing Legionella bacteria or micro-aspiration of ingested water contaminated with Legionella
	Pontiac fever	Influenza-like illness (headache, chills, muscle aches, a dry cough and fever) without manifestations of pneumonia	

Infection or presence of pathogen may be identified by:

- Culture
- Urinary antigen detection
- PCR
- Serology: Detection of antibodies by IF, ELISA

2. INTENDED USE

The Legionella pneumophila IgM ELISA is intended for the qualitative determination of IgM class antibodies against Legionella pneumophila in human serum or plasma (citrate, heparin).

3. PRINCIPLE OF THE ASSAY

The qualitative immunoenzymatic determination of specific antibodies is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique.
Microtiterplates are coated with specific antigens to bind corresponding antibodies of the sample. After washing the wells to remove all unbound sample material a horseradish peroxidase (HRP) labelled conjugate is added. This conjugate binds to the captured antibodies. In a second washing step unbound conjugate is removed. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product.
The intensity of this product is proportional to the amount of specific antibodies in the sample. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450/620 nm is read using an ELISA Microtiterplate reader.

4. MATERIALS

4.1. Reagents supplied

- **Microtiterplate:** 12 break-apart 8-well snap-off strips coated with Legionella pneumophila antigens; in resealable aluminium foil.
- **IgM Sample Dilution Buffer:** 1 bottle containing 100 mL of phosphate buffer (10 mM) for sample dilution; pH 7.2 ± 0.2; anti-human IgG (RF Absorbent); coloured green; ready to use; white cap; ≤ 0.0015% (v/v) CMIT/ MIT (3:1).
- **Stop Solution:** 1 bottle containing 15 mL sulphuric acid, 0.2 mol/L; ready to use; red cap.
- **Washing Buffer (20x conc.):** 1 bottle containing 50 mL of a 20-fold concentrated phosphate buffer (0.2 M), pH 7.2 ± 0.2, for washing the wells; white cap.
- **Conjugate:** 1 bottle containing 20 mL of peroxidase labelled antibody to human IgM in phosphate buffer (10 mM); coloured red; ready to use; black cap.
- **TMB Substrate Solution:** 1 bottle containing 15 mL 3,3',5,5'-tetramethylbenzidine (TMB), < 0.1 %; ready to use; yellow cap.
- **Positive Control:** 1 vial containing 2 mL control; coloured yellow; ready to use; red cap; ≤ 0.02% (v/v) MIT.
- **Cut-off Control:** 1 vial containing 3 mL control; coloured yellow; ready to use; green cap; ≤ 0.02% (v/v) MIT.
- **Negative Control:** 1 vial containing 2 mL control; coloured yellow; ready to use; blue cap; ≤ 0.0015% (v/v) CMIT/ MIT (3:1).

For hazard and precautionary statements see 12.1
For potential hazardous substances please check the safety data sheet.

4.2. Materials supplied

- 1 Cover foil
- 1 Instruction for use (IFU)
- 1 Plate layout

4.3. Materials and Equipment needed

- ELISA Microtiterplate reader, equipped for the measurement of absorbance at 450/620 nm
- Incubator 37 °C
- Manual or automatic equipment for rinsing Microtiterplates
- Pipettes to deliver volumes between 10 and 1000 µL
- Vortex tube mixer
- Distilled water
- Disposable tubes

5. STABILITY AND STORAGE

Store the kit at 2...8 °C. The opened reagents are stable up to the expiry date stated on the label when stored at 2...8 °C.

6. REAGENT PREPARATION

It is very important to bring all reagents and samples to room temperature (20…25 °C) and mix them before starting the test run!

6.1. Microplate

The break-apart snap-off strips are coated with Legionella pneumophila antigens. Immediately after removal of the strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2...8 °C.

6.2. Washing Buffer (20x conc.)

Dilute Washing Buffer 1 + 19; e. g. 10 mL Washing Buffer + 190 mL distilled water. The diluted buffer is stable for 5 days at room temperature (20…25 °C). In case crystals appear in the concentrate, warm up the solution to 37 °C e.g. in a water bath. Mix well before dilution.

6.3. TMB Substrate Solution

The reagent is ready to use and has to be stored at 2...8 °C, away from the light. The solution should be colourless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away.

7. SAMPLE COLLECTION AND PREPARATION

Use human serum or plasma (citrate, heparin) samples with this assay. If the assay is performed within 5 days after sample collection, the samples should be kept at 2...8 °C; otherwise they should be aliquoted and stored deep-frozen (-70…-20 °C). If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing.
Heat inactivation of samples is not recommended.

7.1. Sample Dilution

Before assaying, all samples should be diluted 1+100 with IgM Sample Dilution Buffer. Dispense 10 µL sample and 1 mL IgM Sample Dilution Buffer into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

8. ASSAY PROCEDURE

Please read the instruction for use carefully **before** performing the assay. Result reliability depends on strict adherence to the instruction for use as described. The following test procedure is only validated for manual procedure. If performing the test on ELISA automatic systems we recommend increasing the washing steps from three up to five and the volume of Washing Buffer from 300 µL to 350 µL to avoid washing effects. Pay attention to chapter 12. Prior to commencing the assay, the distribution and identification plan for all samples and standards/controls (duplicates recommended) should be carefully established on the plate layout supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder.

Perform all assay steps in the order given and without any delays.

A clean, disposable tip should be used for dispensing each standard/control and sample.

Adjust the incubator to 37 ± 1 °C.

1. Dispense 100 µL standards/controls and diluted samples into their respective wells. Leave well A1 for the Substrate Blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 1 hour ± 5 min at 37 ± 1 °C.**
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µL of Washing Buffer. Avoid overflows from the reaction wells. The interval between washing und aspiration should be > 5 sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!

   Note: Washing is important! Insufficient washing results in poor precision and false results.
5. Dispense 100 µL Conjugate into all wells except for the Substrate Blank well A1.
6. **Incubate for 30 min at room temperature (20...25 °C).** Do not expose to direct sunlight.
7. Repeat step 4.
8. Dispense 100 µL TMB Substrate Solution into all wells.
9. **Incubate for exactly 15 min at room temperature (20...25 °C) in the dark.** A blue colour occurs due to an enzymatic reaction.
10. Dispense 100 µL Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution, thereby a colour change from blue to yellow occurs.
11. Measure the absorbance at 450/620 nm within 30 min after addition of the Stop Solution.

8.1. Measurement

Adjust the ELISA Microtiterplate reader **to zero** using the **Substrate Blank**.

If - due to technical reasons - the ELISA Microtiterplate reader cannot be adjusted to zero using the Substrate Blank, subtract its absorbance value from all other absorbance values measured in order to obtain reliable results!

Measure the absorbance of all wells at **450 nm** and record the absorbance values for each standard/control and sample in the plate layout.

Bichromatic measurement using a reference wavelength of 620 nm is recommended.

Where applicable calculate the mean absorbance values of all duplicates.

9. RESULTS

9.1. Run Validation Criteria

In order for an assay run to be considered valid, these Instructions for Use have to be strictly followed and the following criteria must be met:

- **Substrate Blank**: Absorbance value **< 0.100**
- **Negative Control**: Absorbance value **< 0.200 and < Cut-off**
- **Cut-off Control**: Absorbance value **0.150 – 1.300**
- **Positive Control**: Absorbance value **> Cut-off**

If these criteria are not met, the test is not valid and must be repeated.

9.2. Calculation of Results

The Cut-off is the mean absorbance value of the Cut-off Control determinations.

Example: Absorbance value Cut-off Control 0.44 + absorbance value Cut-off control 0.42 = 0.86 / 2 = 0.43
Cut-off = 0.43

9.2.1. Results in Units [NTU]

$$\frac{\text{Sample (mean) absorbance value} \times 10}{\text{Cut-off}} = \text{[NovaTec Units = NTU]}$$

Example: $\frac{1.591 \times 10}{0.43} = 37$ NTU (Units)

9.3. Interpretation of Results

Cut-off	10 NTU	-
Positive	> 11 NTU	Antibodies against the pathogen are present. There has been a contact with the antigen (pathogen resp. vaccine).
Equivocal	9 – 11 NTU	Antibodies against the pathogen could not be detected clearly. It is recommended to repeat the test with a fresh sample in 2 to 4 weeks. If the result is equivocal again the sample is judged as **negative**.
Negative	< 9 NTU	The sample contains no antibodies against the pathogen. A previous contact with the antigen (pathogen resp. vaccine) is unlikely.
Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as serological data. In immunocompromised patients and newborns serological data only have restricted value.		

9.3.1. Antibody Isotypes and State of Infection

Serology	Significance
IgM	Characteristic of the primary antibody response High IgM titer with low IgG titer: → suggests a current or very recent infection Rare: → persisting IgM
IgG	Characteristic of the secondary antibody response May persist for several years High IgG titer with low IgM titer: → may indicate a past infection

10. SPECIFIC PERFORMANCE CHARACTERISTICS

The results refer to the groups of samples investigated; these are not guaranteed specifications.

For further information about the specific performance characteristics please contact NovaTec Immundiagnostica GmbH.

10.1. Precision

Intraassay	n	Mean (E)	CV (%)
#1	24	0.461	4.23
#2	24	1.003	2.12
#3	24	0.862	2.65

Interassay	n	Mean (NTU)	CV (%)
#1	12	21.35	5.10
#2	12	15.46	7.62
#3	12	4.22	11.86

10.2. Diagnostic Specificity

The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte.
It is 95.65% (95% confidence interval: 85.16% - 99.47%).

10.3. Diagnostic Sensitivity

The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte.
It is 100% (95% confidence interval: 66.37% - 100%).

10.4. Interferences

Interferences with hemolytic, lipemic or icteric samples are not observed up to a concentration of 10 mg/mL hemoglobin, 5 mg/mL triglycerides and 0.5 mg/mL bilirubin.

10.5. Cross Reactivity

Investigation of a sample panel with antibody activities to potentially cross-reacting parameters did not reveal significant evidence of false-positive results due to cross-reactions.

11. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or repeated freeze-thaw cycles of the sample may affect the absorbance values.

12. PRECAUTIONS AND WARNINGS

- The test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the testkits with analyzers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.
- Only for in-vitro diagnostic use.
- All materials of human or animal origin should be regarded and handled as potentially infectious.
- All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive.
- Do not interchange reagents or Microtiterplates of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- Use only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and standard/control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense reagents without splashing accurately into the wells.
- The ELISA is only designed for qualified personnel following the standards of good laboratory practice (GLP).
- For further internal quality control each laboratory should additionally use known samples.

12.1. Safety note for reagents containing hazardous substances

Reagents may contain CMIT/MIT (3:1) or MIT (refer to 4.1)
Therefore, the following hazard and precautionary statements apply.

Warning	H317	May cause an allergic skin reaction.
	P261	Avoid breathing spray.
	P280	Wear protective gloves/ protective clothing.
	P302+P352	IF ON SKIN: Wash with plenty of soap and water.
	P333+P313	If skin irritation or rash occurs: Get medical advice/ attention.
	P362+P364	Take off contaminated and Wash it before reuse.

Further information can be found in the safety data sheet.

12.2. Disposal Considerations

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

13. ORDERING INFORMATION

Prod. No.: LEGM0650 Legionella pneumophila IgM ELISA (96 Determinations)

NovaTec Immundiagnostica GmbH

NovaLisa®

Legionella pneumophila IgG

ELISA

CE

Only for in-vitro diagnostic use

Product Number: LEGG0650 (96 Determinations)

ENGLISH

1. INTRODUCTION

Legionellae are aerobic gram-negative facultative intracellular parasites of certain protozoa. They are found in freshwater environments worldwide and can cause respiratory disease (legionellosis) in humans.
Legionella was first identified after an outbreak of pneumonia involving delegates of the 1976 American Legion Convention at a Philadelphia hotel.
The genus Legionella currently has at least 50 species comprising 70 distinct serogroups. One species of Legionella, L. pneumophila, is the aetiological agent of approximately 90 % of legionellosis cases, and serogroup 1 (Sg1) accounts for about 84 % of these cases.
L. pneumophila multiplies itself at temperatures between 25 and 42 °C, with an optimal growth temperature of 35 °C. Legionella thrives in warm, stagnant water in the environment and in artificial systems such as cooling towers, evaporative condensers, hot and cold water systems and spa pools that mimic the natural environment in which the organism thrives. These systems also provide the means by which aerosols/droplets are generated and the organism dispersed into the atmosphere.
Legionellosis can be acquired by the inhalation of aerosols containing Legionella bacteria or by micro-aspiration of ingested water contaminated with Legionella. Person-to-person transmission is not thought to be a risk.
The likelihood of contracting Legionnaires' disease depends on the level of contamination in the water source, the susceptibility of the person exposed, and the intensity of exposure. Legionnaires' disease is characterized as an "opportunistic" disease that attacks individuals who have an underlying illness or a weakened immune system. Predisposing risks include increasing age, being male, heavy smoking, alcohol abuse, chronic lung disease, immunosuppressive therapy, cancer chemotherapy, organ or bone marrow transplant, and corticosteroid therapy.
Legionellosis can appear in two distinct clinical presentations: Legionella pneumonia (Legionnaires' disease) with an incubation period of approx. 2-10 days (may extend up to 16-20 days) and Pontiac fever (incubation period: normally 12-48 hours).
Legionella pneumonia (Legionnaires' disease) is a serious form of pneumonia that carries with it a case-fatality ratio of 10-15 %. Legionnaires' disease patients initially present with cough, fever and nonspecific symptoms including malaise, myalgia and headache. Some patients develop shaking chills, chest pain, diarrhea, delirium or other neurologic symptoms. Extra pulmonary involvement is rare.
Pontiac fever is a milder form of the disease without manifestations of pneumonia and presents as an influenza-like illness. Symptoms may include headache, chills, muscle aches, a dry cough and fever. It is usually self-limiting and typically does not require treatment. The attack rate is much higher than for Legionnaires' disease (up to 95 % of those exposed).

Species	Disease	Symptoms (e.g.)	Transmission route
Legionella pneumophila	Legionella pneumonia (Legionnaires' disease) Pontiac fever	Cough, fever and nonspecific symptoms (malaise, myalgia, headache). Some patients develop shaking chills, chest pain, diarrhea, delirium or other neurologic symptoms Influenza-like illness (headache, chills, muscle aches, a dry cough and fever) without manifestations of pneumonia	Inhalation of aerosols containing Legionella bacteria or micro-aspiration of ingested water contaminated with Legionella

Infection or presence of pathogen may be identified by:

- Culture
- Urinary antigen detection
- PCR
- Serology: Detection of antibodies by ELISA

2. INTENDED USE

The Legionella pneumophila IgG ELISA is intended for the qualitative determination of IgG class antibodies against Legionella pneumophila in human serum or plasma (citrate, heparin).

3. PRINCIPLE OF THE ASSAY

The qualitative immunoenzymatic determination of specific antibodies is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique.
Microtiterplates are coated with specific antigens to bind corresponding antibodies of the sample. After washing the wells to remove all unbound sample material a horseradish peroxidase (HRP) labelled conjugate is added. This conjugate binds to the captured antibodies. In a second washing step unbound conjugate is removed. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product.
The intensity of this product is proportional to the amount of specific antibodies in the sample. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450/620 nm is read using an ELISA Microtiterplate reader.

4. MATERIALS

4.1. Reagents supplied

- **Microtiterplate:** 12 break-apart 8-well snap-off strips coated with Legionella pneumophila antigens; in resealable aluminium foil.
- **IgG Sample Dilution Buffer:** 1 bottle containing 100 mL of phosphate buffer (10 mM) for sample dilution; pH 7.2 ± 0.2; coloured yellow; ready to use; white cap; ≤ 0.0015% (v/v) CMIT/ MIT (3:1).
- **Stop Solution:** 1 bottle containing 15 mL sulphuric acid, 0.2 mol/L; ready to use; red cap.
- **Washing Buffer (20x conc.):** 1 bottle containing 50 mL of a 20-fold concentrated phosphate buffer (0.2 M), pH 7.2 ± 0.2, for washing the wells; white cap.
- **Conjugate:** 1 bottle containing 20 mL of peroxidase labelled antibody to human IgG in phosphate buffer (10 mM); coloured blue; ready to use; black cap.
- **TMB Substrate Solution:** 1 bottle containing 15 mL 3,3',5,5'-tetramethylbenzidine (TMB), < 0.1 %; ready to use; yellow cap.
- **Positive Control:** 1 vial containing 2 mL control; coloured yellow; ready to use; red cap; ≤ 0.02% (v/v) MIT.
- **Cut-off Control:** 1 vial containing 3 mL control; coloured yellow; ready to use; green cap; ≤ 0.02% (v/v) MIT.
- **Negative Control:** 1 vial containing 2 mL control; coloured yellow; ready to use; blue cap; ≤ 0.0015% (v/v) CMIT/ MIT (3:1).

For hazard and precautionary statements see 12.1
For potential hazardous substances please check the safety data sheet.

4.2. Materials supplied

- 1 Cover foil
- 1 Instruction for use (IFU)
- 1 Plate layout

4.3. Materials and Equipment needed

- ELISA Microtiterplate reader, equipped for the measurement of absorbance at 450/620 nm
- Incubator 37°C
- Manual or automatic equipment for rinsing Microtiterplates
- Pipettes to deliver volumes between 10 and 1000 µL
- Vortex tube mixer
- Distilled water
- Disposable tubes

5. STABILITY AND STORAGE

Store the kit at 2...8 °C. The opened reagents are stable up to the expiry date stated on the label when stored at 2...8 °C.

6. REAGENT PREPARATION

It is very important to bring all reagents and samples to room temperature (20…25 °C) and mix them before starting the test run!

6.1. Microtiterplate

The break-apart snap-off strips are coated with Legionella pneumophila antigens. Immediately after removal of the strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2...8 °C.

6.2. Washing Buffer (20x conc.)

Dilute Washing Buffer 1 + 19; e. g. 10 mL Washing Buffer + 190 mL distilled water. The diluted buffer is stable for 5 days at room temperature (20…25 °C). In case crystals appear in the concentrate, warm up the solution to 37°C e.g. in a water bath. Mix well before dilution.

6.3. TMB Substrate Solution

The reagent is ready to use and has to be stored at 2...8 °C, away from the light. The solution should be colourless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away.

7. SAMPLE COLLECTION AND PREPARATION

Use human serum or plasma (citrate, heparin) samples with this assay. If the assay is performed within 5 days after sample collection, the samples should be kept at 2...8 °C; otherwise they should be aliquoted and stored deep-frozen (-70…-20 °C). If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing.
Heat inactivation of samples is not recommended.

7.1. Sample Dilution

Before assaying, all samples should be diluted 1+100 with IgG Sample Dilution Buffer. Dispense 10 µL sample and 1 mL IgG Sample Dilution Buffer into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

8. ASSAY PROCEDURE

Please read the instruction for use carefully **before** performing the assay. Result reliability depends on strict adherence to the instruction for use as described. The following test procedure is only validated for manual procedure. If performing the test on ELISA automatic systems we recommend increasing the washing steps from three up to five and the volume of Washing Buffer from 300 µL to 350 µL to avoid washing effects. Pay attention to chapter 12. Prior to commencing the assay, the distribution and identification plan for all samples and standards/controls (duplicates recommended) should be carefully established on the plate layout supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder.

Perform all assay steps in the order given and without any delays.

A clean, disposable tip should be used for dispensing each standard/control and sample.

Adjust the incubator to 37 ± 1 °C.

1. Dispense 100 µL standards/controls and diluted samples into their respective wells. Leave well A1 for the Substrate Blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 1 hour ± 5 min at 37 ± 1 °C.**
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µL of Washing Buffer. Avoid overflows from the reaction wells. The interval between washing und aspiration should be > 5 sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!

   Note: Washing is important! Insufficient washing results in poor precision and false results.
5. Dispense 100 µL Conjugate into all wells except for the Substrate Blank well A1.
6. **Incubate for 30 min at room temperature (20...25 °C).** Do not expose to direct sunlight.
7. Repeat step 4.
8. Dispense 100 µL TMB Substrate Solution into all wells.
9. **Incubate for exactly 15 min at room temperature (20...25 °C) in the dark.** A blue colour occurs due to an enzymatic reaction.
10. Dispense 100 µL Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution, thereby a colour change from blue to yellow occurs.
11. Measure the absorbance at 450/620 nm within 30 min after addition of the Stop Solution.

8.1. Measurement

Adjust the ELISA Microtiterplate reader **to zero** using the **Substrate Blank**.

If - due to technical reasons - the ELISA Microtiterplate reader cannot be adjusted to zero using the Substrate Blank, subtract its absorbance value from all other absorbance values measured in order to obtain reliable results!

Measure the absorbance of all wells at **450 nm** and record the absorbance values for each standard/control and sample in the plate layout.

Bichromatic measurement using a reference wavelength of 620 nm is recommended.

Where applicable calculate the mean absorbance values of all duplicates.

9. RESULTS

9.1. Run Validation Criteria

In order for an assay run to be considered valid, these Instructions for Use have to be strictly followed and the following criteria must be met:

- **Substrate Blank**: Absorbance value **< 0.100**
- **Negative Control**: Absorbance value **< 0.200 and < Cut-off**
- **Cut-off Control**: Absorbance value **0.150 – 1.300**
- **Positive Control**: Absorbance value **> Cut-off**

If these criteria are not met, the test is not valid and must be repeated.

9.2. Calculation of Results

The Cut-off is the mean absorbance value of the Cut-off Control determinations.

Example: Absorbance value Cut-off Control 0.44 + absorbance value Cut-off control 0.42 = 0.86 / 2 = 0.43
Cut-off = 0.43

9.2.1. Results in Units [NTU]

$$\frac{\text{Sample (mean) absorbance value} \times 10}{\text{Cut-off}} = \text{[NovaTec Units = NTU]}$$

Example: $\frac{1.591 \times 10}{0.43} = 37$ NTU (Units)

9.3. Interpretation of Results

Cut-off	10 NTU	-
Positive	> 11 NTU	Antibodies against the pathogen are present. There has been a contact with the antigen (pathogen resp. vaccine).
Equivocal	9 – 11 NTU	Antibodies against the pathogen could not be detected clearly. It is recommended to repeat the test with a fresh sample in 2 to 4 weeks. If the result is equivocal again the sample is judged as **negative**.
Negative	< 9 NTU	The sample contains no antibodies against the pathogen. A previous contact with the antigen (pathogen resp. vaccine) is unlikely.
Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as serological data. In immunocompromised patients and newborns serological data only have restricted value.		

9.3.1. Antibody Isotypes and State of Infection

Serology	Significance
IgM	Characteristic of the primary antibody response High IgM titer with low IgG titer: → suggests a current or very recent infection Rare: → persisting IgM
IgG	Characteristic of the secondary antibody response May persist for several years High IgG titer with low IgM titer: → may indicate a past infection

10. SPECIFIC PERFORMANCE CHARACTERISTICS

The results refer to the groups of samples investigated; these are not guaranteed specifications.

For further information about the specific performance characteristics please contact NovaTec Immundiagnostica GmbH.

10.1. Precision

Intraassay	**n**	**Mean (E)**	**CV (%)**
#1	24	0.275	9.88
#2	24	0.474	7.96
#3	24	1.722	5.05
Interassay	**n**	**Mean (NTU)**	**CV (%)**
#1	12	22.35	9.56
#2	12	62.64	7.20
#3	12	1.88	14.30

10.2. Diagnostic Specificity

The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte.
It is 100% (95% confidence interval: 88.78% - 100%).

10.3. Diagnostic Sensitivity

The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte.
It is 90.0% (95% confidence interval: 68.3% - 98.77%).

10.4. Interferences

Interferences with hemolytic, lipemic or icteric samples are not observed up to a concentration of 10 mg/mL hemoglobin, 5 mg/mL triglycerides and 0.5 mg/mL bilirubin.

10.5. Cross Reactivity

Investigation of a sample panel with antibody activities to potentially cross-reacting parameters did not reveal evidence of false-positive results due to cross-reactions.

11. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or repeated freeze-thaw cycles of the sample may affect the absorbance values.

12. PRECAUTIONS AND WARNINGS

- The test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the testkits with analyzers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.
- Only for in-vitro diagnostic use.
- All materials of human or animal origin should be regarded and handled as potentially infectious.
- All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive.
- Do not interchange reagents or Microtiterplates of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- Use only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and standard/control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense reagents without splashing accurately into the wells.
- The ELISA is only designed for qualified personnel following the standards of good laboratory practice (GLP).
- For further internal quality control each laboratory should additionally use known samples.

12.1. Safety note for reagents containing hazardous substances

Reagents may contain CMIT/MIT (3:1) or MIT (refer to 4.1)
Therefore, the following hazard and precautionary statements apply.

Warning

H317	May cause an allergic skin reaction.
P261	Avoid breathing spray
P280	Wear protective gloves/ protective clothing.
P302+P352	IF ON SKIN: Wash with plenty of soap and water.
P333+P313	If skin irritation or rash occurs: Get medical advice/ attention.
P362+P364	Take off contaminated and Wash it before reuse.

Further information can be found in the safety data sheet.

12.2. Disposal Considerations

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

13. ORDERING INFORMATION

Prod. No.: LEGG0650 Legionella pneumophila IgG ELISA (96 Determinations)

DEUTSCH

1. EINLEITUNG

Legionellen sind aerobe, gram-negative, fakultativ intrazelluläre Parasiten bestimmter Protozoen. Sie sind weltweit in Süßwasser anzutreffen und können beim Menschen respiratorische Erkrankungen (Legionellose) hervorrufen.
Legionella (L.) pneumophila wurde zum ersten Mal nach einem Ausbruch von Lungenentzündung bei einem Treffen der US-Kriegsveteranenvereinigung „American Legion State Convention“, das 1976 in einem Hotel in Philadelphia stattfand, identifiziert.
Das Genus Legionella umfasst aktuell mindestens 50 Spezies, die aus 70 verschiedenen Serogruppen bestehen. L. pneumophila ist der Erreger von etwa 90 % der Legionellose-Fälle, wobei Serogruppe 1 für etwa 84 % der Fälle verantwortlich ist.
L. pneumophila vermehrt sich bei Temperaturen zwischen 25 und 42 °C; die optimale Wachstumstemperatur beträgt 35 °C. Legionella gedeiht in warmem, stehendem Wasser, sowohl in der Umwelt, als auch in künstlichen Systemen wie Kühltürmen, Verdunstungskondensatoren, Kalt-und Warmwassersystemen und Spa-Pools, die die natürliche Umgebung des Organismus nachahmen. Durch diese Systeme kann es auch zur Bildung von Aerosolen/Tröpfchen kommen, über die der Organismus fein verteilt in die Luft abgegeben wird.
Eine Legionellose kann durch die Inhalation von Aerosolen oder durch Mikroaspiration von kontaminiertem Wasser erworben werden. Eine Übertragung von Mensch zu Mensch gilt als unwahrscheinlich.
Die Wahrscheinlichkeit an der Legionärskrankheit zu erkranken, ist abhängig vom Grad der Kontamination der Wasserquelle, der Empfänglichkeit der exponierten Person und der Expositionsintensität. Die Legionella Pneumonie ist eine "opportunistische" Krankheit, die Individuen mit einer bereits bestehenden Grunderkrankung oder mit einem geschwächten Immunsystem befällt. Prädisponierende Faktoren sind z. B. ein hohes Alter, exzessiver Nikotin- und Alkoholmissbrauch, chronische Lungenerkrankungen, immunsuppressive Therapie, zytostatische Behandlungen, Organ- oder Knochenmarkstransplantationen und Corticosteroid-Therapie. Männer erkranken häufiger als Frauen.
Die Legionellose kann zwei unterschiedliche klinische Erscheinungsformen annehmen: die Legionella Pneumonie (Legionärskrankheit) mit einer Inkubationszeit von etwa 2-10 Tagen (bis zu 16-20 Tage) und das Pontiac-Fieber (Inkubationszeit gewöhnlich 12-48 Stunden).
Die Legionärskrankheit ist eine schwere Form der Lungenentzündung mit einer Todesfallrate von 10-15 %. Die Erkrankung beginnt mit Husten, Fieber und unspezifischen Symptomen wie Unwohlsein sowie Muskel- und Kopfschmerzen. Bei einigen Patienten treten Schüttelfrost, Schmerzen in der Brust, Durchfall, Delirium oder andere neurologische Symptome auf. Extrapulmonale Entzündungen sind selten.
Das Pontiac-Fieber ist eine leichtere Form der Erkrankung ohne Pneumonie mit leichten grippalen Symptomen wie Kopf- und Muskelschmerzen, Frösteln, trockenem Husten und Fieber. Die Krankheit ist gewöhnlich selbst-limitierend und erfordert keine Behandlung. Die Erkrankungsrate ist sehr viel höher als bei der Legionärskrankheit (bis zu 95 % der exponierten Personen).

Spezies	**Erkrankung**	**Symptome (z.B.)**	**Infektionsweg**
Legionella pneumophila	Legionella Pneumonie (Legionärskrankheit) Pontiac-Fieber	Husten, Fieber und unspezifische Symptome (Unwohlsein, Muskel- und Kopfschmerzen); teilweise Schüttelfrost, Brustschmerzen, Durchfall, neurologische Symptome; Influenza-ähnlich ohne Pneumonie: Kopf- und Muskelschmerzen, Frösteln, trockener Husten und Fieber	mit Legionellen belastetes Wasser (Inhalation von Aerosolen oder Mikroaspiration)

Nachweis des Erregers bzw. der Infektion durch:

- Kultur
- Antigennachweis im Urin
- PCR
- Serologie: Nachweis spezifischer Antikörper mittels IF, ELISA

2. VERWENDUNGSZWECK

Der Legionella pneumophila IgG ELISA ist für den qualitativen Nachweis spezifischer IgG-Antikörper gegen Legionella pneumophila in humanem Serum oder Plasma (Citrat, Heparin) bestimmt.

3. TESTPRINZIP

Die qualitative immunenzymatische Bestimmung von spezifischen Antikörpern beruht auf der ELISA (Enzyme-linked Immunosorbent Assay) Technik.
Die Mikrotiterplatten sind mit spezifischen Antigenen beschichtet, an welche die korrespondierenden Antikörper aus der Probe binden. Ungebundenes Probenmaterial wird durch Waschen entfernt. Anschließend erfolgt die Zugabe eines Meerettich-Peroxidase (HRP) Konjugates. Dieses Konjugat bindet an die an der Mikrotiterplatte gebundenen spezifischen Antikörper. In einem zweiten Waschschritt wird ungebundenes Konjugat entfernt. Die Immunkomplexe, die durch die Bindung des Konjugates entstanden sind, werden durch die Zugabe von Tetramethylbenzidin (TMB)-Substratlösung und eine resultierende Blaufärbung nachgewiesen.
Die Intensität des Reaktionsproduktes ist proportional zur Menge der spezifischen Antikörper in der Probe. Die Reaktion wird mit Schwefelsäure gestoppt, wodurch ein Farbumschlag von blau nach gelb erfolgt. Die Absorption wird bei 450/620 nm mit einem Mikrotiterplatten-Photometer gemessen.

NovaTec Immundiagnostica GmbH

NovaLisa®

Leptospira IgM

ELISA

CE

Only for in-vitro diagnostic use

Product Number: LEPM0660 (96 Determinations)

ENGLISH

1. INTRODUCTION

Leptospirosis (also known as Weil's syndrome) is probably the most widespread zoonosis in the world. It is caused by infection with spirochete bacteria of the genus Leptospira and affects humans as well as a broad spectrum of animal hosts. The incidence is significantly higher in warm climate countries than in temperate regions. The disease is seasonal, with peak incidence occurring in summer or fall in temperate regions, where temperature is the limiting factor in survival of leptospires, and during rainy seasons in warm climate regions, where rapid desiccation would otherwise prevent survival.

Natural reservoirs for the pathogenic Leptospira interrogans include rodents as well as a large variety of domesticated mammals (e. g. pigs, cattle and dogs). Leptospires occupy the lumen of nephritic tubules in their natural host and are shed into the urine.

Transmission can occur when humans are directly or indirectly exposed to the urine of infected animals or a urine-polluted environment. Leptospires gain entry into the human blood stream via cuts, skin abrasions or mucous membranes through contact with moist soil, vegetation, and contaminated waters; handling infected animal tissues; and ingestion of food and water. Leptospires are rarely transmitted from human to human.

The incubation period is usually 5-14 days, with a range of 2-30 days.

The spectrum of clinical symptoms is extremely wide. The vast majority of leptospiral infections are either subclinical or result in very mild illness and recover without any complications Clinical manifestations of leptospirosis range from mild influenza-like symptoms to severe life-threatening disease forms, characterized by jaundice, renal failure, bleeding and severe pulmonary hemorrhage.

The clinical presentation of leptospirosis is biphasic, with the acute or septicemic phase lasting about a week, followed by the immune phase, characterized by antibody production and excretion of leptospires in the urine. Most of the complications of leptospirosis are associated with localization of leptospires within the tissues during the immune phase and thus occur during the second week of the illness. The classical syndrome of Weil's disease represents only the most severe presentation. It is characterized by jaundice, renal failure, hemorrhage and myocarditis with arrhythmias.

Species	Disease	Symptoms (e.g.)	Transmission route
Leptospira spp.	Leptospirosis	Fever accompanied by chills, intense headache, severe myalgia (muscle ache), abdominal pain, conjunctival suffusion (red eye), and occasionally a skin rash	Direct or indirect contact with the urine of an infected animal (via cuts, skin abrasions or mucous membranes)
	Weil's disease	jaundice, renal failure, meningitis, haemorrhage and myocarditis with arrhythmias	

Infection or presence of pathogen may be identified by:

- Microscopy
- PCR
- Serology: e.g. ELISA

2. INTENDED USE

The Leptospira IgM ELISA is intended for the qualitative determination of IgM class antibodies against Leptospira spp. in human serum or plasma (heparin).

3. PRINCIPLE OF THE ASSAY

The qualitative immunoenzymatic determination of specific antibodies is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique.
Microtiterplates are coated with specific antigens to bind corresponding antibodies of the sample. After washing the wells to remove all unbound sample material a horseradish peroxidase (HRP) labelled conjugate is added. This conjugate binds to the captured antibodies. In a second washing step unbound conjugate is removed. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product.
The intensity of this product is proportional to the amount of specific antibodies in the sample. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450/620 nm is read using an ELISA Microtiterplate reader.

4. MATERIALS

4.1. Reagents supplied

- **Microtiterplate:** 12 break-apart 8-well snap-off strips coated with Leptospira antigens; in resealable aluminium foil.
- **IgM Sample Dilution Buffer:** 1 bottle containing 100 mL of phosphate buffer (10 mM) for sample dilution; pH 7.2 ± 0.2; anti-human IgG (RF Absorbent); coloured green; ready to use; white cap; ≤ 0.0015% (v/v) CMIT/ MIT (3:1).
- **Stop Solution:** 1 bottle containing 15 mL sulphuric acid, 0.2 mol/L; ready to use; red cap.
- **Washing Buffer (20x conc.):** 1 bottle containing 50 mL of a 20-fold concentrated phosphate buffer (0.2 M), pH 7.2 ± 0.2, for washing the wells; white cap.
- **Conjugate:** 1 bottle containing 20 mL of peroxidase labelled antibody to human IgM in phosphate buffer (10 mM); coloured red; ready to use; black cap.
- **TMB Substrate Solution:** 1 bottle containing 15 mL 3,3',5,5'-tetramethylbenzidine (TMB), < 0.1 %; ready to use; yellow cap.
- **Positive Control:** 1 vial containing 2 mL control; coloured yellow; ready to use; red cap; ≤ 0.02% (v/v) MIT.
- **Cut-off Control:** 1 vial containing 3 mL control; coloured yellow; ready to use; green cap; ≤ 0.02% (v/v) MIT.
- **Negative Control:** 1 vial containing 2 mL control; coloured yellow; ready to use; blue cap; ≤ 0.0015% (v/v) CMIT/ MIT (3:1).

For hazard and precautionary statements see 12.1
For potential hazardous substances please check the safety data sheet.

4.2. Materials supplied

- 1 Cover foil
- 1 Instruction for use (IFU)
- 1 Plate layout

4.3. Materials and Equipment needed

- ELISA Microtiterplate reader, equipped for the measurement of absorbance at 450/620 nm
- Incubator 37 °C
- Manual or automatic equipment for rinsing Microtiterplates
- Pipettes to deliver volumes between 10 and 1000 µL
- Vortex tube mixer
- Distilled water
- Disposable tubes

5. STABILITY AND STORAGE

Store the kit at 2...8 °C. The opened reagents are stable up to the expiry date stated on the label when stored at 2...8 °C.

6. REAGENT PREPARATION

It is very important to bring all reagents and samples to room temperature (20…25 °C) and mix them before starting the test run!

6.1. Microtiterplate

The break-apart snap-off strips are coated with Leptospira antigens. Immediately after removal of the strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2...8 °C.

6.2. Washing Buffer (20x conc.)

Dilute Washing Buffer 1 + 19; e. g. 10 mL Washing Buffer + 190 mL distilled water. The diluted buffer is stable for 5 days at room temperature (20…25 °C). In case crystals appear in the concentrate, warm up the solution to 37 °C e.g. in a water bath. Mix well before dilution.

6.3. TMB Substrate Solution

The reagent is ready to use and has to be stored at 2...8 °C, away from the light. The solution should be colourless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away.

7. SAMPLE COLLECTION AND PREPARATION

Use human serum or plasma (heparin) samples with this assay. If the assay is performed within 5 days after sample collection, the samples should be kept at 2...8 °C; otherwise they should be aliquoted and stored deep-frozen (-70…-20 °C). If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing.
Heat inactivation of samples is not recommended.

7.1. Sample Dilution

Before assaying, all samples should be diluted 1+100 with IgM Sample Dilution Buffer. Dispense 10 µL sample and 1 mL IgM Sample Dilution Buffer into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

8. ASSAY PROCEDURE

Please read the instruction for use carefully **before** performing the assay. Result reliability depends on strict adherence to the instruction for use as described. The following test procedure is only validated for manual procedure. If performing the test on ELISA automatic systems we recommend increasing the washing steps from three up to five and the volume of Washing Buffer from 300 µL to 350 µL to avoid washing effects. Pay attention to chapter 12. Prior to commencing the assay, the distribution and identification plan for all samples and standards/controls (duplicates recommended) should be carefully established on the plate layout supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder.

Perform all assay steps in the order given and without any delays.

A clean, disposable tip should be used for dispensing each standard/control and sample.

Adjust the incubator to 37 ± 1 °C.

1. Dispense 100 µL standards/controls and diluted samples into their respective wells. Leave well A1 for the Substrate Blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 1 hour ± 5 min at 37 ± 1 °C.**
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µL of Washing Buffer. Avoid overflows from the reaction wells. The interval between washing and aspiration should be > 5 sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!

   Note: Washing is important! Insufficient washing results in poor precision and false results.
5. Dispense 100 µL Conjugate into all wells except for the Substrate Blank well A1.
6. **Incubate for 30 min at room temperature (20...25 °C).** Do not expose to direct sunlight.
7. Repeat step 4.
8. Dispense 100 µL TMB Substrate Solution into all wells.
9. **Incubate for exactly 15 min at room temperature (20...25 °C) in the dark.** A blue colour occurs due to an enzymatic reaction.
10. Dispense 100 µL Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution, thereby a colour change from blue to yellow occurs.
11. Measure the absorbance at 450/620 nm within 30 min after addition of the Stop Solution.

8.1. Measurement

Adjust the ELISA Microtiterplate reader **to zero** using the **Substrate Blank**.

If - due to technical reasons - the ELISA Microtiterplate reader cannot be adjusted to zero using the Substrate Blank, subtract its absorbance value from all other absorbance values measured in order to obtain reliable results!

Measure the absorbance of all wells at **450 nm** and record the absorbance values for each standard/control and sample in the plate layout.

Bichromatic measurement using a reference wavelength of 620 nm is recommended.

Where applicable calculate the mean absorbance values of all duplicates.

9. RESULTS

9.1. Run Validation Criteria

In order for an assay run to be considered valid, these Instructions for Use have to be strictly followed and the following criteria must be met:

- **Substrate Blank**: Absorbance value **< 0.100**
- **Negative Control**: Absorbance value **< 0.200 and < Cut-off**
- **Cut-off Control**: Absorbance value **0.150 – 1.300**
- **Positive Control**: Absorbance value **> Cut-off**

If these criteria are not met, the test is not valid and must be repeated.

9.2. Calculation of Results

The Cut-off is the mean absorbance value of the Cut-off Control determinations.

Example: Absorbance value Cut-off Control 0.44 + absorbance value Cut-off control 0.42 = 0.86 / 2 = 0.43
Cut-off = 0.43

9.2.1. Results in Units [NTU]

$$\frac{\text{Sample (mean) absorbance value} \times 10}{\text{Cut-off}} = \text{[NovaTec Units = NTU]}$$

Example: $\frac{1.591 \times 10}{0.43} = 37$ NTU (Units)

9.3. Interpretation of Results

Cut-off	10 NTU	-
Positive	> 11 NTU	Antibodies against the pathogen are present. There has been a contact with the antigen (pathogen resp. vaccine).
Equivocal	9 – 11 NTU	Antibodies against the pathogen could not be detected clearly. It is recommended to repeat the test with a fresh sample in 2 to 4 weeks. If the result is equivocal again the sample is judged as **negative**.
Negative	< 9 NTU	The sample contains no antibodies against the pathogen. A previous contact with the antigen (pathogen resp. vaccine) is unlikely.
Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as serological data. In immunocompromised patients and newborns serological data only have restricted value.		

9.3.1. Antibody Isotypes and State of Infection

Serology	Significance
IgM	Characteristic of the primary antibody response High IgM titer with low IgG titer: → suggests a current or very recent infection Rare: → persisting IgM
IgG	Characteristic of the secondary antibody response May persist for several years High IgG titer with low IgM titer: → may indicate a past infection

10. SPECIFIC PERFORMANCE CHARACTERISTICS

The results refer to the groups of samples investigated; these are not guaranteed specifications.

For further information about the specific performance characteristics please contact NovaTec Immundiagnostica GmbH.

10.1. Precision

Intraassay	**n**	**Mean (E)**	**CV (%)**
#1	24	0.531	5.34
#2	24	1.070	3.32
#3	24	1.900	2.80

Interassay	**n**	**Mean (NTU)**	**CV (%)**
#1	12	20.74	4.35
#2	12	37.86	4.97
#3	12	6.79	8.10

10.2. Diagnostic Specificity

The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte.
It is 96.0% (95% confidence interval: 79.65% - 99.9%).

10.3. Diagnostic Sensitivity

The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte.
It is 100% (95% confidence interval: 93.51% - 100%).

10.4. Interferences

Interferences with hemolytic, lipemic or icteric samples are not observed up to a concentration of 10 mg/mL hemoglobin, 5 mg/mL triglycerides and 0.5 mg/mL bilirubin.

10.5. Cross Reactivity

It cannot be excluded that Cytomegalovirus, Treponema pallidum and Coxiella specimens may result in false-positive IgM antibody results. In addition, it should be noted that IgM class antibodies directed against Leptospira generally remain detectable for months or even years but at low titer.

11. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or repeated freeze-thaw cycles of the sample may affect the absorbance values.

12. PRECAUTIONS AND WARNINGS

- The test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the testkits with analyzers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.
- Only for in-vitro diagnostic use.
- All materials of human or animal origin should be regarded and handled as potentially infectious.
- All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive.
- Do not interchange reagents or Microtiterplates of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- Use only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and standard/control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense reagents without splashing accurately into the wells.
- The ELISA is only designed for qualified personnel following the standards of good laboratory practice (GLP).
- For further internal quality control each laboratory should additionally use known samples.

12.1. Safety note for reagents containing hazardous substances

Reagents may contain CMIT/MIT (3:1) or MIT (refer to 4.1)
Therefore, the following hazard and precautionary statements apply.

Warning		
	H317	May cause an allergic skin reaction.
	P261	Avoid breathing spray.
	P280	Wear protective gloves/ protective clothing.
	P302+P352	IF ON SKIN: Wash with plenty of soap and water.
	P333+P313	If skin irritation or rash occurs: Get medical advice/ attention.
	P362+P364	Take off contaminated and Wash it before reuse.

Further information can be found in the safety data sheet.

12.2. Disposal Considerations

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

13. ORDERING INFORMATION

Prod. No.: LEPM0660 Leptospira IgM ELISA (96 Determinations)

DEUTSCH

1. EINLEITUNG

Die Leptospirose (Morbus Weil) ist weltweit die wahrscheinlich am weitesten verbreitete Zoonose. Sie wird durch Spirochäten der Gattung Leptospira verursacht, die sowohl den Menschen, als auch ein breites Spektrum an tierischen Wirten infizieren können. Die Leptospirose tritt in tropischen und subtropischen Ländern deutlich häufiger auf als in Regionen mit gemäßigtem Klima.

Die Erkrankung zeigt einen saisonalen Verlauf: in gemäßigten Breiten, in denen die Temperatur den limitierenden Faktor für das Überleben der Leptospiren darstellt, ist der Höhepunkt der Erkrankungsfälle im Sommer oder Herbst; in warmen Klimaten findet man die höchste Inzidenz während der Regensaison, da ansonsten eine rasche Austrocknung das Überleben der Leptospiren verhindern würde.

Natürliche Reservoire der pathogenen Spezies Leptospira interrrogans umfassen Nager sowie eine Vielzahl domestizierter Säugetiere (z. B. Schweine, Rinder und Hunde). Leptospiren besiedeln in ihrem natürlichen Wirt das Lumen der Nierentubuli und werden mit dem Urin ausgeschieden.

Der Mensch kann sich durch direkten oder indirekten Kontakt mit dem Urin infizierter Tiere anstecken. Leptospiren erhalten Zugang zum menschlichen Blutkreislauf über kleinere Hautverletzungen (Schnitte, Abschürfungen) oder über die Schleimhäute bei Kontakt mit feuchter Erde, Vegetation und kontaminiertem Wasser, beim Umgang mit Geweben infizierter Tiere und über Nahrung und Trinkwasser. Eine Übertragung von Mensch zu Mensch ist nur in seltenen Fällen beschrieben worden.

Die Inkubationszeit der Leptospirose beträgt gewöhnlich 5-14 Tage, mit einer Spannweite von 2-30 Tagen.

Das Spektrum klinischer Symptome ist äußerst vielfältig. Die überwiegende Mehrheit der Leptospira-Infektionen ist entweder subklinisch oder verläuft sehr mild und heilt ohne Komplikationen aus. Klinische Manifestationen der Leptospirose reichen von milden Grippeähnlichen Symptomen bis hin zu schweren, lebensbedrohlichen Formen, charakterisiert durch Gelbsucht, Nierenversagen und schwere pulmonale Hämorrhagien.

Häufig wird ein biphasischer Krankheitsverlauf beobachtet. Eine akute oder septikämische Phase geht nach ungefähr einer Woche in eine Immunphase über, die durch Antikörperproduktion und Ausscheidung der Leptospiren im Urin gekennzeichnet ist. Die meisten Komplikationen der Leptospirose sind mit der Lokalisierung der Leptospiren innerhalb der Gewebe während der Immunphase assoziiert und liegen in der Immunantwort des Körpers begründet. Das klassische Syndrom der Weil-Krankheit stellt nur die schwerste Ausprägung dar. Sie ist gekennzeichnet durch Gelbsucht, Nierenversagen, Hämorrhagie und Myokarditiden mit Arrhythmien.

Spezies	**Erkrankung**	**Symptome (z.B.)**	**Infektionsweg**
Leptospira spp.	Leptospirose	Fieber begleitet von Schüttelfrost, intensiven Kopfschmerzen, starker Myalgie (Muskelschmerzen), Bauchschmerzen, Bindehautentzündung (rote Augen),und gelegentlich Hautausschlag,	Direkter oder indirekter Kontakt mit dem Urin infizierter Tiere (z. B. über kleine Hautverletzungen, Schleimhäute)
	Morbus Weil	Gelbsucht, Nierenversagen, Meningitis, Hämorrhagie und Myokarditis mit Arrhythmien	

Nachweis des Erregers bzw. der Infektion durch:

- Mikroskopie
- PCR
- Serologie: z.B. ELISA

2. VERWENDUNGSZWECK

Der Leptospira IgM ELISA ist für den qualitativen Nachweis spezifischer IgM-Antikörper gegen Leptospira spp. in humanem Serum oder Plasma (Heparin) bestimmt.

3. TESTPRINZIP

Die qualitative immunenzymatische Bestimmung von spezifischen Antikörpern beruht auf der ELISA (Enzyme-linked Immunosorbent Assay) Technik.
Die Mikrotiterplatten sind mit spezifischen Antigenen beschichtet, an welche die korrespondierenden Antikörper aus der Probe binden. Ungebundenes Probenmaterial wird durch Waschen entfernt. Anschließend erfolgt die Zugabe eines Meerettich-Peroxidase (HRP) Konjugates. Dieses Konjugat bindet an die an der Mikrotiterplatte gebundenen spezifischen Antikörper. In einem zweiten Waschschritt wird ungebundenes Konjugat entfernt. Die Immunkomplexe, die durch die Bindung des Konjugates entstanden sind, werden durch die Zugabe von Tetramethylbenzidin (TMB)-Substratlösung und eine resultierende Blaufärbung nachgewiesen.
Die Intensität des Reaktionsproduktes ist proportional zur Menge der spezifischen Antikörper in der Probe. Die Reaktion wird mit Schwefelsäure gestoppt, wodurch ein Farbumschlag von blau nach gelb erfolgt. Die Absorption wird bei 450/620 nm mit einem Mikrotiterplatten-Photometer gemessen.

NovaTec Immundiagnostica GmbH

NovaLisa®

Leptospira IgG

ELISA

CE

Only for in-vitro diagnostic use

Product Number: LEPG0660 (96 Determinations)

ENGLISH

1. INTRODUCTION

Leptospirosis (also known as Weil's syndrome) is probably the most widespread zoonosis in the world. It is caused by infection with spirochete bacteria of the genus Leptospira and affects humans as well as a broad spectrum of animal hosts. The incidence is significantly higher in warm climate countries than in temperate regions. The disease is seasonal, with peak incidence occurring in summer or fall in temperate regions, where temperature is the limiting factor in survival of leptospires, and during rainy seasons in warm climate regions, where rapid desiccation would otherwise prevent survival.

Natural reservoirs for the pathogenic Leptospira interrogans include rodents as well as a large variety of domesticated mammals (e. g. pigs, cattle and dogs). Leptospires occupy the lumen of nephritic tubules in their natural host and are shed into the urine.

Transmission can occur when humans are directly or indirectly exposed to the urine of infected animals or a urine-polluted environment. Leptospires gain entry into the human blood stream via cuts, skin abrasions or mucous membranes through contact with moist soil, vegetation, and contaminated waters; handling infected animal tissues; and ingestion of food and water. Leptospires are rarely transmitted from human to human.

The incubation period is usually 5-14 days, with a range of 2-30 days.

The spectrum of clinical symptoms is extremely wide. The vast majority of leptospiral infections are either subclinical or result in very mild illness and recover without any complications Clinical manifestations of leptospirosis range from mild influenza-like symptoms to severe life-threatening disease forms, characterized by jaundice, renal failure, bleeding and severe pulmonary hemorrhage.

The clinical presentation of leptospirosis is biphasic, with the acute or septicemic phase lasting about a week, followed by the immune phase, characterized by antibody production and excretion of leptospires in the urine. Most of the complications of leptospirosis are associated with localization of leptospires within the tissues during the immune phase and thus occur during the second week of the illness. The classical syndrome of Weil's disease represents only the most severe presentation. It is characterized by jaundice, renal failure, hemorrhage and myocarditis with arrhythmias.

Species	Disease	Symptoms (e.g.)	Transmission route
Leptospira spp.	Leptospirosis	Fever accompanied by chills, intense headache, severe myalgia (muscle ache), abdominal pain, conjunctival suffusion (red eye), and occasionally a skin rash	Direct or indirect contact with the urine of an infected animal (via cuts, skin abrasions or mucous membranes)
	Weil's disease	jaundice, renal failure, meningitis, haemorrhage and myocarditis with arrhythmias	

Infection or presence of pathogen may be identified by:

- Microscopy
- PCR
- Serology: e.g. ELISA

2. INTENDED USE

The Leptospira IgG ELISA is intended for the qualitative determination of IgG class antibodies against Leptospira spp. in human serum or plasma (heparin).

3. PRINCIPLE OF THE ASSAY

The qualitative immunoenzymatic determination of specific antibodies is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique.
Microtiterplates are coated with specific antigens to bind corresponding antibodies of the sample. After washing the wells to remove all unbound sample material a horseradish peroxidase (HRP) labelled conjugate is added. This conjugate binds to the captured antibodies. In a second washing step unbound conjugate is removed. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product.
The intensity of this product is proportional to the amount of specific antibodies in the sample. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450/620 nm is read using an ELISA Microtiterplate reader.

4. MATERIALS

4.1. Reagents supplied

- **Microtiterplate:** 12 break-apart 8-well snap-off strips coated with Leptospira antigens; in resealable aluminium foil.
- **IgG Sample Dilution Buffer:** 1 bottle containing 100 mL of phosphate buffer (10 mM) for sample dilution; pH 7.2 ± 0.2; coloured yellow; ready to use; white cap; ≤ 0.0015% (v/v) CMIT/ MIT (3:1).
- **Stop Solution:** 1 bottle containing 15 mL sulphuric acid, 0.2 mol/L; ready to use; red cap.
- **Washing Buffer (20x conc.):** 1 bottle containing 50 mL of a 20-fold concentrated phosphate buffer (0.2 M), pH 7.2 ± 0.2, for washing the wells; white cap.
- **Conjugate:** 1 bottle containing 20 mL of peroxidase labelled antibody to human IgG in phosphate buffer (10 mM); coloured blue; ready to use; black cap.
- **TMB Substrate Solution:** 1 bottle containing 15 mL 3,3',5,5'-tetramethylbenzidine (TMB), < 0.1 %; ready to use; yellow cap.
- **Positive Control:** 1 vial containing 2 mL control; coloured yellow; ready to use; red cap; ≤ 0.02% (v/v) MIT.
- **Cut-off Control:** 1 vial containing 3 mL control; coloured yellow; ready to use; green cap; ≤ 0.02% (v/v) MIT.
- **Negative Control:** 1 vial containing 2 mL control; coloured yellow; ready to use; blue cap; ≤ 0.0015% (v/v) CMIT/ MIT (3:1).

For hazard and precautionary statements see 12.1
For potential hazardous substances please check the safety data sheet.

4.2. Materials supplied

- 1 Cover foil
- 1 Instruction for use (IFU)
- 1 Plate layout

4.3. Materials and Equipment needed

- ELISA Microtiterplate reader, equipped for the measurement of absorbance at 450/620 nm
- Incubator 37 °C
- Manual or automatic equipment for rinsing Microtiterplates
- Pipettes to deliver volumes between 10 and 1000 µL
- Vortex tube mixer
- Distilled water
- Disposable tubes

5. STABILITY AND STORAGE

Store the kit at 2...8 °C. The opened reagents are stable up to the expiry date stated on the label when stored at 2...8 °C.

6. REAGENT PREPARATION

It is very important to bring all reagents and samples to room temperature (20…25 °C) and mix them before starting the test run!

6.1. Microtiterplate

The break-apart snap-off strips are coated with Leptospira antigens. Immediately after removal of the strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2...8 °C.

6.2. Washing Buffer (20x conc.)

Dilute Washing Buffer 1 + 19; e. g. 10 mL Washing Buffer + 190 mL distilled water. The diluted buffer is stable for 5 days at room temperature (20…25 °C). In case crystals appear in the concentrate, warm up the solution to 37 °C e.g. in a water bath. Mix well before dilution.

6.3. TMB Substrate Solution

The reagent is ready to use and has to be stored at 2...8 °C, away from the light. The solution should be colourless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away.

7. SAMPLE COLLECTION AND PREPARATION

Use human serum or plasma (heparin) samples with this assay. If the assay is performed within 5 days after sample collection, the samples should be kept at 2...8 °C; otherwise they should be aliquoted and stored deep-frozen (-70…-20 °C). If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing.
Heat inactivation of samples is not recommended.

7.1. Sample Dilution

Before assaying, all samples should be diluted 1+100 with IgG Sample Dilution Buffer. Dispense 10 µL sample and 1 mL IgG Sample Dilution Buffer into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

8. ASSAY PROCEDURE

Please read the instruction for use carefully **before** performing the assay. Result reliability depends on strict adherence to the instruction for use as described. The following test procedure is only validated for manual procedure. If performing the test on ELISA automatic systems we recommend increasing the washing steps from three up to five and the volume of Washing Buffer from 300 µL to 350 µL to avoid washing effects. Pay attention to chapter 12. Prior to commencing the assay, the distribution and identification plan for all samples and standards/controls (duplicates recommended) should be carefully established on the plate layout supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder.

Perform all assay steps in the order given and without any delays.

A clean, disposable tip should be used for dispensing each standard/control and sample.

Adjust the incubator to 37 ± 1 °C.

1. Dispense 100 µL standards/controls and diluted samples into their respective wells. Leave well A1 for the Substrate Blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 1 hour ± 5 min at 37 ± 1 °C.**
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µL of Washing Buffer. Avoid overflows from the reaction wells. The interval between washing and aspiration should be > 5 sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!

   Note: Washing is important! Insufficient washing results in poor precision and false results.
5. Dispense 100 µL Conjugate into all wells except for the Substrate Blank well A1.
6. **Incubate for 30 min at room temperature (20...25 °C).** Do not expose to direct sunlight.
7. Repeat step 4.
8. Dispense 100 µL TMB Substrate Solution into all wells.
9. **Incubate for exactly 15 min at room temperature (20...25 °C) in the dark.** A blue colour occurs due to an enzymatic reaction.
10. Dispense 100 µL Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution, thereby a colour change from blue to yellow occurs.
11. Measure the absorbance at 450/620 nm within 30 min after addition of the Stop Solution.

8.1. Measurement

Adjust the ELISA Microtiterplate reader **to zero** using the **Substrate Blank**.

If - due to technical reasons - the ELISA Microtiterplate reader cannot be adjusted to zero using the Substrate Blank, subtract its absorbance value from all other absorbance values measured in order to obtain reliable results!

Measure the absorbance of all wells at **450 nm** and record the absorbance values for each standard/control and sample in the plate layout.

Bichromatic measurement using a reference wavelength of 620 nm is recommended.

Where applicable calculate the mean absorbance values of all duplicates.

9. RESULTS

9.1. Run Validation Criteria

In order for an assay run to be considered valid, these Instructions for Use have to be strictly followed and the following criteria must be met:

- **Substrate Blank**: Absorbance value **< 0.100**
- **Negative Control**: Absorbance value **< 0.200 and < Cut-off**
- **Cut-off Control**: Absorbance value **0.150 – 1.300**
- **Positive Control**: Absorbance value **> Cut-off**

If these criteria are not met, the test is not valid and must be repeated.

9.2. Calculation of Results

The Cut-off is the mean absorbance value of the Cut-off Control determinations.

Example: Absorbance value Cut-off Control 0.44 + absorbance value Cut-off control 0.42 = 0.86 / 2 = 0.43
Cut-off = 0.43

9.2.1. Results in Units [NTU]

$$\frac{\text{Sample (mean) absorbance value} \times 10}{\text{Cut-off}} = \text{[NovaTec Units = NTU]}$$

Example: $\frac{1.591 \times 10}{0.43} = 37$ NTU

9.3. Interpretation of Results

Cut-off	10 NTU	-
Positive	> 11 NTU	Antibodies against the pathogen are present. There has been a contact with the antigen (pathogen resp. vaccine).
Equivocal	9 – 11 NTU	Antibodies against the pathogen could not be detected clearly. It is recommended to repeat the test with a fresh sample in 2 to 4 weeks. If the result is equivocal again the sample is judged as **negative**.
Negative	< 9 NTU	The sample contains no antibodies against the pathogen. A previous contact with the antigen (pathogen resp. vaccine) is unlikely.
Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as serological data. In immunocompromised patients and newborns serological data only have restricted value.		

9.3.1. Antibody Isotypes and State of Infection

Serology	Significance
IgM	Characteristic of the primary antibody response High IgM titer with low IgG titer: → suggests a current or very recent infection Rare: → persisting IgM
IgG	Characteristic of the secondary antibody response May persist for several years High IgG titer with low IgM titer: → may indicate a past infection

10. SPECIFIC PERFORMANCE CHARACTERISTICS

The results refer to the groups of samples investigated; these are not guaranteed specifications.

For further information about the specific performance characteristics please contact NovaTec Immundiagnostica GmbH.

10.1. Precision

Intraassay	n	Mean (E)	CV (%)
#1	24	0.470	2.95
#2	24	0.782	6.50
#3	24	0.460	3.49

Interassay	n	Mean (NTU)	CV (%)
#1	12	19.60	4.87
#2	12	12.72	9.64
#3	12	3.99	4.85

10.2. Diagnostic Specificity

The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte.
It is 97.37% (95% confidence interval: 86.19% - 99.93%).

10.3. Diagnostic Sensitivity

The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte.
It is 100% (95% confidence interval: 93,4% - 100%).

10.4. Interferences

Interferences with hemolytic, lipemic or icteric samples are not observed up to a concentration of 10 mg/mL hemoglobin, 5 mg/mL triglycerides and 0.5 mg/mL bilirubin.

10.5. Cross Reactivity

It cannot be excluded that polyclonal B-cell activation induced by Epstein-Barr virus (EBV) or the presence of Rheumatoid Factors may result in false-positive Leptospira IgG antibody results.

11. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or repeated freeze-thaw cycles of the sample may affect the absorbance values.

12. PRECAUTIONS AND WARNINGS

- The test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the testkits with analyzers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.
- Only for in-vitro diagnostic use.
- All materials of human or animal origin should be regarded and handled as potentially infectious.
- All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive.
- Do not interchange reagents or Microtiterplates of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- Use only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and standard/control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense reagents without splashing accurately into the wells.
- The ELISA is only designed for qualified personnel following the standards of good laboratory practice (GLP).
- For further internal quality control each laboratory should additionally use known samples.

12.1. Safety note for reagents containing hazardous substances

Reagents may contain CMIT/MIT (3:1) or MIT (refer to 4.1)
Therefore, the following hazard and precautionary statements apply.

Warning	H317	May cause an allergic skin reaction.
	P261	Avoid breathing spray.
	P280	Wear protective gloves/ protective clothing.
	P302+P352	IF ON SKIN: Wash with plenty of soap and water.
	P333+P313	If skin irritation or rash occurs: Get medical advice/ attention.
	P362+P364	Take off contaminated and Wash it before reuse.

Further information can be found in the safety data sheet.

12.2. Disposal Considerations

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

13. ORDERING INFORMATION

Prod. No.: LEPG0660 Leptospira IgG ELISA (96 Determinations)

NovaTec Immundiagnostica GmbH

NovaLisa®

Yersinia enterocolitica IgG

ELISA

CE

Only for in-vitro diagnostic use

Instructions for use

REF YERG0990 (96 Determinations)

ENGLISH

1. INTENDED USE

The Yersinia enterocolitica IgG ELISA is intended for the qualitative determination of IgG class antibodies against antigens of the 70 kb virulence plasmid of Yersinia enterocolitica in human serum or plasma (citrate, heparin). The ELISA is intended for use as an aid in identifying individuals with an adaptive immune response to Yersinia enterocolitica. The determination of increased antibody levels contributes to diagnosis of Yersinia-induced reactive arthritis. The test is not intended for diagnosing acute enteric diseases.

2. PRINCIPLE OF THE ASSAY

The qualitative immunoenzymatic determination of specific antibodies is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique.
Microtiterplates are coated with specific antigens to bind corresponding antibodies of the sample. After washing the wells to remove all unbound sample material a horseradish peroxidase (HRP) labelled conjugate is added. This conjugate binds to the captured antibodies. In a second washing step unbound conjugate is removed. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product.
The intensity of this product is proportional to the amount of specific antibodies in the sample. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450/620 nm is read using an ELISA Microtiterplate reader.

3. MATERIALS

3.1. Reagents supplied

- **Microtiterplate:** 12 break-apart 8-well snap-off strips coated with specific antigen; in resealable aluminium foil.
- **DIL:** 1 bottle containing 100 mL of phosphate buffer (10 mM) for sample dilution; pH 7.2 ± 0.2; coloured yellow; ready to use; white cap; ≤ 0.0015 % (v/v) CMIT/MIT (3:1).
- **SOLN STOP:** 1 bottle containing 15 mL sulphuric acid, 0.2 mol/L; ready to use; red cap.
- **WASH BUF 20x:** 1 bottle containing 50 mL of a 20-fold concentrated phosphate buffer (0.2 M), pH 7.2 ± 0.2, for washing the wells; white cap; 0.2% (w/v) 5-Bromo-5-nitro-1,3-dioxane.
- **Conjugate:** 1 bottle containing 20 mL of peroxidase labelled antibody to human IgG in phosphate buffer (10 mM); coloured blue; ready to use; black cap.
- **SUB TMB:** 1 bottle containing 15 mL 3,3',5,5'-tetramethylbenzidine (TMB), < 0.1 %; ready to use; yellow cap.
- **Positive Control:** 1 vial containing 2 mL control; coloured yellow; ready to use; red cap; ≤ 0.02 % (v/v) MIT.
- **Cut-off Control:** 1 vial containing 3 mL control; coloured yellow; ready to use; green cap; ≤ 0.02 % (v/v) MIT.
- **Negative Control:** 1 vial containing 2 mL control; coloured yellow; ready to use; blue cap; ≤ 0.0015 % (v/v) CMIT/MIT (3:1).

For hazard and precautionary statements see 11.1

3.2. Materials supplied

- 1 Cover foil
- 1 Instruction for use (IFU)
- 1 Plate layout

3.3. Materials and Equipment needed

- ELISA Microtiterplate reader, equipped for the measurement of absorbance at 450/620 nm
- Incubator 37 °C
- Manual or automatic equipment for rinsing Microtiterplates
- Pipettes to deliver volumes between 10 and 1000 µL
- Vortex tube mixer
- Distilled water
- Disposable tubes

4. STABILITY AND STORAGE

Store the kit at 2...8 °C. The opened reagents are stable up to the expiry date stated on the label when stored at 2...8 °C.

5. REAGENT PREPARATION

It is very important to bring all reagents and samples to room temperature (20…25 °C) and mix them before starting the test run!

5.1. Microtiterplate

The break-apart snap-off strips are coated with specific antigen. Immediately after removal of the strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2...8 °C.

5.2. WASH | BUF | 20x

Dilute **WASH | BUF | 20x** 1 + 19; e. g. 10 mL **WASH | BUF | 20x** + 190 mL distilled water. The diluted buffer (**WASH | BUF | 1x**) is stable for 5 days at room temperature (20…25 °C). In case crystals appear in the concentrate, warm up the solution to 37 °C e.g., in a water bath. Mix well before dilution.

5.3. SUB | TMB

The reagent is ready to use and has to be stored at 2...8 °C, away from the light. The solution should be colourless or could have a slight blue tinge. If **SUB | TMB** turns into blue, it may have become contaminated and should be thrown away.

6. SAMPLE COLLECTION AND PREPARATION

Use human serum or plasma (citrate, heparin) samples with this assay. If the assay is performed within 5 days after sample collection, the samples should be kept at 2...8 °C; otherwise, they should be aliquoted and stored deep-frozen (-70…-20 °C). If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing.
Heat inactivation of samples is not recommended.

6.1. Sample Dilution

Before assaying, all samples should be diluted 1+100 with **DIL**. Dispense 10 µL sample and 1 mL **DIL** into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

7. ASSAY PROCEDURE

Please read the instruction for use carefully **before** performing the assay. Result reliability depends on strict adherence to the instruction for use as described. The following test procedure is only validated for manual procedure. If performing the test on ELISA automatic systems, we recommend increasing the washing steps from three up to five and the volume of Washing Buffer from 300 µL to 350 µL to avoid washing effects. Pay attention to chapter 11. Prior to commencing the assay, the distribution and identification plan for all samples and standards/controls (duplicates recommended) should be carefully established on the plate layout supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder.

Perform all assay steps in the order given and without any delays.

A clean, disposable tip should be used for dispensing each standard/control and sample.

Adjust the incubator to 37 ± 1 °C.

1. Dispense 100 µL standards/controls and diluted samples into their respective wells. Leave well A1 for the Substrate Blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 1 hour ± 5 min at 37 ± 1 °C.**
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µL of **WASH | BUF | 1x**. Avoid overflows from the reaction wells. The interval between washing and aspiration should be > 5 sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!

   Note: Washing is important! Insufficient washing results in poor precision and false results.
5. Dispense 100 µL Conjugate into all wells except for the Substrate Blank well A1.
6. **Incubate for 30 min at room temperature (20...25 °C).** Do not expose to direct sunlight.
7. Repeat step 4.
8. Dispense 100 µL **SUB | TMB** into all wells.
9. **Incubate for exactly 15 min at room temperature (20...25 °C) in the dark.** A blue colour occurs due to an enzymatic reaction.
10. Dispense 100 µL **SOLN | STOP** into all wells in the same order and at the same rate as for the **SUB | TMB**, thereby a colour change from blue to yellow occurs.
11. Measure the absorbance at 450/620 nm within 30 min after addition of the **SOLN | STOP**.

7.1. Measurement

Adjust the ELISA Microtiterplate reader **to zero** using the **Substrate Blank**.

If - due to technical reasons - the ELISA Microtiterplate reader cannot be adjusted to zero using the Substrate Blank, subtract its absorbance value from all other absorbance values measured in order to obtain reliable results!

Measure the absorbance of all wells at **450 nm** and record the absorbance values for each standard/control and sample in the plate layout.

Bichromatic measurement using a reference wavelength of 620 nm is recommended.

Where applicable calculate the mean absorbance values of all duplicates.

8. RESULTS

8.1. Run Validation Criteria

In order for an assay run to be considered valid, these Instructions for Use have to be strictly followed and the following criteria must be met:

- **Substrate Blank**: Absorbance value **< 0.100**
- **Negative Control**: Absorbance value **< 0.200 and < Cut-off**
- **Cut-off Control**: Absorbance value **0.150 – 1.300**
- **Positive Control**: Absorbance value **> Cut-off**

If these criteria are not met, the test is not valid and must be repeated.

8.2. Calculation of Results

The Cut-off is the mean absorbance value of the Cut-off Control determinations.

Example: Absorbance value Cut-off Control 0.44 + absorbance value Cut-off control 0.42 = 0.86 / 2 = 0.43
Cut-off = 0.43

8.2.1. Results in Units [NTU]

$$\frac{\text{Sample (mean) absorbance value} \times 10}{\text{Cut-off}} = \text{[NovaTec Units = NTU]}$$

Example: $\frac{1.591 \times 10}{0.43} = 37$ NTU (Units)

8.3. Interpretation of Results

Cut-off	10 NTU	-
Positive	> 11 NTU	Antibodies against the pathogen are present. There has been a contact with the antigen (pathogen resp. vaccine).
Equivocal	9 – 11 NTU	Antibodies against the pathogen could not be detected clearly. It is recommended to repeat the test with a fresh sample in 2 to 4 weeks.
Negative	< 9 NTU	The sample contains no antibodies against the pathogen. A previous contact with the antigen (pathogen resp. vaccine) is unlikely.
Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as serological data. In immunocompromised patients and newborns serological data only have restricted value.		

8.3.1. Antibody Isotypes and State of Infection

Serology	Significance
IgG	Characteristic of the secondary antibody response May persist for several years High IgG titer with low IgM titer: → may indicate a past infection
IgA	Produced in mucosal linings throughout the body (⇒ protective barrier) Usually produced early in the course of the infection

9. SPECIFIC PERFORMANCE CHARACTERISTICS

The results refer to the groups of samples investigated; these are not guaranteed specifications.

For further information about the specific performance characteristics please contact NovaTec Immundiagnostica GmbH.

9.1. Precision

Evaluation of precision of the assay was performed according to “CLSI. *Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline - Third Edition.* CLSI document EP05-A3. Wayne, PA: Clinical and Laboratory Standards Institute; 2014”.

9.1.1. Single-Site Study

The precision study was performed at a single site. A negative, a high negative, a low positive, and a moderate positive sample were run in 4 replicates, two times per day for 12 days for a total of 96 results.

Sample	n	Mean (NTU)	Repeatability		Between Run		Within Day		Between Day		Within lab	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
moderate positive	96	21.50	0.8883	4.1	1.6690	7.8	1.8907	8.8	0.9469	4.4	2.1145	9.8
low positive	96	15.28	0.6923	4.5	1.0913	7.1	1.2924	8.5	0.0000	0.0	1.2924	8.5
high negative	96	8.56	0.5784	6.8	0.7467	8.7	0.9445	11.0	0.2395	2.8	0.9744	11.4
negative	96	4.60	0.4439	9.7	0.3461	7.5	0.5629	12.2	0.1438	3.1	0.5810	12.6

9.1.2. Multisite-Study

The precision study was performed at two different sites. A negative, a high negative, a low positive, and a moderate positive sample were run in 5 replicates, once a day for 5 days for a total of 50 results.

Sample	n	Mean (NTU)	Repeatability		Within Site		Reproducibility	
			SD	%CV	SD	%CV	SD	%CV
moderate positive	50	21.68	0.6338	2.9	1.4163	6.5	2.1946	10.1
low positive	50	14.58	0.5295	3.6	1.1098	7.6	1.2922	8.9
high negative	50	8.60	0.4094	4.8	0.9157	10.6	1.2857	14.9
negative	50	3.93	0.3859	9.8	0.7162	18.2	0.9696	24.7

9.2. Diagnostic Specificity

The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte. It is 98.82 % (95 % confidence interval: 95.81 % - 99.86 %).

9.3. Diagnostic Sensitivity

The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte. It is 95.65 % (95 % confidence interval: 85.16 % - 99.47 %).

9.4. Interferences

The assay was evaluated for interferences according to guideline EP07-A3 (“Interference Testing in Clinical Chemistry” from the Clinical and Laboratory Standards Institute). Three samples, covering the relevant measuring range, were spiked with high levels of interferents and were tested along with the unspiked sample. The following table shows the tested substances added to patient samples at the indicated concentrations. These correspond to the recommendations in the CLSI guideline to represent pathological elevated concentrations in patient samples.

Interferent	**Concentration tested**
Albumin	60 mg/mL
Bilirubin, unconjugated	0.4 mg/mL
Bilirubin, conjugated	0.4 mg/mL
Cholesterol	4 mg/mL
Hemoglobin	10 mg/mL
Triglycerides	15 mg/mL

No clinically significant interference effect was found for all tested substances.

9.5. Cross Reactivity

A minimum of 5 samples with antibody activities to potentially cross-reacting parameters (Adenovirus, Borrelia burgdorferi, Brucella, Campylobacter jejuni, Chlamydia pneumoniae, Enterovirus, Epstein-Barr virus, Helicobacter pylori, Parvovirus B19) or samples positive for ANA or rheumatoid factors, and samples from pregnant women were tested to evaluate the cross reactivity of the assay. Positive findings were additionally analyzed with a CE-marked reference assay. The results are shown in the following table.

Pathogen/Condition	Samples tested	Number of positive samples	
		Yersinia enterocolitica IgG	CE-marked reference assay
Adenovirus	15	2	4
Antinuclear antibodies (ANA)	15	3	2
Borrelia burgdorferi	15	4	4
Brucella	10	9	9
Campylobacter jejuni	8	4	5
Chlamydia pneumoniae	12	4	2
Enterovirus	15	7	5
Epstein-Barr virus (EBV)	15	3	3
Helicobacter pylori	14	6	6
Parvovirus B19	14	6	1
Pregnancy samples	14	1	0
Rheumatoid factor (RF)	13	4	5

Cross-reactions with antibodies against Adenovirus, Borrelia burgdorferi, Brucella, Campylobacter jejuni, Chlamydia pneumoniae, Enterovirus, Epstein-Barr virus, Helicobacter pylori, Parvovirus B19 as well as with samples from pregnant women or samples positive for antinuclear antibodies (ANA) or rheumatoid factor (RF) cannot be excluded.
Based on the prevalence values of 4-35 % for anti-Yersinia enterocolitica IgG antibodies reported in the literature, it cannot be excluded that some of the tested samples are correctly positive[1,2].

References

1. Granfors K, Isomäki H, Essen R von, Maatela J, Kalliomäki JL, Toivanen A. 1983. Yersinia antibodies in inflammatory joint diseases. Clin Exp Rheumatol 1:215–218.

2. Strieder TGA, Wenzel BE, Prummel MF, Tijssen JGP, Wiersinga WM. 2003. Increased prevalence of antibodies to enteropathogenic Yersinia enterocolitica virulence proteins in relatives of patients with autoimmune thyroid disease. Clin Exp Immunol 132:278–282. doi:10.1046/j.1365-2249.2003.02139.x.

10. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or repeated freeze-thaw cycles of the sample may affect the absorbance values.

11. PRECAUTIONS AND WARNINGS

- The test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the testkits with analyzers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.
- Only for in-vitro diagnostic use.
- All materials of human or animal origin should be regarded and handled as potentially infectious.
- All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive.
- Do not interchange reagents or Microtiterplates of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- Use only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and standard/control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense reagents without splashing accurately into the wells.
- The ELISA is only designed for qualified personnel following the standards of good laboratory practice (GLP).
- For further internal quality control each laboratory should additionally use known samples.

11.1. Safety note for reagents containing hazardous substances

Reagents may contain CMIT/MIT (3:1) or MIT (refer to 3.1).
Therefore, the following hazard and precautionary statements apply.

Warning	H317	May cause an allergic skin reaction.
	P261	Avoid breathing spray.
	P280	Wear protective gloves/ protective clothing.
	P302+P352	IF ON SKIN: Wash with plenty of soap and water.
	P333+P313	If skin irritation or rash occurs: Get medical advice/ attention.
	P362+P364	Take off contaminated and Wash it before reuse.

Reagents may contain 5-Bromo-5-nitro-1,3-dioxane (refer to 3.1)
Therefore, the following hazard and precautionary statements apply.

Warning	H315	Causes skin irritation.
	H317	May cause an allergic skin reaction.
	P280	Wear protective gloves/ protective clothing.
	P302+P352	IF ON SKIN: Wash with plenty of soap and water.
	P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
	P337+P313	If eye irritation persists: Get medical advice/attention.

Further information can be found in the safety data sheet.

11.2. Disposal Considerations

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

12. ORDERING INFORMATION

REF	YERG0990	Yersinia enterocolitica IgG ELISA	(96 Determinations)

ВЕКТОР БЕСТ

Набор реагентов для иммуноферментного выявления иммуноглобулинов класса М к возбудителям иерсиниозов

ИНСТРУКЦИЯ ПО ПРИМЕНЕНИЮ

Утверждена 28.08.2013
Приказом Росздравнадзора № 4415-Пр/13

НАБОР РЕАГЕНТОВ
D-3206

Иерсиния – IgM – ИФА – БЕСТ

1. НАЗНАЧЕНИЕ

1.1. Набор **«Иерсиния-IgM-ИФА-БЕСТ»** предназначен для выявления иммуноглобулинов класса М к патогенным иерсиниям *(Yersinia enterocolitica и Yersinia pseudotuberculosis)* в сыворотке *(плазме)* крови человека методом иммуноферментного анализа.

1.2. Набор рассчитан на проведение 96 анализов, включая контроли. Возможны 12 независимых постановок ИФА по 8 анализов каждая, включая контроли.

2. ХАРАКТЕРИСТИКА НАБОРА

2.1. Принцип действия.

Набор реагентов **«Иерсиния-IgM-ИФА-БЕСТ»** представляет собой набор реагентов, основой которого является смесь рекомбинантных антигенов иерсиний, сорбированных на поверхности лунок разборного полистиролового планшета.

Основным свойством набора является способность выявлять в сыворотке/плазме крови человека иммуноглобулины класса М к *Yersinia enterocolitica* и *Yersinia pseudotuberculosis* за счёт их взаимодействия с иммобилизованными **антигенами (АГ)**. АГ представляют собой рекомбинантные белки внешней мембраны иерсиний (YOPs). Данные АГ высокоспецифичны

для *Yersinia*, не обнаружены ни у каких других бактерий и характерны только для штаммов *Yersinia*, патогенных для человека. Образование комплекса антиген-антитело выявляют с помощью иммуноферментного конъюгата. Во время инкубации с раствором тетраметилбензидина происходит окрашивание раствора в лунках, содержащих комплексы «антиген-антитело». Реакцию останавливают добавлением стоп-реагента. Результаты ИФА регистрируют с помощью спектрофотометра, измеряя **оптическую плотность (ОП)** в двухволновом режиме: основной фильтр – 450 нм, референс-фильтр – в диапазоне 620–655 нм. Допустима регистрация результатов только с фильтром 450 нм. После измерения ОП в лунках на основании рассчитанного значения $ОП_{крит.}$ результат для каждого анализируемого образца оценивается как положительный или отрицательный.

2.2. Состав набора:

- планшет разборный с иммобилизованными антигенами иерсиний – 1 шт.;
- положительный контрольный образец ($К^+$), инактивированный – 1 фл., 0,5 мл;
- отрицательный контрольный образец ($К^-$), инактивированный – 1 фл., 0,5 мл;

- конъюгат *(антитела к IgM человека, меченные пероксидазой хрена)* – 1 фл. или 2 фл.;
- раствор для разведения сывороток (РРС) – 1 фл., 13 мл;
- раствор для разведения конъюгата (РК) – 1 фл., 13 мл;
- раствор для предварительного разведения (РПР) – 1 фл., 3 мл;
- 25-кратный концентрат фосфатно-солевого буферного раствора с твином (ФСБ-Т×25) – 1 фл., 28 мл;
- раствор тетраметилбензидина (раствор ТМБ) – 1 фл., 13 мл;
- стоп-реагент – 1 фл., 12 мл.

Набор дополнительно комплектуется:

- плёнками для заклеивания планшета – 3 шт.;
- ванночками для реагентов – 2 шт.;
- наконечниками для пипетки на 4–200 мкл – 16 шт.

3. АНАЛИТИЧЕСКИЕ И ДИАГНОСТИЧЕСКИЕ ХАРАКТЕРИСТИКИ

3.1. Результат качественного определения набором иммуноглобулинов класса М к возбудителям иерсиниозов должен соответствовать требованиям стандартной панели предприятия *(рег. № 05-2-439)*, аттестованной ОБТК **АО «Вектор-Бест»,** включающей образцы, содержащие иммуноглобулины класса М к возбудителям иерсиниозов: **чувствительность** по IgM к возбудителю иерсиний – 100%.

3.2. Результат качественного определения набором иммуноглобулинов класса М к возбудителям иерсиниозов должен соответствовать требованиям стандартной панели предприятия *(рег. № 05-2-439)*, аттестованной ОБТК **АО «Вектор-Бест»,** включающей образцы, не содержащие иммуноглобулины класса М к возбудителям иерсиниозов: **специфичность** по IgM к возбудителю иерсиний – 100%.

4. МЕРЫ ПРЕДОСТОРОЖНОСТИ

Потенциальный риск применения набора – класс 2б *(ГОСТ Р 51609-2000)*.

При работе с исследуемыми сыворотками и контрольными образцами следует соблюдать меры предосторожности, принятые при работе с потенциально инфекционным материалом:

* работать в резиновых перчатках;
* не пипетировать растворы ртом;
* все использованные материалы дезинфицировать в соответствии с требованиями с СП 1.3.2322-08 и МУ-287-113-00 от 29.10.98.

5. ОБОРУДОВАНИЕ И МАТЕРИАЛЫ

– Спектрофотометр, позволяющий проводить измерения оптической плотности растворов в лунках планшета при длине волны 450 нм и/или в двухволновом режиме при основной

длине волны 450 нм и длине волны сравнения в диапазоне 620–655 нм;

– термостат, поддерживающий температуру (37±1) °С;

– термошейкер, поддерживающий температуру (37±1) °С, интенсивность перемешивания 400–800 об/мин;

– холодильник бытовой, поддерживающий температуру (2–8) °С;

– пипетки полуавтоматические одноканальные с переменным объёмом со сменными наконечниками, позволяющие отбирать объёмы жидкости от 5 до 1000 мкл, аттестованные по значению средней дозы и сходимости результатов пипетирования *(погрешность не более 5%);*

– пипетки полуавтоматические многоканальные со сменными наконечниками, позволяющая отбирать объёмы жидкостей от 5 мкл до 350 мкл, аттестованная по значению средней дозы и сходимости результатов пипетирования *(погрешность не более 5%)*;

– промывочное устройство для планшетов;

– перчатки резиновые хирургические;

– бумага фильтровальная лабораторная;

– цилиндр мерный 2-го класса точности вместимостью 1000 мл;

– вода дистиллированная;
– дезинфицирующие средства, разрешенные к применению в соответствии с указанием СП 1.3.2322-08 и МУ-287-113-00.

6. АНАЛИЗИРУЕМЫЕ ОБРАЗЦЫ

– Допускается использование образцов, хранившихся при температуре (2–8)°С не более 5 суток, либо при температуре минус (20±4) °С, если необходимо более длительное хранение.
– Сыворотки, содержащие взвешенные частицы, могут дать неправильный результат. Такие образцы перед использованием следует центрифугировать при 3000 об/мин в течение 10–15 минут.
– Нельзя использовать проросшие, гемолизированные, гиперлипидные сыворотки или подвергавшиеся многократному замораживанию и оттаиванию.

7. ПРОВЕДЕНИЕ АНАЛИЗА

7.1. ВНИМАНИЕ! Тщательное соблюдение описанных ниже требований позволит избежать искажения результатов ИФА.

– Перед постановкой реакции все компоненты набора необходимо выдержать при температуре (18–25) °С не менее 30 минут.

– Для приготовления растворов и проведения ИФА следует использовать чистую мерную посуду и автоматические пипетки с погрешностью измерения объёмов не более 5%.

– Лиофилизированные компоненты должны быть восстановлены, как минимум, за 15 минут до их использования.

– После отбора необходимого количества стрипов оставшиеся сразу упаковать в пакет с осушителем. Упакованные стрипы, плотно закрытые флаконы с исходными компонентами сразу после постановки реакции поместить в холодильник при температуре (2–8) °С.

– Раствор конъюгата в рабочем разведении готовить непосредственно перед использованием.

– Необходимо исключить воздействие прямого света на раствор ТМБ.

– При промывке лунки *(стрипа, планшета)* заполнять полностью, не допуская переливания промывочного раствора через край лунок,

и не касаясь лунок наконечником пипетки. Время между заполнением и опорожнением лунок должно быть не менее 30 секунд.

– При использовании автоматического или ручного промывателя необходимо следить за состоянием ёмкости для промывочного раствора и соединительных шлангов: в них не должно быть «заростов». Раз в неделю желательно ёмкость для промывочного раствора и шланги промывать 70% спиртом.
– Не допускать высыхания лунок планшета между отдельными операциями.
– При постановке ИФА нельзя использовать компоненты из наборов разных серий или смешивать их при приготовлении растворов, кроме неспецифических компонентов *(ФСБ-Т×25, стоп-реагент, раствор ТМБ),* которые взаимозаменяемы в наборах АО «Вектор-Бест».
– Наконечники для пипеток *(автоматических дозаторов)* следует использовать однократно.
– Запрещается повторное использование планшета для предварительного нанесения сывороток.
– Посуду *(ванночки)*, используемые для работы с растворами конъюгата и ТМБ, не обрабаты-

вать дезинфицирующими растворами и моющими средствами.

– В случае повторного использования посуду *(ванночки)* для раствора конъюгата промыть проточной водой и тщательно ополоснуть дистиллированной водой, посуду *(ванночки)* для раствора ТМБ сразу после работы необходимо промыть 50% раствором этилового спирта, а затем дистиллированной водой.
– Для дезинфекции посуды и материалов, контактировавших с исследуемыми и контрольными образцами, рекомендуем использовать дезинфицирующие средства, не оказывающие негативного воздействия на качество ИФА, не содержащие активный кислород и хлор, например, комбинированные средства на основе ЧАС, спиртов, третичных аминов (*МУ-231-113-00 от 29.10.98).*
– Пипетки и рабочие поверхности обрабатывать только 70% раствором этилового спирта. Не использовать перекись водорода, хлорамин и т.д.

7.2. Приготовление реагентов.

7.2.1. Промывочный раствор.

Взболтать содержимое флакона с ФСБ-Т×25. При выпадении осадка солей в концентрате прогреть его перед разведением до полного растворения осадка.

Таблица расхода реагентов

	Количество используемых стрипов											
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Промывочный раствор												
ФСБ-Т×25, мл	2	4	6	8	10	12	14	16	18	20	22	24
Дистиллированная вода, мл	до 50	до 100	до 150	до 200	до 250	до 300	до 350	до 400	до 450	до 500	до 550	до 600
Раствор конъюгата в рабочем разведении												
Конъюгат (концентрат), мкл	α*	2×а	3×а	4×а	5×а	6×а	7×а	8×а	9×а	10×а	11×а	12×а
РК, мл	1,0	2,0	3,0	4,0	5,0	6,0	7,0	8,0	9,0	10,0	11,0	12,0
	Раствор ТМБ											
Раствор ТМБ, мл	1,0	2,0	3,0	4,0	5,0	6,0	7,0	8,0	9,0	10,0	11,0	12,0

* α = ▲▲▲ **мкл** *(значение α указано в инструкции и в паспорте на данную серию наборов).*

В соответствии с числом используемых стрипов отобрать необходимое количество ФСБ-Т×25 *(см. таблицу)* и развести дистиллированной водой до указанного в таблице объёма или содержимое 1 флакона – до **700 мл**.

Хранение: при температуре (2–8) °С до 5 суток.

7.2.2. Растворы конъюгата.

Внимание! *Для работы с конъюгатом рекомендуем использовать* ***одноразовые*** *наконечники для пипеток.*

7.2.2.1. Приготовить концентрированный раствор конъюгата путём растворения содержимого флакона с конъюгатом в **1,0 мл РПР**.

Хранение: концентрированный раствор конъюгата – до 3 месяцев при (2–8) °С или до 12 месяцев при минус (20±4) °С. Допускается 3-кратное замораживание концентрированного раствора конъюгата.

Внимание! *Раствор конъюгата в рабочем разведении готовить в пластиковой ванночке, входящей в состав набора, непосредственно перед использованием!*

7.2.2.2. Тщательно взболтать содержимое флакона РК. Затем в ванночку для реагентов отобрать необходимое количество *(см. таблицу)* концентрированного раствора конъюгата, доба-

вить соответствующее количество РК, тщательно перемешать пипетированием.

Хранение: конъюгат в рабочем разведении хранению не подлежит.

7.2.3. Раствор ТМБ.

Внимание! *Раствор ТМБ готов к использованию. Необходимо исключить воздействие света на раствор ТМБ.*

Рекомендуем выделить наконечники для пипеток, которые использовать ***только*** *для работы с раствором ТМБ.*

Непосредственно перед использованием отобрать в пластиковую ванночку только необходимое в соответствии с числом используемых стрипов количество раствора ТМБ *(см. таблицу)*. Остатки раствора ТМБ из ванночки утилизировать *(не сливать во флакон с исходным раствором ТМБ)*.

Хранение: при температуре (2–8) °С в течение всего срока годности набора.

7.3. Проведение анализа.

7.3.1. Подготовить необходимое количество стрипов к работе. Оставшиеся – сразу упаковать во избежание губительного воздействия влаги. Для этого стрипы поместить в цефленовый пакет с влагопоглотителем, тщательно закрыть пакет пластиковой застёжкой. Упакованные та-

ким образом стрипы хранить при температуре (2–8) °С до конца срока годности набора.

Приготовить промывочный раствор *(п. 7.2.1)*, концентрированный раствор конъюгата *(п. 7.2.2.1)*.

7.3.2. Во все лунки стрипов внести по **90 мкл РРС**. В одну лунку внести **10 мкл К$^+$**, в 2 другие лунки – по **10 мкл К$^-$**. Во все остальные лунки стрипов внести по **10 мкл исследуемых образцов**. При этом цвет раствора в лунках изменится на синий.

Внесение сывороток должно сопровождаться тщательным перемешиванием (пипетирование не менее 4 раз).

Лунки заклеить плёнкой и инкубировать при (37±1) °С в термошейкере при 500 об/мин **30 минут.**

За 5 минут до окончания инкубации приготовить раствор конъюгата в рабочем разведении *(п. 7.2.2.2)*.

7.3.3. По окончании инкубации содержимое лунок собрать в сосуд с дезинфицирующим раствором, промыть лунки 5 раз промывочным раствором и тщательно удалить влагу.

Внимание! *Каждую лунку при промывке необходимо заполнять полностью* ***(400 мкл рабочего промывочного раствора)***. *Необходимо добиваться полного опорожнения лунок*

после каждого их заполнения. Время между заполнением и опорожнением лунок должно быть не менее 30 секунд.

По окончании промывки необходимо тщательно удалить влагу из лунок, постукивая перевёрнутыми стрипами по сложенной в несколько слоёв фильтровальной бумаге. Не допускать высыхания лунок стрипов между отдельными операциями при постановке реакции.

7.3.4. Внести во все лунки по **100 мкл раствора конъюгата в рабочем разведении**.

Внимание*! Для внесения раствора конъюгата в рабочем разведении использовать пластиковую ванночку и* ***одноразовые*** *наконечники, входящие в состав набора.*

Лунки заклеить плёнкой и инкубировать при температуре (37±1) °С **30 минут**.

7.3.5. По окончании инкубации содержимое лунок собрать в сосуд с дезинфицирующим раствором и промыть 5 раз промывочным раствором, удалить влагу как описано выше.

7.3.6. Внести в каждую лунку по **100 мкл раствора ТМБ** *(п. 7.2.3).*

Внимание! *Для внесения раствора ТМБ использовать пластиковую ванночку и одноразовые наконечники, прилагаемые к набору.*

Стрипы поместить в защищённое от света место при температуре (18–25) °С на **25 минут**.

7.3.7. Остановить реакцию добавлением в лунки по **100 мкл стоп-реагента** и через 2–3 минуты измерить ОП.

Следует избегать попадания стоп-реагента на одежду и открытые участки тела. При попадании – промыть большим количеством воды.

8. РЕГИСТРАЦИЯ РЕЗУЛЬТАТОВ

Результаты ИФА регистрировать с помощью спектрофотометра, измеряя ОП в двухволновом режиме: основной фильтр – 450 нм, референс-фильтр – в диапазоне 620–655 нм. Допустима регистрация результатов только с фильтром 450 нм.

Выведение спектрофотометра на нулевой уровень *(«бланк»)* осуществлять по воздуху.

9. УЧЁТ РЕЗУЛЬТАТОВ РЕАКЦИИ

9.1. Результаты исследований учитывать только при соблюдении следующих условий:

– среднее значение ОП в лунках с $К^-$ не более 0,2 ($ОП_{ср}К^- \leq 0,2$).

– значение ОП в лунке с $К^+$ не менее 0,6 ($ОПК^+ \geq 0,6$);

Вычислить критическое значение ОП **($ОП_{крит}$)** по формуле:

$$ОП_{крит} = ОП_{ср}(К^{-}) + 0{,}3,$$

где $ОП_{ср}(К^{-})$ – среднее арифметическое значение ОП в лунках с отрицательным контрольным образцом.

Если $ОП_{ср}$ $(К^{-})$ имеет отрицательное значение, считать её равной нулю.

Для интерпретации результатов исследования сывороток использовать **коэффициент позитивности (КП)**:

$$КП = ОП_{иссл.\ сыв.} / ОП_{крит.}$$

При КП < 1,0 результат расценивать как **отрицательный**.

КП ≥ 1,0 – результат **положительный.**

10. УСЛОВИЯ ХРАНЕНИЯ И ЭКСПЛУАТАЦИИ НАБОРА

10.1. Транспортирование набора должно проводиться при температуре (2–8) °С. Допускается транспортирование при температуре до 25 °С не более 10 суток. Замораживание не допускается.

10.2. Хранение набора в упаковке предприятия-изготовителя должно производиться при температуре (2–8) °С. Замораживание не допускается.

10.3. Срок годности набора реагентов – 12 месяцев со дня выпуска.

10.4. Дробное использование набора может быть реализовано в течение всего срока годности. В случае дробного использования набора:

– неиспользованные стрипы можно хранить в плотно закрытом пакете при температуре (2–8) °С в течение всего срока годности набора;
– контрольные образцы, ФСБ-Т×25, РРС, РК, РПР, раствор ТМБ и стоп-реагент после вскрытия флаконов можно хранить в плотно закрытых флаконах при температуре (2–8) °С в течение всего срока годности набора;
– концентрированный раствор конъюгата можно хранить в плотно закрытых флаконах при температуре (2–8) °С в течение 3 месяцев с момента приготовления или при температуре минус (20±4) °С в течение всего срока годности набора;

– промывочный раствор можно хранить при температуре (2–8) °С до 5 суток с момента притовления.

По вопросам, касающимся качества набора ***«Иерсиния-IgM-ИФА-БЕСТ»****, следует обращаться в* ***АО «Вектор-Бест»*** *по адресу:*
630117, г. Новосибирск-117, а/я 492,
тел.: (383) 332-92-49, 227-60-30;
тел./факс: (383) 332-94-47, 332-94-44;
E-mail: plkobtk@vector-best.ru

Консультацию специалиста по работе с набором можно получить по тел.: (383) 227-68-23.

ДОПОЛНИТЕЛЬНАЯ ИНФОРМАЦИЯ ДЛЯ ПОТРЕБИТЕЛЕЙ:

– Набор реагентов предназначен для профессионального применения и должен использоваться обученным персоналом;

– При использовании набора образуются отходы классов А, Б и Г, которые классифицируются и уничтожаются *(утилизируются)* в соответствии с СанПиН 2.1.7.2790-10 «Санитарно-эпидемиологические требования к обращению с медицинскими отходами». Дезинфекцию наборов следует проводить по МУ-287-113 «Методические указания по дезинфекции, предстерилизационной очистке и стерилизации изделий медицинского назначения»;

– Требования безопасности к медицинским лабораториям приведены в ГОСТ Р 52905-2007;

– Не применять набор реагентов по назначению после окончания срока годности;

– Транспортирование должно проводиться всеми видами крытого транспорта в соответствии с правилами перевозок, действующими на транспорте данного вида.

– Производитель гарантирует соответствие выпускаемых изделий требованиям нормативной и технической документации;

– Безопасность и качество изделия гарантируются в течение всего срока годности;
– Производитель отвечает за недостатки изделия, за исключением дефектов, возникших вследствие нарушения правил пользования, условий транспортирования и хранения, либо действия третьих лиц, либо непреодолимой силы.
– Производитель обязуется за свой счёт заменить изделие, технические и функциональные характеристики *(потребительские свойства)* которого не соответствуют нормативной и технической документации, если указанные недостатки явились следствием скрытого дефекта материалов или некачественного изготовления изделия производителем.

ПРИЛОЖЕНИЕ

Набор **«Иерсиния – IgM – ИФА – БЕСТ»** рекомендуется для:
– диагностики иерсиниозов;
– наблюдения в процессе лечения и последующей диспансеризации;
– обследование контактных лиц.

Согласно литературным данным **антитела (АТ)** класса G к *Yersinia* встречаются у 10–40%

населения, АТ класса А – до 11%, АТ класса М – до 2%. Эти цифры отражают распространенность иерсиниозов среди взрослого населения.

Для правильной интерпретации результатов, разграничения острой, недавно перенесённой, давно перенесённой инфекции, прогнозирования исхода заболевания *(выздоровление или хронизация процесса)* и последующего выбора тактики лечения важно включать в исследование определение IgA, IgG и IgM.

После перенесённой инфекции IgG могут циркулировать в крови несколько месяцев или лет.

Иммуноглобулины классов А и М при благоприятном исходе иерсиниозов исчезают из сыворотки крови в течении 2–6 месяцев. Сохранение в крови IgA и/или IgM более 6-ти месяцев свидетельствует о хроническом течении заболевания и персистенции возбудителя.

Увеличение или уменьшение КП в динамике свидетельствует о нарастании или снижении, соответственно, титра АТ.

Достоверным считать изменение КП в 2 раза и более.

При динамическом наблюдении пациента для получения результатов, адекватно отражающих изменения концентрации

иммуноглобулинов класса G к иерсиниям в крови, необходимо использовать наборы реагентов одного наименования *(одного предприятия-изготовителя).*

Рекомендуется исследовать парные сыворотки с интервалом 3–4 недели.

При интерпретации результатов можно руководствоваться приведённой ниже таблицей:

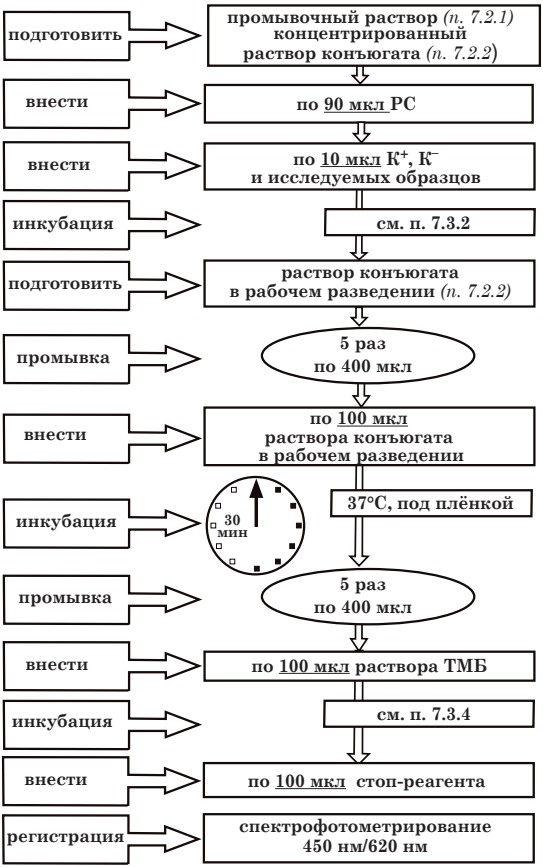
IgG	**IgM**	**IgA**	**Интерпретация**
+	—	—	перенесённая в прошлом инфекция
+	+, но КП снижается в течении 3–-6 мес.	+, но КП снижается в течении 3–6 мес.	недавно перенесённая инфекция с исходом в выздоровление
+ или —	— или +, но КП снижается в динамике	+ более 6 мес.	персистенция возбудителя. Артриты, узловатая эритема, миокардиты и др. осложнения инфекционно-аллергического характера
+ или —	+ более 6 мес.	+ или —	хронические илеиты, тифлиты, колиты и др.

При обследовании больного с подозрением на иерсиниозную инфекцию представляется

важным тесное сотрудничество между лечащим врачом и врачом-лаборантом, особенно если получены слабоположительные результаты при тестировании.

Окончательный диагноз формулируется на основании клинико-анамнестических данных, эпиданамнеза и результатов комплекса лабора торных исследований.

Схема анализа D-3206



ГРАФИЧЕСКИЕ СИМВОЛЫ

REF	Номер по каталогу	IVD	Медицинское изделие для диагностики *in vitro*
Σ n	Содержимого достаточно для проведения n количества тестов	NON STERILE	Не стерильно
LOT	Код партии		Температурный диапазон
	Дата изготовления: XXXX-XX-XX Формат даты: год-месяц-число		Изготовитель
	Использовать до: XXXX-XX-XX Формат даты: год-месяц-число		Обратитесь к Инструкции по применению
	Осторожно! Обратитесь к Инструкции по применению		

26.02.16

АКЦИОНЕРНОЕ ОБЩЕСТВО
«ВЕКТОР-БЕСТ»

Международный сертификат
ISO 13485

Наш адрес: 630117, Новосибирск-117, а/я 492

Тел.: (383) 332-37-58, 332-37-10, 332-36-34,
332-67-49, 332-67-52

Тел./факс: (383) 227-73-60 (многоканальный)

E-mail: vbmarket@vector-best.ru
Internet: www.vector-best.ru

NovaTec Immundiagnostica GmbH

NovaLisa®

Yersinia enterocolitica IgA

ELISA

CE

Only for in-vitro diagnostic use

Instructions for use

REF YERA0990 (96 Determinations)

ENGLISH

1. INTENDED USE

The Yersinia enterocolitica IgA ELISA is intended for the qualitative determination of IgA class antibodies against antigens of the 70 kb virulence plasmid of Yersinia enterocolitica in human serum or plasma (citrate, heparin). The ELISA is intended for use as an aid in identifying individuals with an adaptive immune response to Yersinia enterocolitica. The determination of increased antibody levels contributes to diagnosis of Yersinia-induced reactive arthritis. The test is not intended for diagnosing acute enteric diseases.

2. PRINCIPLE OF THE ASSAY

The qualitative immunoenzymatic determination of specific antibodies is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique.
Microtiterplates are coated with specific antigens to bind corresponding antibodies of the sample. After washing the wells to remove all unbound sample material a horseradish peroxidase (HRP) labelled conjugate is added. This conjugate binds to the captured antibodies. In a second washing step unbound conjugate is removed. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product.
The intensity of this product is proportional to the amount of specific antibodies in the sample. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450/620 nm is read using an ELISA Microtiterplate reader.

3. MATERIALS

3.1. Reagents supplied

- **Microtiterplate:** 12 break-apart 8-well snap-off strips coated with specific antigen; in resealable aluminium foil.
- **DIL:** 1 bottle containing 100 mL of phosphate buffer (10 mM) for sample dilution; pH 7.2 ± 0.2; coloured yellow; ready to use; white cap; ≤ 0.0015 % (v/v) CMIT/MIT (3:1).
- **SOLN STOP:** 1 bottle containing 15 mL sulphuric acid, 0.2 mol/L; ready to use; red cap.
- **WASH BUF 20x:** 1 bottle containing 50 mL of a 20-fold concentrated phosphate buffer (0.2 M), pH 7.2 ± 0.2, for washing the wells; white cap; 0.2 % (w/v) 5-Bromo-5-nitro-1,3-dioxane.
- **Conjugate:** 1 bottle containing 20 mL of peroxidase labelled antibody to human IgA in phosphate buffer (10 mM); coloured violet; ready to use; black cap.
- **SUB TMB:** 1 bottle containing 15 mL 3,3',5,5'-tetramethylbenzidine (TMB), < 0.1 %; ready to use; yellow cap.
- **Positive Control:** 1 vial containing 2 mL control; coloured yellow; ready to use; red cap; ≤ 0.02 % (v/v) MIT.
- **Cut-off Control:** 1 vial containing 3 mL control; coloured yellow; ready to use; green cap; ≤ 0.02 % (v/v) MIT.
- **Negative Control:** 1 vial containing 2 mL control; coloured yellow; ready to use; blue cap; ≤ 0.0015 % (v/v) CMIT/MIT (3:1).

For hazard and precautionary statements see 11.1

3.2. Materials supplied

- 1 Cover foil
- 1 Instruction for use (IFU)
- 1 Plate layout

3.3. Materials and Equipment needed

- ELISA Microtiterplate reader, equipped for the measurement of absorbance at 450/620 nm
- Incubator 37 °C
- Manual or automatic equipment for rinsing Microtiterplates
- Pipettes to deliver volumes between 10 and 1000 µL
- Vortex tube mixer
- Distilled water
- Disposable tubes

4. STABILITY AND STORAGE

Store the kit at 2...8 °C. The opened reagents are stable up to the expiry date stated on the label when stored at 2...8 °C.

5. REAGENT PREPARATION

It is very important to bring all reagents and samples to room temperature (20…25 °C) and mix them before starting the test run!

5.1. Microtiterplate

The break-apart snap-off strips are coated with specific antigen. Immediately after removal of the strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2...8 °C.

5.2. WASH | BUF | 20x

Dilute **WASH | BUF | 20x** 1 + 19; e. g. 10 mL **WASH | BUF | 20x** + 190 mL distilled water. The diluted buffer (**WASH | BUF | 1x**) is stable for 5 days at room temperature (20…25 °C). In case crystals appear in the concentrate, warm up the solution to 37 °C e.g., in a water bath. Mix well before dilution.

5.3. SUB | TMB

The reagent is ready to use and has to be stored at 2...8 °C, away from the light. The solution should be colourless or could have a slight blue tinge. If **SUB | TMB** turns into blue, it may have become contaminated and should be thrown away.

6. SAMPLE COLLECTION AND PREPARATION

Use human serum or plasma (citrate, heparin) samples with this assay. If the assay is performed within 5 days after sample collection, the samples should be kept at 2...8 °C; otherwise, they should be aliquoted and stored deep-frozen (-70…-20 °C). If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing.
Heat inactivation of samples is not recommended.

6.1. Sample Dilution

Before assaying, all samples should be diluted 1+100 with **DIL**. Dispense 10 µL sample and 1 mL **DIL** into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

7. ASSAY PROCEDURE

Please read the instruction for use carefully **before** performing the assay. Result reliability depends on strict adherence to the instruction for use as described. The following test procedure is only validated for manual procedure. If performing the test on ELISA automatic systems, we recommend increasing the washing steps from three up to five and the volume of **WASH | BUF | 1x** from 300 µL to 350 µL to avoid washing effects. Pay attention to chapter 11. Prior to commencing the assay, the distribution and identification plan for all samples and standards/controls (duplicates recommended) should be carefully established on the plate layout supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder.

Perform all assay steps in the order given and without any delays.

A clean, disposable tip should be used for dispensing each standard/control and sample.

Adjust the incubator to 37 ± 1 °C.

1. Dispense 100 µL standards/controls and diluted samples into their respective wells. Leave well A1 for the Substrate Blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 1 hour ± 5 min at 37 ± 1 °C.**
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µL of **WASH | BUF | 1x**. Avoid overflows from the reaction wells. The interval between washing and aspiration should be > 5 sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!

   Note: Washing is important! Insufficient washing results in poor precision and false results.
5. Dispense 100 µL Conjugate into all wells except for the Substrate Blank well A1.
6. **Incubate for 30 min at room temperature (20...25 °C).** Do not expose to direct sunlight.
7. Repeat step 4.
8. Dispense 100 µL **SUB | TMB** into all wells.
9. **Incubate for exactly 15 min at room temperature (20...25 °C) in the dark.** A blue colour occurs due to an enzymatic reaction.
10. Dispense 100 µL **SOLN | STOP** into all wells in the same order and at the same rate as for the **SUB | TMB**, thereby a colour change from blue to yellow occurs.
11. Measure the absorbance at 450/620 nm within 30 min after addition of the **SOLN | STOP**.

7.1. Measurement

Adjust the ELISA Microtiterplate reader **to zero** using the **Substrate Blank**.

If - due to technical reasons - the ELISA Microtiterplate reader cannot be adjusted to zero using the Substrate Blank, subtract its absorbance value from all other absorbance values measured in order to obtain reliable results!

Measure the absorbance of all wells at **450 nm** and record the absorbance values for each standard/control and sample in the plate layout.

Bichromatic measurement using a reference wavelength of 620 nm is recommended.

Where applicable calculate the mean absorbance values of all duplicates.

8. RESULTS

8.1. Run Validation Criteria

In order for an assay run to be considered valid, these Instructions for Use have to be strictly followed and the following criteria must be met:

- **Substrate Blank**: Absorbance value **< 0.100**
- **Negative Control**: Absorbance value **< 0.200 and < Cut-off**
- **Cut-off Control**: Absorbance value **0.150 – 1.300**
- **Positive Control**: Absorbance value **> Cut-off**

If these criteria are not met, the test is not valid and must be repeated.

8.2. Calculation of Results

The Cut-off is the mean absorbance value of the Cut-off Control determinations.

Example: Absorbance value Cut-off Control 0.44 + absorbance value Cut-off control 0.42 = 0.86 / 2 = 0.43
Cut-off = 0.43

8.2.1. Results in Units [NTU]

$$\frac{\text{Sample (mean) absorbance value} \times 10}{\text{Cut-off}} = \text{[NovaTec Units = NTU]}$$

Example: $\frac{1.591 \times 10}{0.43} = 37$ NTU (Units)

8.3. Interpretation of Results

Cut-off	10 NTU	-
Positive	> 11 NTU	Antibodies against the pathogen are present. There has been a contact with the antigen (pathogen resp. vaccine).
Equivocal	9 – 11 NTU	Antibodies against the pathogen could not be detected clearly. It is recommended to repeat the test with a fresh sample in 2 to 4 weeks.
Negative	< 9 NTU	The sample contains no antibodies against the pathogen. A previous contact with the antigen (pathogen resp. vaccine) is unlikely.
Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as serological data. In immunocompromised patients and newborns serological data only have restricted value.		

8.3.1. Antibody Isotypes and State of Infection

Serology	Significance
IgG	Characteristic of the secondary antibody response May persist for several years High IgG titer with low IgM titer: → may indicate a past infection
IgA	Produced in mucosal linings throughout the body (⇒ protective barrier) Usually produced early in the course of the infection

9. SPECIFIC PERFORMANCE CHARACTERISTICS

The results refer to the groups of samples investigated; these are not guaranteed specifications.

For further information about the specific performance characteristics please contact NovaTec Immundiagnostica GmbH.

9.1. Precision

Evaluation of precision of the assay was performed according to “CLSI. *Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline - Third Edition.* CLSI document EP05-A3. Wayne, PA: Clinical and Laboratory Standards Institute; 2014”.

9.1.1. Single-Site Study

The precision study was performed at a single site. A negative, a high negative, an equivocal, and a positive sample were run in 4 replicates, two times per day for 12 days for a total of 96 results.

Sample	n	Mean (NTU)	Repeatability		Between Run		Within Day		Between Day		Within lab	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
positive	96	25.03	1.3174	5.3	1.3537	5.4	1.8889	7.5	0.8140	3.3	2.0568	8.2
equivocal	96	10.01	0.7302	7.3	0.7926	7.9	1.0777	10.8	0.4165	4.2	1.1554	11.5
high negative	96	6.81	0.6429	9.4	0.0912	1.3	0.6494	9.5	0.3696	5.4	0.7472	11.0
negative	96	2.82	0.4468	15.8	0.1387	4.9	0.4678	16.6	0.2013	7.1	0.5093	18.0

9.1.2. Multisite-Study

The precision study was performed at three different sites. A negative, a high negative, an equivocal, and a moderate positive sample were run in 5 replicates, once a day for 5 days for a total of 75 results.

Sample	n	Mean (NTU)	Repeatability		Within Site		Reproducibility	
			SD	CV	SD	CV	SD	CV
moderate positive	75	23.10	1.0818	4.7	1.8064	7.8	2.0543	8.9
equivocal	75	9.98	0.6410	6.4	0.9572	9.6	1.3167	13.2
high negative	75	6.13	0.4330	7.1	0.6231	10.2	0.6688	10.9
negative	75	2.14	0.2894	13.5	0.4488	20.9	0.6611	30.8

9.2. Diagnostic Specificity

The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte. It is 97.32 % (95 % confidence interval: 92.37 % - 99.44 %).

9.3. Diagnostic Sensitivity

The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte. It is 93.94 % (95 % confidence interval: 79.77 % - 99.26 %).

9.4. Interferences

The assay was evaluated for interferences according to guideline EP07-A3 (“Interference Testing in Clinical Chemistry” from the Clinical and Laboratory Standards Institute). Three samples, covering the relevant measuring range, were spiked with high levels of interferents and were tested along with the unspiked sample. The following table shows the tested substances added to patient samples at the indicated concentrations. These correspond to the recommendations in the CLSI guideline to represent pathological elevated concentrations in patient samples.

Interferent	**Concentration tested**
Albumin	60 mg/mL
Bilirubin, unconjugated	0.4 mg/mL
Bilirubin, conjugated	0.4 mg/mL
Cholesterol	4 mg/mL
Hemoglobin	10 mg/mL
Triglycerides	15 mg/mL

No clinically significant interference effect was found for all tested substances.

9.5. Cross Reactivity

A minimum of 5 samples with antibody activities to potentially cross-reacting parameters (Adenovirus, Borrelia burgdorferi, Brucella, Campylobacter jejuni, Chlamydia pneumoniae, Enterovirus, Epstein-Barr virus, Helicobacter pylori, Parvovirus B19, Salmonella typhi) or samples positive for ANA or rheumatoid factors, and samples from pregnant women were tested to evaluate the cross reactivity of the assay. Positive findings were additionally analyzed with a CE-marked reference assay. The results are shown in the following table.

Pathogen/Condition	Samples tested	Number of positive samples	
		Yersinia enterocolitica IgA	CE-marked reference assay
Adenovirus	15	3	0
Antinuclear antibodies (ANA)	15	5	1
Borrelia burgdorferi	10	1	1
Brucella	10	6	5
Campylobacter jejuni	9	1	0
Chlamydia pneumoniae	12	4	2
Enterovirus	7	2	2
Epstein-Barr virus (EBV)	13	4	0
Helicobacter pylori	13	2	1
Pregnancy samples	13	2	0
Rheumatoid factor (RF)	11	3	0
Salmonella typhi	9	3	1

Cross-reactions with antibodies against Borrelia burgdorferi, Chlamydia pneumoniae, Epstein-Barr virus, Helicobacter pylori, Enterovirus, Adenovirus, Campylobacter jejuni, Salmonella typhi, Brucella as well as with samples from pregnant women or samples positive for rheumatoid factor (RF) or antinuclear antibodies (ANA) cannot be excluded.

References

1. Granfors K, Isomäki H, Essen R von, Maatela J, Kalliomäki JL, Toivanen A. 1983. Yersinia antibodies in inflammatory joint diseases. Clin Exp Rheumatol 1:215–218.

2. Strieder TGA, Wenzel BE, Prummel MF, Tijssen JGP, Wiersinga WM. 2003. Increased prevalence of antibodies to enteropathogenic Yersinia enterocolitica virulence proteins in relatives of patients with autoimmune thyroid disease. Clin Exp Immunol 132:278–282. doi:10.1046/j.1365-2249.2003.02139.x.

10. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or repeated freeze-thaw cycles of the sample may affect the absorbance values.

11. PRECAUTIONS AND WARNINGS

- The test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the testkits with analyzers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.
- Only for in-vitro diagnostic use.
- All materials of human or animal origin should be regarded and handled as potentially infectious.
- All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive.
- Do not interchange reagents or Microtiterplates of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- Use only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and standard/control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense reagents without splashing accurately into the wells.
- The ELISA is only designed for qualified personnel following the standards of good laboratory practice (GLP).
- For further internal quality control each laboratory should additionally use known samples.

11.1. Safety note for reagents containing hazardous substances

Reagents may contain CMIT/MIT (3:1) or MIT (refer to 3.1).
Therefore, the following hazard and precautionary statements apply.

Warning	H317	May cause an allergic skin reaction.
	P261	Avoid breathing spray.
	P280	Wear protective gloves/ protective clothing.
	P302+P352	IF ON SKIN: Wash with plenty of soap and water.
	P333+P313	If skin irritation or rash occurs: Get medical advice/ attention.
	P362+P364	Take off contaminated and Wash it before reuse.

Reagents may contain 5-Bromo-5-nitro-1,3-dioxane (refer to 3.1)
Therefore, the following hazard and precautionary statements apply.

Warning	H315	Causes skin irritation.
	H317	May cause an allergic skin reaction.
	P280	Wear protective gloves/ protective clothing.
	P302+P352	IF ON SKIN: Wash with plenty of soap and water.
	P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
	P337+P313	If eye irritation persists: Get medical advice/attention.

Further information can be found in the safety data sheet.

11.2. Disposal Considerations

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

12. ORDERING INFORMATION

REF	YERA0990	Yersinia enterocolitica IgA ELISA	(96 Determinations)

NovaTec Immundiagnostica GmbH

NovaLisa®

Taenia solium IgG

ELISA

CE

Only for in-vitro diagnostic use

Product Number: TAEG0420 (96 Determinations)

ENGLISH

1. INTRODUCTION

Taenia solium is a tapeworm of 2-7 m in length which resides in the small intestine of humans but also other animal species (monkeys, hamsters). The tapeworms produce proglottids (less than 1,000, and each with 50,000 eggs) which mature, become gravid, detach from the tapeworm, and migrate to the anus or are passed in the stool. The eggs contained in the gravid proglottids and passed with the faeces can survive for months to years in the environment. After ingestion of a suitable intermediate host (pigs and other animals, including humans) the eggs release the oncosphere, invade the intestinal wall and migrate to the striated muscles, into the brain, liver and other tissues of the host where they develop in cysticerci. In the human intestine, a cysticercus develops over 2 months into an adult tapeworm, which can survive for up to 25 years. The important parasitic infection caused by Taenia solium is cysticercosis which may involve the eye and the central nervous system. The swine tapeworm Taenia solium is worldwide in distribution. Prevalence is higher in poorer communities where humans live in close contact with pigs and eat undercooked pork, and is very rare in Muslim countries. The main symptom of Taeniasis (only mild) is often the passage (passive) of proglottids.

The most important feature of Taeniasis solium is the risk of development of Cysticercosis.

Species	Disease	Symptoms (e.g.)	Transmission route
Taenia solium	Taeniasis	Abdominal pain; Nausea; Weakness and fatigue; Weight loss; Flatulence (gases); Diarrhea or constipation; Appetite changes (too much hunger or loss of appetite)	Ingestion of undercooked pork meat containing cysticerci or ingestion of Taenia solium eggs via fecally contaminated food or water
	Cysticercosis (Neurocysticercosis)	Cysticerci in the brain may cause increased cranial pressure, convulsions and altered mental states	

Infection or presence of pathogen may be identified by:

- Histology
- Microscopy
- PCR
- Serology: e.g. ELISA

2. INTENDED USE

The Taenia solium IgG ELISA is intended for the qualitative determination of IgG class antibodies against Taenia solium in human serum or plasma (citrate, heparin).

3. PRINCIPLE OF THE ASSAY

The qualitative immunoenzymatic determination of specific antibodies is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique.
Microtiterplates are coated with specific antigens to bind corresponding antibodies of the sample. After washing the wells to remove all unbound sample material a horseradish peroxidase (HRP) labelled conjugate is added. This conjugate binds to the captured antibodies. In a second washing step unbound conjugate is removed. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product.
The intensity of this product is proportional to the amount of specific antibodies in the sample. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450/620 nm is read using an ELISA Microtiterplate reader.

4. MATERIALS

4.1. Reagents supplied

- **Microtiterplate:** 12 break-apart 8-well snap-off strips coated with Taenia solium antigens; in resealable aluminium foil.
- **IgG Sample Dilution Buffer:** 1 bottle containing 100 mL of phosphate buffer (10 mM) for sample dilution; pH 7.2 ± 0.2; coloured yellow; ready to use; white cap; ≤ 0.0015% (v/v) CMIT/ MIT (3:1).
- **Stop Solution:** 1 bottle containing 15 mL sulphuric acid, 0.2 mol/L; ready to use; red cap.
- **Washing Buffer (20x conc.):** 1 bottle containing 50 mL of a 20-fold concentrated phosphate buffer (0.2 M), pH 7.2 ± 0.2, for washing the wells; white cap.
- **Conjugate:** 1 bottle containing 20 mL of peroxidased Protein A in phosphate buffer (10 mM); coloured blue; ready to use; black cap.
- **TMB Substrate Solution:** 1 bottle containing 15 mL 3,3',5,5'-tetramethylbenzidine (TMB), < 0.1 %; ready to use; yellow cap.
- **Positive Control:** 1 vial containing 2 mL control; coloured yellow; ready to use; red cap; ≤ 0.02% (v/v) MIT.
- **Cut-off Control:** 1 vial containing 3 mL control; coloured yellow; ready to use; green cap; ≤ 0.02% (v/v) MIT.
- **Negative Control:** 1 vial containing 2 mL control; coloured yellow; ready to use; blue cap; ≤ 0.0015% (v/v) CMIT/ MIT (3:1).

For hazard and precautionary statements see 12.1
For potential hazardous substances please check the safety data sheet.

4.2. Materials supplied

- 1 Cover foil
- 1 Instruction for use (IFU)
- 1 Plate layout

4.3. Materials and Equipment needed

- ELISA Microtiterplate reader, equipped for the measurement of absorbance at 450/620 nm
- Incubator 37 °C
- Manual or automatic equipment for rinsing Microtiterplate
- Pipettes to deliver volumes between 10 and 1000 µL
- Vortex tube mixer
- Distilled water
- Disposable tubes

5. STABILITY AND STORAGE

Store the kit at 2...8 °C. The opened reagents are stable up to the expiry date stated on the label when stored at 2...8 °C.

6. REAGENT PREPARATION

It is very important to bring all reagents and samples to room temperature (20…25 °C) and mix them before starting the test run!

6.1. Microtiterplate

The break-apart snap-off strips are coated with Taenia solium antigens. Immediately after removal of the strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2...8 °C.

6.2. Washing Buffer (20x conc.)

Dilute Washing Buffer 1 + 19; e. g. 10 mL Washing Buffer + 190 mL distilled water. The diluted buffer is stable for 5 days at room temperature (20…25 °C). In case crystals appear in the concentrate, warm up the solution to 37°C e.g. in a water bath. Mix well before dilution.

6.3. TMB Substrate Solution

The reagent is ready to use and has to be stored at 2...8 °C, away from the light. The solution should be colourless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away.

7. SAMPLE COLLECTION AND PREPARATION

Use human serum or plasma (citrate, heparin) samples with this assay. If the assay is performed within 5 days after sample collection, the samples should be kept at 2...8 °C; otherwise they should be aliquoted and stored deep-frozen (-70…-20 °C). If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing.
Heat inactivation of samples is not recommended.

7.1. Sample Dilution

Before assaying, all samples should be diluted 1+100 with IgG Sample Dilution Buffer. Dispense 10 µL sample and 1 mL IgG Sample Dilution Buffer into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

8. ASSAY PROCEDURE

Please read the instruction for use carefully **before** performing the assay. Result reliability depends on strict adherence to the instruction for use as described. The following test procedure is only validated for manual procedure. If performing the test on ELISA automatic systems we recommend increasing the washing steps from three up to five and the volume of Washing Buffer from 300 µL to 350 µL to avoid washing effects. Pay attention to chapter 12. Prior to commencing the assay, the distribution and identification plan for all samples and standards/controls (duplicates recommended) should be carefully established on the plate layout supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder.

Perform all assay steps in the order given and without any delays.

A clean, disposable tip should be used for dispensing each standard/control and sample.

Adjust the incubator to 37 ± 1 °C.

1. Dispense 100 µL standards/controls and diluted samples into their respective wells. Leave well A1 for the Substrate Blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 1 hour ± 5 min at 37 ± 1 °C.**
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µL of Washing Buffer. Avoid overflows from the reaction wells. The interval between washing and aspiration should be > 5 sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!

   Note: Washing is important! Insufficient washing results in poor precision and false results.
5. Dispense 100 µL Protein A conjugate into all wells except for the Substrate Blank well A1.
6. **Incubate for 30 min at room temperature (20...25 °C).** Do not expose to direct sunlight.
7. Repeat step 4.
8. Dispense 100 µL TMB Substrate Solution into all wells.
9. **Incubate for exactly 15 min at room temperature (20...25 °C) in the dark.** A blue colour occurs due to an enzymatic reaction.
10. Dispense 100 µL Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution, thereby a colour change from blue to yellow occurs.
11. Measure the absorbance at 450/620 nm within 30 min after addition of the Stop Solution.

8.1. Measurement

Adjust the ELISA Microtiterplate reader **to zero** using the **Substrate Blank**.

If - due to technical reasons - the ELISA Microtiterplate reader cannot be adjusted to zero using the Substrate Blank, subtract its absorbance value from all other absorbance values measured in order to obtain reliable results!

Measure the absorbance of all wells at **450 nm** and record the absorbance values for each standard/control and sample in the plate layout.

Bichromatic measurement using a reference wavelength of 620 nm is recommended.

Where applicable calculate the mean absorbance values of all duplicates.

9. RESULTS

9.1. Run Validation Criteria

In order for an assay run to be considered valid, these Instructions for Use have to be strictly followed and the following criteria must be met:

- **Substrate Blank**: Absorbance value **< 0.100**
- **Negative Control**: Absorbance value **< 0.200 and < Cut-off**
- **Cut-off Control**: Absorbance value **0.150 – 1.300**
- **Positive Control**: Absorbance value **> Cut-off**

If these criteria are not met, the test is not valid and must be repeated.

9.2. Calculation of Results

The Cut-off is the mean absorbance value of the Cut-off Control determinations.

Example: Absorbance value Cut-off Control 0.44 + absorbance value Cut-off control 0.42 = 0.86 / 2 = 0.43
Cut-off = 0.43

9.2.1. Results in Units [NTU]

$$\frac{\text{Sample (mean) absorbance value} \times 10}{\text{Cut-off}} = \text{[NovaTec Units = NTU]}$$

Example: $\frac{1.591 \times 10}{0.43} = 37$ NTU

9.3. Interpretation of Results

Cut-off	10 NTU	-
Positive	> 11 NTU	Antibodies against the pathogen are present. There has been a contact with the antigen (pathogen resp. vaccine).
Equivocal	9 – 11 NTU	Antibodies against the pathogen could not be detected clearly. It is recommended to repeat the test with a fresh sample in 2 to 4 weeks. If the result is equivocal again the sample is judged as **negative**.
Negative	< 9 NTU	The sample contains no antibodies against the pathogen. A previous contact with the antigen (pathogen resp. vaccine) is unlikely.
Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as serological data. In immunocompromised patients and newborns serological data only have restricted value.		

10. SPECIFIC PERFORMANCE CHARACTERISTICS

The results refer to the groups of samples investigated; these are not guaranteed specifications.

For further information about the specific performance characteristics please contact NovaTec Immundiagnostica GmbH.

10.1. Precision

Intraassay	n	Mean (E)	CV (%)
#1	24	0.460	4.89
#2	24	0.782	6.40
#3	24	0.790	8.29

Interassay	n	Mean (NTU)	CV (%)
#1	12	17.20	5.23
#2	12	20.32	7.84
#3	12	3.70	13.87

10.2. Diagnostic Specificity

The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte.
It is 100% (95% confidence interval: 96.67% - 100%).

10.3. Diagnostic Sensitivity

The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte.
It is 100% (95% confidence interval: 78.2% - 100%).

10.4. Interferences

Interferences with hemolytic, lipemic or icteric samples are not observed up to a concentration of 10 mg/mL hemoglobin, 5 mg/mL triglycerides and 0.5 mg/mL bilirubin.

10.5. Cross Reactivity

Cross reaction of the antigens with antibodies against Echinococcus and Entamoeba is possible.

11. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or repeated freeze-thaw cycles of the sample may affect the absorbance values.

12. PRECAUTIONS AND WARNINGS

- The test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the testkits with analyzers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.
- Only for in-vitro diagnostic use.
- All materials of human or animal origin should be regarded and handled as potentially infectious.
- All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive.
- Do not interchange reagents or Microtiterplates of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- Use only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and standard/control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense reagents without splashing accurately into the wells.
- The ELISA is only designed for qualified personnel following the standards of good laboratory practice (GLP).
- For further internal quality control each laboratory should additionally use known samples.

12.1. Safety note for reagents containing hazardous substances

Reagents may contain CMIT/MIT (3:1) or MIT (refer to 4.1)
Therefore, the following hazard and precautionary statements apply.

Warning	H317	May cause an allergic skin reaction.
	P261	Avoid breathing spray.
	P280	Wear protective gloves/ protective clothing.
	P302+P352	IF ON SKIN: Wash with plenty of soap and water.
	P333+P313	If skin irritation or rash occurs: Get medical advice/ attention.
	P362+P364	Take off contaminated and Wash it before reuse.

Further information can be found in the safety data sheet.

12.2. Disposal Considerations

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

13. ORDERING INFORMATION

Prod. No.: TAEG0420 Taenia solium IgG ELISA (96 Determinations)

EC DECLARATION OF CONFORMITY

ZAO "Vector-Best" hereby ensures under own responsibility and declares that the products listed on pages 2-4 are in conformity with applicable provisions and fulfill the essential requirements of Annex I Directive 98/79/EC of 27 October 1998 regarding in vitro diagnostic medical devices.

Classification of products:	Other devices (all devices except Annex II and self-testing devices)
Conformity assessment procedure:	Annex III (not including section 6).
Manufacturer:	ZAO "Vector-Best" Address: AHC, Koltsovo, Novosibirsk Region, 630559, Russia, Tel. +7 (383) 363 20 60, Fax: +7 (383) 363 35 55
European authorized representative:	Bioron GmbH, Rheinhorststr. 18, D-67071 Ludwigshafen, Germany. tel.: +49 (0) 621 5720 915, fax: +49 (0) 621 5720 916

Date: 2013/04/12

Murat Khusainov
General Director ZAO «Vector-Best»

No.	Product name	Identification data	REF
1.	Vectohep A-IgM	ELISA kit for determination of IgM to hepatitis A virus	D-0352
2.	Vectohep A-IgG	ELISA kit for quantitative and qualitative determination of IgG to hepatitis A virus	D-0362
3.	Vectohep TTV-IgG	ELISA kit for determination of IgG to TT virus	D-0802
4.	Vectohep E-IgG	ELISA kit for determination of IgG to hepatitis E virus	D-1056
5.	Vectohep E-IgM	ELISA kit for determination of IgM to hepatitis E virus	D-1058
6.	Vectohep G-IgG	ELISA kit for determination of IgG to hepatitis G virus	D-1252
7.	LymeBest-IgG	ELISA kit for determination of IgG to infectious borreliosis agents	D-1452
8.	LymeBest-IgM	ELISA kit for determination of IgM to infectious borreliosis agents	D-1454
9.	RecombiBest antipallidum-IgG	ELISA kit for determination of IgG to Treponema pallidum	D-1852
10.	RecombiBest antipallidum-total antibodies	ELISA kit for determination of total antibodies to Treponema pallidum	D-1856
11.	RecombiBest antipallidum-IgM	ELISA kit for determination of IgM to Treponema pallidum	D-1858
12.	RecombiBest antipallidum-total antibodies	ELISA kit for determination of total antibodies to Treponema pallidum	D-1857
13.	VectoHSV-1,2 - IgG	ELISA kit for determination of IgG to herpes simplex virus types 1 and 2	D-2152
14.	VectoHSV - IgM	ELISA kit for determination of IgM to herpes simplex virus types 1 and 2	D-2154
15.	VectoHHV-8 - IgG	ELISA kit for determination of IgG to human herpes virus type 8	D-2160
16.	VectoHHV-6 - IgG	ELISA kit for determination of IgG to human herpes virus type 6	D-2166
17.	Ureaplasma urealyticum – IgG-EIA-BEST	ELISA kit for determination of IgG to Ureaplasma urealyticum antigens	D-2254
18.	Ureaplasma urealyticum – IgA-EIA-BEST	ELISA kit for determination of IgA to Ureaplasma urealyticum antigens	D-2258
19.	VectoParotitis-IgG	ELISA kit for determination of IgG to parotitis virus	D-2602
20.	VectoParotitis-IgM	ELISA kit for determination of IgM to parotitis virus	D-2604
21.	Toxocara-IgG-EIA-BEST	ELISA kit for determination of IgG to toxocara antigens	D-2752
22.	Opisthorchiasis – IgG-EIA-BEST	ELISA kit for determination of IgG to opisthorchiasis antigens	D-2952
23.	Echinococcus-IgG-EIA-BEST	ELISA kit for determination of IgG to Echinococcus	D-3356

		antigens	
24.	Ascarid-IgG-EIA-BEST	ELISA kit for determination of IgG to Ascaris lumbricoides	D-3452
25.	Lamblia-antibodies-EIA-BEST	ELISA kit for determination of IgG, IgM and IgA to Lamblia antibodies	D-3552
26.	Lamblia-IgM-EIA-BEST	ELISA kit for determination of IgM to Lamblia antibodies	D-3554
27.	Lamblia-antigen-EIA-BEST	ELISA kit for determination of Lamblia antigen	D-3556
28.	Helicobacter pylori-CagA-antigen-EIA-BEST	ELISA kit for determination of total antibodies to CagA Helicobacter pylori	D-3752
29.	TSH-EIA-BEST	ELISA kit for determination of concentration of thyroid-stimulating hormone	X-3952
30.	T3 total-EIA-BEST	ELISA kit for determination of concentration of total triiodothyronine	X-3954
31.	T4 total-EIA-BEST	ELISA kit for determination of concentration of total thyroxine	X-3956
32.	Anti-TPO-EIA-BEST	ELISA kit for determination of antibody concentration to thyroperoxidase	X-3968
33.	PAPP-A-EIA-BEST	ELISA kit for determination of concentration of pregnancy-associated plasma protein A	D-4160
34.	Mycoplasma hominis-IgG-EIA-BEST	ELISA kit for determination of IgG to Mycoplasma hominis	D-4352
35.	Mycoplasma hominis-IgA-EIA-BEST	ELISA kit for determination of IgA to Mycoplasma hominis	D-4358
36.	Mycoplasma pneumoniae-IgG-EIA-BEST	ELISA kit for determination of IgG to Mycoplasma pneumoniae	D-4362
37.	Mycoplasma pneumoniae-IgM-EIA-BEST	ELISA kit for determination of IgM to Mycoplasma pneumoniae	D-4366
38.	Vectocrimean – CHF – IgG	ELISA kit for determination of IgG to Crimean-Congo hemorrhagic fever virus	D-5052
39.	Vectocrimean – CHF – IgM	ELISA kit for determination of IgM to Crimean-Congo hemorrhagic fever virus	D-5054
40.	CEA-EIA-BEST	ELISA kit for determination of concentration of carcinoembryonic antigen	T-8454
41.	AFP-EIA-BEST	ELISA kit for determination of concentration of Alpha-Fetal Protein	T-8456
42.	CA-125-EIA-BEST	ELISA kit for determination of concentration of oncomarker CA-125	T-8466
43.	CA 19-9-EIA-BEST	ELISA kit for determination of concentration of CA 19-9	T-8470
44.	CA 15-3-EIA-BEST	ELISA kit for determination of concentration of oncomarker CA 15-3	T-8472
45.	NSE-EIA-BEST	ELISA kit for determination of concentration of neuron specific enolase	T-8476

46.	Ferritin-EIA-BEST	ELISA kit for determination of concentration of ferritin	T-8552
47.	IgE total-EIA-BEST	ELISA kit for determination of concentration of total IgE	A-8660
48.	IgG total-EIA-BEST	ELISA kit for determination of concentration of total IgG	A-8662
49.	IgM total-EIA-BEST	ELISA kit for determination of concentration of total IgM	A-8664
50.	IgA total-EIA-BEST	ELISA kit for determination of concentration of total IgA	A-8666
51.	Gamma-Interferon-EIA-BEST	ELISA kit for determination of concentration of gamma-interferon	A-8752
52.	Interleukine-4-EIA-BEST	ELISA kit for determination of concentration of Interleukine-4	A-8754
53.	Alpha-TNF-EIA-BEST	ELISA kit for determination of concentration of alpha-tumor necrosis factor	A-8756
54.	Alpha-Interferon-EIA-BEST	ELISA kit for determination of concentration of alpha-interferon	A-8758
55.	Interleukine-6-EIA-BEST	ELISA kit for determination of concentration of Interleukine-6	A-8768
56.	Interleukine-2-EIA-BEST	ELISA kit for determination of concentration of Interleukine-2	A-8772
57.	Procalcitonin-EIA-BEST	ELISA kit for determination of concentration of procalcitonin	A-9004
58.	NTproBNP-EIA-BEST	ELISA kit for determination of concentration of N-terminal prohormone of brain natriuretic peptide	A-9102
59.	Troponin I-EIA-BEST	ELISA kit for determination of concentration of troponin I	A-9106

Certificate

mdc medical device certification GmbH
certifies that

VECTOR BEST

AO Vector-Best
Research and Production Area
Building 36,Office 211, Koltsovo
630559 Novosibirsk region
Russian Federation

with the locations listed in the attachment

for the scope

Design and development, production and distribution of medical devices for in vitro diagnostics (PCR, ELISA, Biochemistry)

has introduced and applies a

Quality Management System

The mdc audit has proven that this quality management system meets all requirements of the following standard

EN ISO 13485

Medical devices – Quality management systems – Requirements for regulatory purposes

EN ISO 13485:2016 + AC:2016 - ISO 13485:2016

Valid from	2020-07-04
Valid until	2023-07-03
Registration no.	D1213100019
Report no.	P20-00568-173687
Stuttgart	2020-06-02

Head of Certification Body

medical device certification mdc

DAkkS Deutsche Akkreditierungsstelle D-ZM-16002-06-00

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Attachment of the certificate

No. D1213100019 date 2020-06-02

Location	Scope
AO Vector-Best Arbuzova str. 1/1, 630117 Novosibirsk Russian Federation	design and development, production and distribution of medical devices for in vitro diagnostics
AO Vector-Best Research and Production area, building 36, Koltsovo, 630559 Novosibirsk region Russian Federation	design and development, production of medical devices for in vitro diagnostics
AO Vector-Best Pasechnaya str, 3, 630117 Novosibirsk Russian Federation	design and development, production of medical devices for in vitro diagnostics

medical device certification mdc

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ВЕКТОР

БЕСТ

ВектоКрым-КГЛ-IgG

Набор реагентов
для иммуноферментного выявления
иммуноглобулинов класса G
к вирусу Крымской-Конго
геморрагической лихорадки

НАБОР РЕАГЕНТОВ
D-5052

ИНСТРУКЦИЯ ПО ПРИМЕНЕНИЮ

Утверждена 04.05.2010
Приказом Росздравнадзора № 3682-Пр/10

1. НАЗНАЧЕНИЕ

1.1. Набор предназначен для выявления иммуноглобулинов класса G к вирусу Крымской-Конго геморрагической лихорадки (К-КГЛ) в сыворотке (плазме) крови человека методом иммуноферментного анализа и может быть использован с целью дифференциальной диагностики К-КГЛ в клинических и эпидемиологических исследованиях.

1.2. Набор рассчитан на проведение 96 анализов, включая контроли. Для исследования небольшой партии проб возможны 12 независимых постановок ИФА по 8 анализов каждая, включая контрольные образцы.

2. ХАРАКТЕРИСТИКИ НАБОРА

2.1. Принцип метода

Метод определения основан на твердофазном иммуноферментном анализе.

В лунках полистироловых планшетов иммобилизованы поликлональные антитела к вирусу К-КГЛ, связанные с антигенами вируса К-КГЛ. При инкубации в лунках исследуемых образцов сывороток происходит связывание специфических антител к вирусу К-КГЛ с антигенами вируса К-КГЛ. Несвязавшийся материал отмывают, добавляют в лунки конъюгат, состоящий из моноклональных антител против IgG человека, конъюгированных с пероксидазой хрена, после инкубации которого лунки промывают и

вносят раствор тетраметилбензидина (хромоген) и перекись водорода (субстрат пероксидазы). В результате ферментативной реакции образуется окрашенный продукт, интенсивность окраски которого пропорциональна концентрации в лунках специфических антител к вирусу К-КГЛ.

Реакцию останавливают добавлением стоп-реагента. Результаты ИФА регистрируют с помощью спектрофотометра, измеряя оптическую плотность (ОП) в двухволновом режиме: основной фильтр – 450 нм, референс-фильтр – в диапазоне 620–650 нм. Допустима регистрация результатов только с фильтром 450 нм.

После измерения оптической плотности раствора в лунках на основании рассчитанного значения $ОП_{крит.}$ анализируемые образцы оцениваются как положительные или отрицательные.

2.2. Состав набора

В состав набора входят:

- планшет разборный с иммобилизованным антигеном вируса Крымской-Конго геморрагической лихорадки (К-КГЛ) – 1 шт.;
- положительный контрольный образец ($К^+$), инактивированный – на основе инактивированной сыворотки крови человека, содержащей IgG к вирусу К-КГЛ – прозрачная жидкость красного цвета – 1 флакон (1,5 мл);
- отрицательный контрольный образец ($К^-$), инактивированный – на основе инактивированной

сыворотки крови человека, не содержащей IgG к вирусу К-КГЛ – прозрачная бесцветная или бледно-желтого цвета жидкость – 1 флакон (3,0 мл);
- конъюгат – моноклональные антитела против IgG человека, меченные пероксидазой хрена, готовый для использования – прозрачная жидкость синего цвета – 1 флакон (13 мл);
- 25-кратный концентрат фосфатно-солевого буферного раствора с твином (ФСБ-Т×25) – прозрачная или слегка опалесцирующая бесцветная жидкость, возможно выпадение осадка солей, растворяющегося при нагревании до (30–40)°С – 2 флакона (по 28 мл);
- раствор для предварительного разведения сывороток (РПРС) – прозрачная жидкость малинового цвета – 1 флакон (10 мл);
- раствор для разведения сывороток (РРС) – прозрачная бесцветная или бледно-желтого цвета жидкость – 1 флакон (12 мл);
- раствор тетраметилбензидина (раствор ТМБ), готовый для использования – прозрачная бесцветная или светло-желтого цвета жидкость – 1 флакон (13 мл);
- стоп-реагент – прозрачная бесцветная жидкость – 1 флакон (12 мл).

Набор дополнительно комплектуется:
- планшетом для предварительного разведения исследуемых образцов – 1 шт.;
- пленкой для заклеивания планшета – 2 шт.;
- ванночками для реагентов – 2 шт.;
- наконечниками для пипетки на 4–200 мкл – 16 шт.

3. АНАЛИТИЧЕСКИЕ И ДИАГНОСТИЧЕСКИЕ ХАРАКТЕРИСТИКИ

3.1. Чувствительность набора, определенная по сывороткам стандартной панели предприятия, содержащим IgG к вирусу К-КГЛ (СПП 05-2-229), составляет 100 %.

3.2. Специфичность набора, определенная по сывороткам стандартной панели предприятия, не содержащим IgG к вирусу К-КГЛ (СПП 05-2-229), составляет 100 %.

4. МЕРЫ ПРЕДОСТОРОЖНОСТИ

Потенциальный риск применения набора – класс 2а (ГОСТ Р 51609-2000, приказ Минздравсоцразвития России №735 от 30 октября 2006 г.).

Все компоненты набора являются нетоксичными. Стоп-реагент (0,5 М раствор серной кислоты) обладает раздражающим действием. Избегать разбрызгивания и попадания на кожу и слизистые. В случае попадания стоп-реагента на кожу и слизистые необходимо промыть пораженный участок большим количеством проточной воды.

Дробное использование набора может быть реализовано в течение всего срока годности набора.

Для получения надежных результатов необходимо строгое соблюдение инструкции по применению набора.

При постановке ИФА нельзя использовать компоненты из наборов разных серий или смешивать их при приготовлении растворов, кро-

ме неспецифических компонентов (ФСБ-Т×25, РПРС, стоп-реагент), которые взаимозаменяемы во всех наборах АО «Вектор-Бест».

5. ОБОРУДОВАНИЕ И МАТЕРИАЛЫ, НЕОБХОДИМЫЕ ПРИ РАБОТЕ С НАБОРОМ:

- спектрофотометр, позволяющий проводить измерения оптической плотности растворов в лунках планшета при длине волны 450 нм и/или в двухволновом режиме при основной длине волны 450 нм и длине волны сравнения в диапазоне 620–650 нм;
- термостат, поддерживающий температуру 37°С;
- холодильник бытовой, поддерживающий температуру от 2 до 8°С;
- пипетки полуавтоматические одноканальные с переменным или фиксированным объемом со сменными наконечниками, позволяющие отбирать объемы жидкости от 5 до 1000 мкл;
- пипетка полуавтоматическая многоканальная со сменными наконечниками, позволяющая отбирать объемы жидкостей от 5 мкл до 350 мкл;
- промывочное устройство для планшета;
- перчатки резиновые хирургические;
- бумага фильтровальная лабораторная;
- цилиндр мерный 2-го класса точности вместимостью 1000 мл;
- вода дистиллированная.

6. АНАЛИЗИРУЕМЫЕ ОБРАЗЦЫ

Допускается использование образцов, хранившихся при температуре от 2 до 8°С не более

5 суток, а также хранившихся при минус 20°С не более 3 мес.

Образцы, содержащие взвешенные частицы, могут дать неправильный результат. Такие образцы перед использованием следует центрифугировать 5 мин при 5000–10000 об/мин при температуре от 18 до 25°С .

Нельзя использовать проросшие, гиперлипидные образцы или подвергавшиеся многократному замораживанию и оттаиванию. После размораживания образцы тщательно перемешать.

7. ПРОВЕДЕНИЕ ИММУНОФЕРМЕНТНОГО АНАЛИЗА

7.1. Внимание! *Тщательное соблюдение описанных ниже требований позволит избежать искажения результатов ИФА.*

Для приготовления растворов и проведения ИФА следует использовать чистую мерную посуду и автоматические пипетки с погрешностью измерения объемов не более 5%.

Сразу после постановки реакции неиспользованный планшет и плотно закрытые флаконы с исходными компонентами необходимо поместить в холодильник, поддерживающий температуру от 2 до 8°С.

При промывке лунки (*стрипа, планшета*) заполнять полностью, не допуская переливания промывочного раствора через края лунок, и не касаясь лунок наконечником пипетки. Время между заполнением и опорожнением лунок должно быть не менее 30 с.

При использовании автоматического или ручного промывателя необходимо следить за состоянием емкости для промывочного раствора и соединительных шлангов: в них не должно быть заметного роста микроорганизмов. Раз в неделю емкость для промывочного раствора и шланги следует промывать 70% спиртом.

Не допускать высыхания лунок стрипов между отдельными операциями.

При приготовлении растворов и проведении ИФА следует использовать **одноразовые** наконечники для дозаторов.

В случае повторного использования посуду (*ванночки*) для раствора конъюгата промыть проточной водой и тщательно ополоснуть дистиллированной водой; посуду (*ванночки*) для раствора ТМБ сразу после работы промыть 50% раствором этилового спирта, а затем дистиллированной водой.

Для дезинфекции посуды и материалов, контактирующих с исследуемыми и контрольными образцами, рекомендуем использовать дезинфицирующие средства, не оказывающие негативного воздействия на качество ИФА, не содержащие активный кислород и хлор, например, комбинированные средства на основе четвертичных аммониевых соединений, спиртов, третичных аминов.

Пипетки и рабочие поверхности обрабатывать только 70% раствором этилового спирта. Не использовать во время проведения ИФА перекись водорода, хлорамин и т.д.

7.2. Приготовление реагентов

Перед работой извлечь набор из холодильника, вскрыть упаковку и выдержать все компоненты при температуре от 18 до 25°С в течение 60 мин.

7.2.1. Подготовка планшета

Непосредственно перед использованием вскрыть пакет с планшетом со стороны замка, отступив примерно 1 см. Оставить на рамке необходимое для проведения анализа количество стрипов; остальные стрипы снять с рамки и немедленно поместить вновь в пакет, удалить из него воздух и плотно закрыть замок.

Неиспользованные стрипы после первого вскрытия пакета можно хранить при температуре от 2 до 8°С в течение всего срока годности набора.

7.2.2. Приготовление контрольных образцов ($К^+$, $К^-$)

Контрольные образцы готовы к использованию и не требуют дополнительного разведения.

7.2.3. Подготовка исследуемых образцов

Исследуемые сыворотки развести в 10 раз раствором для предварительного разведения сывороток. Для этого, используя планшет для предварительного разведения образцов, к 90 мкл РПРС добавить 10 мкл цельной сыворотки, тщательно перемешать. При этом цвет раствора дол-

жен измениться с малинового на желтый. Если изменения не произошло, сыворотка для анализа не годится. При разведении плазмы цвет раствора в лунке меняется незначительно.

Приготовленные 10-ти кратно разведенные сыворотки можно хранить до 3 часов при температуре от 18 до 25°С.

7.2.4. Приготовление промывочного раствора

В соответствии с числом стрипов (см. таблицу расхода реагентов) в мерный цилиндр вместимостью 1000 мл внести необходимое количество концентрата ФСБ-Т×25 и довести до необходимого объема дистиллированной водой, тщательно перемешать до полного растворения. При выпадении осадка солей в концентрате необходимо прогреть его при температуре (30–40)°С до полного растворения осадка.

Приготовленный промывочный раствор можно хранить при температуре от 2 до 8°С до 5 суток .

7.2.5. Подготовка конъюгата

Конъюгат готов к использованию. В соответствии с числом используемых стрипов (см. таблицу расхода компонентов) отобрать в чистый флакон или в пластиковую ванночку для реагента необходимое количество конъюгата.

Остатки конъюгата из флакона или ванночки утилизировать ***(не сливать во флакон с исходным конъюгатом).***

7.2.6. Подготовка раствора тетраметилбензидина

Раствор ТМБ готов к использованию. Исключить воздействие прямого света на раствор тетраметилбензидина. Непосредственно перед внесением и в соответствии с числом используемых стрипов (см. таблицу расхода компонентов) отобрать необходимое количество раствора ТМБ в чистый флакон или в пластиковую ванночку для реагента.

Утилизировать раствор тетраметилбензидина, оставшийся в ванночке или флаконе после проведения ИФА ***(не сливать во флакон с исходным раствором).***

Внимание! *Для работы с раствором ТМБ необходимо использовать только одноразовые наконечники. Посуду, предназначенную для растявора ТМБ, нельзя отмывать с применением синтетических моющих средств, поскольку даже их следы ведут к неконтролируемому окислению ТМБ в ходе реакции. После работы посуду ополоснуть водой, промыть 70% этиловым спиртом и тщательно отмыть дистиллированной водой.*

7.2.7. Подготовка раствора для разведения сывороток

Перед использованием тщательно взболтать содержимое флакона с раствором для разведения сывороток.

Таблица расхода компонентов набора

Кол-во используемых стрипов	Конъюгат, мл	Раствор ТМБ, мл	Промывочный раствор	
			ФСБ-Т, концентрат, мл	Дистил. вода, мл
1	1,0	1,0	2,0	до 50
2	2,0	2,0	4,0	до 100
3	3,0	3,0	6,0	до 150
4	4,0	4,0	8,0	до 200
5	5,0	5,0	10,0	до 250
6	6,0	6,0	12,0	до 300
7	7,0	7,0	14,0	до 350
8	8,0	8,0	16,0	до 400
9	9,0	9,0	18,0	до 450
10	10,0	10,0	20,0	до 500
11	11,0	11,0	22,0	до 550
12	12,0	12,0	24,0	до 600

7.3. Проведение анализа

Внимание! *Внесение контрольных и исследуемых образцов проводить достаточно быстро, в течение 5–7 мин, так как при длительном времени внесения образцов в лунки планшета время инкубации первого и последнего образцов значительно отличаются, что может привести к неправильной оценке результатов.*

7.3.1. Внести в две лунки, например, А-1 и В-1, по 100 мкл отрицательного контрольного образца ($К^-$) и в одну лунку, например, С-1, – 100 мкл положительного контрольного образца ($К^+$).

В остальные лунки внести по 90 мкл раствора для разведения сывороток и по 10 мкл предварительно разведенных исследуемых образцов (п. 7.2.3), тщательно перемешать. Таким образом, исследуемый образец в лунке разбавляется в 100 раз.

7.3.2. Планшет заклеить пленкой и инкубировать 1 ч при температуре (37±1)°С.

7.3.3. По окончании инкубации снять липкую пленку и поместить ее в сосуд с дезинфицирующим раствором. С помощью промывочного устройства промыть лунки планшета 5 раз промывочным раствором (п. 7.2.4), чередуя аспирацию и немедленное заполнение лунок каждого стрипа. В каждую лунку вносить не менее 400 мкл жидкости в процессе каждого цикла промывки. Время между заполнением и опорожнением лунок должно быть не менее 30 сек. *Необходимо добиваться полного опорожнения лунок после каждого их заполнения.* По окончании промывки остатки влаги из лунок тщательно удалить, постукивая перевернутым планшетом по фильтровальной бумаге.

7.3.4. В лунки планшета внести по 100 мкл конъюгата (п. 7.2.5).

Для внесения раствора конъюгата использовать ванночку для реагентов и одноразовые наконечники, входящие в комплектацию.

Планшет заклеить пленкой и инкубировать 30 мин при температуре (37±1)°С.

7.3.5. По окончании инкубации содержимое лунок собрать в сосуд с дезинфицирующим раствором и промыть 5 раз промывочным раствором так, как указано в п. 7.3.3.

7.3.6. Внести во все лунки по 100 мкл раствора тетраметилбензидина (см. п. 7.2.6.).

Для внесения раствора тетраметилбензидина использовать пластиковую ванночку и одноразовые наконечники, входящие в состав набора.

Планшет выдержать в защищенном от света месте в течение 25 мин при температуре от 18 до 25°С.

7.3.7. Внести во все лунки с той же скоростью и в той же последовательности, как и раствор тетраметилбензидина, по 100 мкл стоп-реагента.

В случае попадания на кожу раствора тетраметилбензидина или стоп-реагента необходимо немедленно смыть их водой с мылом.

8. РЕГИСТРАЦИЯ РЕЗУЛЬТАТОВ

Результаты ИФА регистрировать с помощью спектрофотометра, измеряя оптическую плотность (ОП) в двухволновом режиме: основной фильтр – 450 нм, референс-фильтр в диапазоне 620–650 нм. Допускается регистрация результатов только с фильтром 450 нм (выведение спектрофотометра на нулевой уровень (бланк) осуществлять по воздуху).

Время между остановкой реакции и измерением оптической плотности не должно превышать 5 мин.

9. УЧЕТ РЕЗУЛЬТАТОВ АНАЛИЗА

9.1. Рассчитать среднее арифметическое значение оптической плотности в лунках с отрицательным контрольным образцом.

На основании полученных данных вычислить критическое значение оптической плотности ($ОП_{крит.}$) по формуле:

$$ОП_{крит.} = ОП_{ср.}\ (К^{-}) + 0{,}2$$

где: $ОП_{ср.}\ (К^{-})$ – среднее арифметическое значение оптической плотности в лунках с отрицательным контрольным образцом.

9.2. Среднее значение оптической плотности в лунках с отрицательным контрольным образцом $ОП_{ср.}\ (К^{-})$ не должно превышать 0,25 ед. опт. плотн.

Значение оптической плотности в лунке с положительным контрольным образцом должно быть не менее 1,0 ед. опт. плотн.

9.3. Результат анализа считают **положительным**, если $ОП_{обр.} \geq ОП_{крит.}$;

Результат анализа считают **сомнительным**, если $0{,}8 < ОП_{обр.} < ОП_{крит.}$;

Результат анализа считают **отрицательным**, если $ОП_{обр.} \leq 0{,}8 \times ОП_{крит.}$;

где $ОП_{обр.}$ – оптическая плотность в лунке с исследуемым образцом.

Если $ОП_{обр.} \geq ОП_{крит.}$, то результат анализа исследуемого образца считают положительным. Присутствие IgG к вирусу К-КГЛ отражает наличие текущей или перенесенной ранее инфекции.

Если $ОП_{обр.}$ попадает в интервал от $0{,}8 \times ОП_{крит.}$ до $ОП_{крит.}$, то результат анализа сомнительный. Рекомендуется повторить анализ такой сыворотки. При повторном сомнительном результате необходимо проанализировать сыворотку, полученную через 10–15 дней, параллельно с 1-м образцом сыворотки, чтобы проследить увеличение концентрации IgG.

Если $ОП_{обр.} \leq 0{,}8 \times ОП_{крит.}$, то результат анализа считают отрицательным, IgG к вирусу К-КГЛ не определены. Но это не означает, что пациент не инфицирован вирусом К-КГЛ. Если кровь взята у больного в начале острой фазы заболевания, IgG в сыворотке крови могут отсутствовать, поэтому при подозрении на наличие инфекции (клинические проявления) рекомендуется исследовать сыворотку на наличие IgM и провести анализ на наличие IgG и IgM сыворотки, взятой у пациента через 10–15 дней.

Если при анализе парных сывороток образец, взятый в острую фазу, негативный, а образец, взятый в фазу реконвалесценции, позитивный, имеет место сероконверсия, что свидетельствует о первичной инфекции.

При анализе парных сывороток для контроля изменения концентрации IgG или нали-

чия сероконверсии оба образца должны быть тестированы в дубликате одномоментно во время одной постановки.

ОПРЕДЕЛЕНИЕ ТИТРА АНТИТЕЛ В ВЫЯВЛЕННЫХ ПОЛОЖИТЕЛЬНЫХ ОБРАЗЦАХ

Если необходимо определить титр антител в выявленных положительных образцах, непосредственно перед основной реакцией проводят титрование исследуемых сывороток следующим образом:

В лунки А-1 – В-1 внести по 100 мкл $К^{-}$, в лунку С-1 внести 100 мкл $К^{+}$. В остальные лунки верхнего ряда А-2 – А-12 внести по 180 мкл раствора для разведения сывороток и по 20 мкл предварительно разведенных исследуемых образцов, перемешать пипетированием. Во все оставшиеся лунки внести по 100 мкл раствора для разведения сывороток.

Многоканальной пипеткой перенести по 100 мкл разведенных образцов сыворотки (плазмы) из лунок верхнего ряда в лунки второго ряда, перемешать (контрольные образцы не титровать). Из лунок второго ряда – в лунки третьего ряда, перемешать. Так же последовательно перенести до последнего ряда. Из последнего ряда удалить по 100 мкл содержимого лунок. Таким образом, в вертикальных рядах получаются последовательные 2-кратные разведения исследуемых образцов.

Планшет закрыть и инкубировать при температуре (37±1)°С в течение 60 мин. Дальнейший ход анализа аналогичен выше описанному.

Результаты анализа оценивают аналогично вышеописанному. Титром считают последнее разведение исследуемой сыворотки, при котором ОП в соответствующей лунке на 0,2 единицы превышает $ОП_{ср.}$ К$^{-}$.

10. УСЛОВИЯ ХРАНЕНИЯ И ПРИМЕНЕНИЯ НАБОРА

10.1. Транспортирование набора – при температуре от 2 до 8°С. Допускается транспортирование при температуре до 25°С не более 10 сут. Замораживание компонентов набора не допускается.

10.2. Хранение набора в упаковке предприятия-изготовителя – при температуре от 2 до 8°С. Замораживание компонентов набора не допускается.

10.3. Срок годности – 12 мес. Набор с истекшим сроком годности применению не подлежит.

По вопросам, касающимся качества набора «ВектоКрым-КГЛ-IgG»,
следует обращаться в АО «Вектор-БЕСТ»
по адресу:
630559, Новосибирская область,
Новосибирский район,
п. Кольцово, а/я 121,
тел. (383) 363-13-46,
E-mail: vbobtk@vector-best.ru

ДОПОЛНИТЕЛЬНАЯ ИНФОРМАЦИЯ ДЛЯ ПОТРЕБИТЕЛЕЙ

Набор предназначен для профессионального применения в клинической лабораторной диагностике обученным персоналом.

Требования безопасности к медицинским лабораториям приведены в ГОСТ Р 52905-2007.

Все реагенты наборов, содержащие в своем составе материалы человеческого происхождения, инактивированы.

Антитела к ВИЧ-1,2 и вирусу гепатита С и HBsAg отсутствуют.

Транспортирование должно проводиться всеми видами крытого транспорта в соответствии с правилами перевозок, действующими на транспорте данного вида.

При динамическом наблюдении пациента для получения результатов, адекватно отражающих изменение концентрации IgG к вирусу К-КГЛ в крови, необходимо использовать наборы реагентов одного наименования (одного предприятия-изготовителя).

1. Гарантийные обязательства

Производитель гарантирует соответствие выпускаемых изделий требованиям нормативной и технической документации.

Безопасность и качество изделия гарантируются в течение всего срока годности.

Производитель отвечает за недостатки изделия, за исключением дефектов, возникших

вследствие нарушения правил пользования, условий транспортирования и хранения, либо действия третьих лиц, либо непреодолимой силы.

Производитель обязуется за свой счет заменить изделие, технические и функциональные характеристики (потребительские свойства) которого не соответствуют нормативной и технической документации, если указанные недостатки явились следствием скрытого дефекта материалов или некачественного изготовления изделия производителем.

2. Обеспечение безопасности персонала

Обращение с материалами, контактирующими с исследуемыми образцами

Материалы, контактирующие с исследуемыми образцами, следует дезинфицировать МУ-287-113 «Методические указания по дезинфекции, предстерилизационной очистке и стерилизации изделий медицинского назначения» (утв. департаментом госсанэпиднадзора Минздрава РФ от 30.12.1998).

Порядок утилизации или уничтожения компонентов набора

При использовании набора образуются отходы классов А, Б и Г, которые классифицируются и уничтожаются (утилизируются) в соответствии с СанПиНом 2.1.7.2790-10 «Санитарно-эпидемиологические требования к обращению с медицинскими отходами». Дезинфекцию наборов

реагентов следует проводить по МУ 287-113 «Методические указания по дезинфекции, предстерилизационной очистке и стерилизации изделий медицинского назначения».

3. Обеспечение получения правильных результатов анализа

Достоверность и воспроизводимость результатов анализа зависят от выполнения следующих основных правил:

– не проводите ИФА в присутствии паров кислот, щелочей, альдегидов или пыли, которые могут менять ферментативную активность конъюгатов;

– ферментативная реакция чувствительна к присутствию ионов металлов, поэтому не допускайте контактов каких-либо металлических предметов с конъюгатом и раствором ТМБ;

– избегайте загрязнения компонентов набора микроорганизмами и химическими примесями, для этого используйте в работе чистую посуду и чистые одноразовые наконечники для каждого реагента, контроля, образца;

– никогда не используйте одну и ту же емкость для конъюгата и раствора ТМБ;

– перед отбором раствора ТМБ из флакона необходимо протереть конус пипетки (внутреннюю и внешнюю поверхности) сначала дистиллированной водой, а затем 70% этиловым спиртом, так как малейшее загрязнение пипе-

ток конъюгатом может привести к контаминации всего содержимого флакона;

– если допущена ошибка при внесении анализируемых образцов, нельзя, опорожнив эту лунку, вносить в нее новый образец; такая лунка бракуется.

Качество промывки лунок планшета играет важную роль для получения правильных результатов анализа:

– Для аспирации анализируемых образцов и последующей промывки рекомендуется использовать автоматическое или ручное промывочное устройство.

– Для предотвращения засорения игл промывочного устройства в конце рабочего дня обязательно выполните процедуру ополаскивания системы подачи жидкости дистиллированной водой.

Ложноположительные результаты могут быть обусловлены:

а) получением неправильного рабочего разведения исследуемых сывороток (например, 1:70; 1:50);

б) контаминацией отрицательных сывороток в рабочем и вспомогательных планшетах положительными сыворотками крови из соседних лунок.

Для получения правильного рабочего разведения исследуемых сывороток необходимо:

а) при отборе 10 мкл сыворотки для предварительного разведения не погружать наконечник

глубоко в сыворотку, чтобы исключить налипание сыворотки на внешнюю поверхность наконечника;

б) тщательно перемешивать сыворотку при предварительном разведении 1:10.

Для исключения взаимной контаминации сывороток во вспомогательном планшете для предварительного разведения сывороток и в рабочем планшете необходима тщательная и аккуратная работа, без разбрызгивания растворов.

4. Оценка анализа по коэффициенту позитивности

Результаты анализа можно оценить по коэффициенту позитивности (КП), рассчитывая отношение ОП в лунке с образцом пациента относительно $ОП_{крит.}$ (п. 9.1).

В случае необходимости оценки результатов по коэффициенту позитивности провести повторное измерение ОП в режиме: основной фильтр – **405 нм**, референс-фильтр – в диапазоне 620–655 нм. Допускается измерение без использования референс-фильтра.

Для расчета коэффициента позитивности образцов, имеющих $\mathbf{ОП_{450} \leq 3{,}5}$ **о.е.**, использовать формулу:

$$КП_{обр.} = \frac{ОП_{450\ обр.}}{ОП_{крит.}},$$

где $\mathbf{ОП_{450\ обр.}}$ – ОП образца, полученная в двухволновом режиме **450/620–655 нм** (или только с фильтром **450 нм**).

Для расчета коэффициента позитивности образцов, имеющих $\mathbf{ОП_{450} > 3{,}5}$ **о.е.**, использовать формулу:

$$\mathbf{КП_{обр.}} = 3{,}2 \times \frac{\mathbf{ОП_{405\ обр.}}}{\mathbf{ОП_{крит.}}},$$

где $\mathbf{ОП_{405\ обр.}}$ – ОП образца, полученная в двухволновом режиме **405/620–655 нм** (или только с фильтром **405 нм**).

Результат анализа **положительный**, если $КП_{обр.} \geq 1$, где $КП_{обр.}$ – коэффициент позитивности исследуемого образца.

Результат анализа **отрицательный**, если $КП_{обр.} \leq 0{,}8$.

Результат анализа **сомнительный**, если соответствующее ему значение $КП_{обр.}$ попадает в интервал от 0,8 до 1,0.

Расчет КП целесообразно проводить для оценки концентрации специфических антител класса G в исследуемых образцах и при наблюдении за изменением концентрации IgG к вирусу К-КГЛ в динамике в парных образцах сывороток.

5. Краткая схема проведения ИФА для набора реагентов «ВектоКрым-КГЛ-IgG»

Использовать только после тщательного ознакомления с инструкцией!

Внести:	по 100 мкл $К^+$, $К^-$; по 90 мкл РРС и по 10 мкл предварительно разведенных анализируемых образцов.
Инкубировать:	60 мин, 37°С.
Промыть:	промывочным раствором, 400 мкл, 5 раз.
Внести:	по 100 мкл конъюгата.
Инкубировать:	30 мин, 37°С.
Промыть:	промывочным раствором, 400 мкл, 5 раз.
Внести:	по 100 мкл раствора тетраметилбензидина.
Инкубировать:	25 мин, 18–25°С, в темноте.
Внести:	по 100 мкл стоп-реагента.
Измерить:	ОП при 450 нм / референсная длина волны 620–655 нм.

6. Графические символы

REF	Номер по каталогу	IVD	Медицинское изделие для диагностики *in vitro*
Σ n	Содержимого достаточно для проведения n-количества тестов	NON STERILE	Не стерильно
LOT	Код партии		Температурный диапазон
	Изготовитель		Дата изготовления
	Использовать до …		Обратитесь к инструкции по применению
	Осторожно! Обратитесь к Инструкции по применению	YYYY-MM-DD YYYY-MM	Дата в формате Год-Месяц-День Год-Месяц

Консультацию специалиста по работе с набором можно получить по тел.: (383) 363-57-95.

29.02.16.

АКЦИОНЕРНОЕ ОБЩЕСТВО
«ВЕКТОР-БЕСТ»

Международный сертификат ISO 13485

Наш адрес: 630117, Новосибирск-117, а/я 492
Тел./факс: (383) 227-73-60 (многоканальный)
Тел.: (383) 332-37-10, 332-37-58, 332-36-34,
332-67-49, 332-67-52
E-mail: vbmarket@vector-best.ru
Internet: www.vector-best.ru

ВЕКТОР

БЕСТ

ВектоКрым-КГЛ-IgM

Набор реагентов
для иммуноферментного выявления
иммуноглобулинов класса М
к вирусу Крымской-Конго
геморрагической лихорадки

ИНСТРУКЦИЯ ПО ПРИМЕНЕНИЮ

Утверждена 04.05.2010
Приказом Росздравнадзора № 3680-Пр/10

НАБОР РЕАГЕНТОВ
D-5054

1. НАЗНАЧЕНИЕ

1.1. Набор предназначен для выявления иммуноглобулинов класса М к вирусу Крымской-Конго геморрагической лихорадки (К-КГЛ) в сыворотке (плазме) крови человека методом «захвата» иммуноферментного анализа.

1.2. Набор рассчитан на проведение 96 анализов, включая контроли. Возможны 12 независимых постановок по 8 анализов каждая, включая контроли.

2. ХАРАКТЕРИСТИКИ НАБОРА

2.1. Принцип метода

Метод определения основан на твердофазном иммуноферментном анализе.

В лунках полистироловых планшетов иммобилизованы моноклональные антитела к IgM человека. Во время первой инкубации, при внесении в лунки планшета исследуемого образца происходит связывание присутствующих в нем иммуноглобулинов класса М с иммобилизованными на внутренней поверхности лунок моноклональными антителами к IgM человека. После удаления промыванием несвязавшихся компонентов сыворотки, в лунки планшета вносят смесь конъюгата и антигена вируса К-КГЛ. Во время второй инкубации, связавшиеся специфические IgM взаимодействуют с антигеном вируса К-КГЛ, находящемся в комплексе с пероксидазным конъюгатом.

После удаления несвязавшихся компонентов реакции, во время инкубации с раствором тетраметилбензидина (хромоген) и перекисью водорода (субстрат пероксидазы), происходит окрашивание раствора в лунках. Степень окраски пропорциональна концентрации IgM к вирусу К-КГЛ в анализируемых образцах.

Реакцию останавливают добавлением стоп-реагента. Результаты ИФА регистрируют с помощью спектрофотометра, измеряя оптическую плотность (ОП) в двухволновом режиме: основной фильтр – 450 нм, референс-фильтр – в диапазоне 620–650 нм. Допустима регистрация результатов только с фильтром 450 нм.

После измерения оптической плотности раствора в лунках на основании рассчитанного значения $ОП_{крит.}$ анализируемые образцы оцениваются как положительные или отрицательные.

2.2. Состав набора

В состав набора входят:

- планшет разборный с иммобилизованными моноклональными антителами к IgM человека – 1 шт.;
- положительный контрольный образец ($К^+$), инактивированный – на основе инактивированной сыворотки крови человека, содержащей IgM к вирусу К-КГЛ – прозрачная красного цвета жидкость – 1 флакон (1,5 мл);
- отрицательный контрольный образец ($К^-$), инактивированный – на основе инактивированной сыво-

ротки крови человека, не содержащей IgM к вирусу К-КГЛ – прозрачная бесцветная или бледно-желтого цвета жидкость – 1 флакон (3,0 мл);
- конъюгат, смесь антигена вируса К-КГЛ и поликлональных мышиных антител к вирусу К-КГЛ с пероксидазой хрена – жидкость синего цвета – 1 флакон (13 мл);
- 25-кратный концентрат фосфатно-солевого буферного раствора с твином (ФСБ-Т×25) – прозрачная или слегка опалесцирующая бесцветная жидкость, возможно выпадение осадка солей, растворяющегося при нагревании до (30–40)°С – 2 флакона (по 28 мл);
- раствор для предварительного разведения сывороток (РПРС) – прозрачная жидкость малинового цвета – 1 флакон (10 мл);
- раствор для разведения сывороток (РРС) – прозрачная бесцветная или бледно-желтого цвета жидкость – 1 флакон (12 мл);
- раствор тетраметилбензидина (раствор ТМБ), готовый для использования – прозрачная бесцветная или светло-желтого цвета жидкость – 1 флакон (13 мл);
- стоп-реагент – прозрачная бесцветная жидкость – 1 флакон (12 мл).

Набор дополнительно комплектуется:
- планшетом для предварительного разведения исследуемых образцов – 1 шт.;
- пленкой для заклеивания планшета – 2 шт.;
- ванночками для реагентов – 2 шт.;
- наконечниками для пипетки на 4–200 мкл – 16 шт.

3. АНАЛИТИЧЕСКИЕ И ДИАГНОСТИЧЕСКИЕ ХАРАКТЕРИСТИКИ

3.1. Чувствительность набора, определенная по сывороткам стандартной панели предприятия, содержащим IgM к вирусу К-КГЛ (СПП 05-2-230), составляет 100%.

3.2. Специфичность набора, определенная по сывороткам стандартной панели предприятия, не содержащим IgM к вирусу К-КГЛ (СПП 05-2-230), составляет 100%.

4. МЕРЫ ПРЕДОСТОРОЖНОСТИ

Потенциальный риск применения набора – класс 2а (ГОСТ Р 51609-2000, приказ Минздравсоцразвития России №735 от 30 октября 2006 г.).

Все компоненты набора являются нетоксичными. Стоп-реагент (0,5 М раствор серной кислоты) обладает раздражающим действием. Избегать разбрызгивания и попадания на кожу и слизистые. В случае попадания стоп-реагента на кожу и слизистые необходимо промыть пораженный участок большим количеством проточной воды.

Дробное использование набора может быть реализовано в течение всего срока годности набора.

Для получения надежных результатов необходимо строгое соблюдение инструкции по применению набора.

При постановке ИФА нельзя использовать компоненты из наборов разных серий или смешивать их при приготовлении растворов, кро-

ме неспецифических компонентов (ФСБ-Т×25, РПРС, стоп-реагент), которые взаимозаменяемы во всех наборах АО «Вектор-Бест».

5. ОБОРУДОВАНИЕ И МАТЕРИАЛЫ, НЕОБХОДИМЫЕ ПРИ РАБОТЕ С НАБОРОМ:

- спектрофотометр, позволяющий проводить измерения оптической плотности растворов в лунках планшета при длине волны 450 нм и/или в двухволновом режиме при основной длине волны 450 нм и длине волны сравнения в диапазоне 620–650 нм;
- термостат, поддерживающий температуру 37°С;
- холодильник бытовой, поддерживающий температуру от 2 до 8°С;
- пипетки полуавтоматические одноканальные с переменным или фиксированным объемом со сменными наконечниками, позволяющие отбирать объемы жидкости от 5 до 1000 мкл;
- пипетка полуавтоматическая многоканальная со сменными наконечниками, позволяющая отбирать объемы жидкостей от 5 мкл до 350 мкл;
- промывочное устройство для планшета;
- перчатки резиновые хирургические;
- бумага фильтровальная лабораторная;
- цилиндр мерный 2-го класса точности вместимостью 1000 мл;
- вода дистиллированная.

6. АНАЛИЗИРУЕМЫЕ ОБРАЗЦЫ

Допускается использование образцов, хранившихся при температуре от 2 до 8°С не более

5 суток, а также хранившихся при минус 20°С не более 3 мес.

Образцы, содержащие взвешенные частицы, могут дать неправильный результат. Такие образцы перед использованием следует центрифугировать 5 мин при 5000–10000 об/мин при температуре от 18 до 25°С .

Нельзя использовать проросшие, гиперлипидные образцы или подвергавшиеся многократному замораживанию и оттаиванию. После размораживания образцы тщательно перемешать.

7. ПРОВЕДЕНИЕ ИММУНОФЕРМЕНТНОГО АНАЛИЗА

7.1. Внимание! *Тщательное соблюдение описанных ниже требований позволит избежать искажения результатов ИФА.*

– Для приготовления растворов и проведения ИФА следует использовать чистую мерную посуду и автоматические пипетки с погрешностью измерения объемов не более 5%.

– Сразу после постановки реакции неиспользованный планшет и плотно закрытые флаконы с исходными компонентами необходимо поместить в холодильник, поддерживающий температуру от 2 до 8°С.

– При промывке лунки *(стрипа, планшета)* заполнять полностью, не допуская переливания промывочного раствора через края лунок, и не касаясь лунок наконечником пипетки. Время между заполнением и опорожнением лунок должно быть не менее 30 с.

– При использовании автоматического или ручного промывателя необходимо следить за состоянием емкости для промывочного раствора и соединительных шлангов: в них не должно быть заметного роста микроорганизмов. Раз в неделю емкость для промывочного раствора и шланги следует промывать 70% спиртом.

– Не допускать высыхания лунок стрипов между отдельными операциями.

– При приготовлении растворов и проведении ИФА следует использовать одноразовые наконечники для дозаторов.

– В случае повторного использования посуду *(ванночки)* для раствора конъюгата промыть проточной водой и тщательно ополоснуть дистиллированной водой; посуду *(ванночки)* для раствора ТМБ сразу после работы промыть 50% раствором этилового спирта, а затем дистиллированной водой.

– Для дезинфекции посуды и материалов, контактирующих с исследуемыми и контрольными образцами, рекомендуем использовать дезинфицирующие средства, не оказывающие негативного воздействия на качество ИФА, не содержащие активный кислород и хлор, например, комбинированные средства на основе четвертичных аммониевых соединений, спиртов, третичных аминов.

– Пипетки и рабочие поверхности обрабатывать только 70% раствором этилового спирта. Не использовать во время проведения ИФА перекись водорода, хлорамин и т.д.

7.2. Приготовление реагентов

Перед работой извлечь набор из холодильника, вскрыть упаковку и выдержать все компоненты при температуре от 18 до 25°С в течение 60 мин.

7.2.1. Подготовка планшета

Непосредственно перед использованием вскрыть пакет с планшетом со стороны замка, отступив примерно 1 см. Оставить на рамке необходимое для проведения анализа количество стрипов; остальные стрипы снять с рамки и немедленно поместить вновь в пакет, удалить из него воздух и плотно закрыть замок.

Неиспользованные стрипы после первого вскрытия пакета можно хранить при температуре от 2 до 8°С в течение всего срока годности набора.

7.2.2. Приготовление контрольных образцов ($К^+$, $К^-$)

Контрольные образцы готовы к использованию и не требуют дополнительного разведения.

7.2.3. Подготовка исследуемых образцов

Исследуемые сыворотки развести в 10 раз раствором для предварительного разведения сывороток. Для этого, используя планшет для предварительного разведения образцов, к 90 мкл РПРС добавить 10 мкл цельной сыворотки, тщательно перемешать. При этом цвет раствора дол-

жен измениться с малинового на желтый. Если изменения не произошло, сыворотка для анализа не годится. При разведении плазмы цвет раствора в лунке меняется незначительно.

Приготовленные 10-ти кратно разведенные сыворотки можно хранить до 3 часов при температуре от 18 до 25°С.

7.2.4. Приготовление промывочного раствора

В соответствии с числом стрипов (см. таблицу расхода реагентов) в мерный цилиндр вместимостью 1000 мл внести необходимое количество концентрата ФСБ-Т×25 и довести до необходимого объема дистиллированной водой, тщательно перемешать до полного растворения. При выпадении осадка солей в концентрате необходимо прогреть его при температуре (30–40)°С до полного растворения осадка.

Приготовленный промывочный раствор можно хранить при температуре от 2 до 8°С до 5 суток .

7.2.5. Подготовка конъюгата

Конъюгат готов к использованию. В соответствии с числом используемых стрипов (см. таблицу расхода компонентов) отобрать в чистый флакон или в пластиковую ванночку для реагента необходимое количество конъюгата.

Остатки конъюгата из флакона или ванночки утилизировать ***(не сливать во флакон с исходным конъюгатом).***

7.2.6. Подготовка раствора тетраметилбензидина

Раствор ТМБ готов к использованию. Исключить воздействие прямого света на раствор тетраметилбензидина. Непосредственно перед внесением и в соответствии с числом используемых стрипов (см. таблицу расхода компонентов) отобрать необходимое количество раствора ТМБ в чистый флакон или в пластиковую ванночку для реагента.

Утилизировать раствор тетраметилбензидина, оставшийся в ванночке или флаконе после проведения ИФА ***(не сливать во флакон с исходным раствором).***

Внимание! *Для работы с раствором ТМБ необходимо использовать только одноразовые наконечники. Посуду, предназначенную для раствора ТМБ, нельзя отмывать с применением синтетических моющих средств, поскольку даже их следы ведут к неконтролируемому окислению ТМБ в ходе реакции. После работы посуду ополоснуть водой, промыть 70% этиловым спиртом и тщательно отмыть дистиллированной водой.*

7.2.7. Подготовка раствора для разведения сывороток

Перед использованием тщательно взболтать содержимое флакона с раствором для разведения сывороток.

Таблица расхода компонентов набора

Кол-во используемых стрипов	Конъюгат, мл	Раствор ТМБ, мл	Промывочный раствор	
			ФСБ-Т, концентрат, мл	Дистил. вода, мл
1	1,0	1,0	2,0	до 50
2	2,0	2,0	4,0	до 100
3	3,0	3,0	6,0	до 150
4	4,0	4,0	8,0	до 200
5	5,0	5,0	10,0	до 250
6	6,0	6,0	12,0	до 300
7	7,0	7,0	14,0	до 350
8	8,0	8,0	16,0	до 400
9	9,0	9,0	18,0	до 450
10	10,0	10,0	20,0	до 500
11	11,0	11,0	22,0	до 550
12	12,0	12,0	24,0	до 600

7.3. Проведение анализа

Внимание! *Внесение контрольных и исследуемых образцов проводить достаточно быстро, в течение 5–7 мин, так как при длительном времени внесения образцов в лунки планшета время инкубации первого и последнего образцов значительно отличаются, что может привести к неправильной оценке результатов.*

7.3.1. Внести в две лунки, например, А-1 и В-1, по 100 мкл отрицательного контрольного образца ($К^-$) и в одну лунку, например, С-1, – 100 мкл положительного контрольного образца ($К^+$).

В остальные лунки внести по 90 мкл раствора для разведения сывороток и по 10 мкл предварительно разведенных исследуемых образцов (п. 7.2.3), тщательно перемешать. Таким образом, исследуемый образец в лунке разбавляется в 100 раз.

7.3.2. Планшет заклеить пленкой и инкубировать 1 ч при температуре (37±1)°С.

7.3.3. По окончании инкубации снять липкую пленку и поместить ее в сосуд с дезинфицирующим раствором. С помощью промывочного устройства промыть лунки планшета 5 раз промывочным раствором (п. 7.2.4), чередуя аспирацию и немедленное заполнение лунок каждого стрипа. В каждую лунку вносить не менее 400 мкл жидкости в процессе каждого цикла промывки. Время между заполнением и опорожнением лунок должно быть не менее 30 сек. *Необходимо добиваться полного опорожнения лунок после каждого их заполнения.* По окончании промывки остатки влаги из лунок тщательно удалить, постукивая перевернутым планшетом по фильтровальной бумаге.

7.3.4. В лунки планшета внести по 100 мкл конъюгата (п. 7.2.5).

Для внесения конъюгата использовать пластиковую ванночку и одноразовые наконечники, входящие в состав набора.

Планшет заклеить пленкой и инкубировать 90 мин при температуре (37±1)°С.

7.3.5. По окончании инкубации содержимое лунок собрать в сосуд с дезинфицирующим раствором и промыть 5 раз промывочным раствором так, как указано в п. 7.3.3.

7.3.6. Внести во все лунки по 100 мкл раствора тетраметилбензидина (см. п. 7.2.6.).

Для внесения раствора тетраметилбензидина использовать пластиковую ванночку и одноразовые наконечники, входящие в состав набора.

Планшет выдержать в защищенном от света месте в течение 25 мин при температуре от 18 до 25°С.

7.3.7. Внести во все лунки с той же скоростью и в той же последовательности, как и раствор тетраметилбензидина, по 100 мкл стоп-реагента.

В случае попадания на кожу раствора тетраметилбензидина или стоп-реагента необходимо немедленно смыть их водой с мылом.

8. РЕГИСТРАЦИЯ РЕЗУЛЬТАТОВ

Результаты ИФА регистрировать с помощью спектрофотометра, измеряя оптическую плотность (ОП) в двухволновом режиме: основной фильтр – 450 нм, референс-фильтр в диапазоне 620–650 нм. Допускается регистрация

результатов только с фильтром 450 нм (выведение спектрофотометра на нулевой уровень (бланк) осуществлять по воздуху).

Время между остановкой реакции и измерением оптической плотности не должно превышать 5 мин.

9. УЧЕТ РЕЗУЛЬТАТОВ АНАЛИЗА

9.1. Рассчитать среднее арифметическое значение оптической плотности в лунках с отрицательным контрольным образцом.

На основании полученных данных вычислить критическое значение оптической плотности (ОПкрит.) по формуле:

$$\mathbf{ОП_{крит.} = ОП_{ср.}\ (К^{-}) + 0{,}2,}$$

где: $ОП_{ср.}$ $(К^{-})$ — среднее арифметическое значение оптической плотности в лунках с отрицательным контрольным образцом.

9.2. Среднее значение оптической плотности в лунках с отрицательным контрольным образцом $ОП_{ср.}$ $(К^{-})$ не должно превышать 0,25 ед. опт. плотн.

Значение оптической плотности в лунке с положительным контрольным образцом должно быть не менее 1,0 ед. опт. плотн.

9.3. Результат анализа считают **положительным**, если $ОП_{обр.} \geq ОП_{крит.}$;

Результат анализа считают **сомнительным**, если $0{,}8 \times ОП_{крит.} < ОП_{обр.} < ОП_{крит.}$;

Результат анализа считают **отрицательным**, если $ОП_{обр.} \leq 0{,}8 \times ОП_{крит.}$;

где $ОП_{обр.}$ – оптическая плотность в лунке с исследуемым образцом.

Если $ОП_{обр.} \geq ОП_{крит.}$, то результат анализа исследуемого образца считают положительным, причем необходимо проведение повторного анализа таких сывороток для исключения ложноположительных результатов, обусловленных случайными, несистемными ошибками при постановке анализа.

Если $ОП_{обр.}$ попадает в интервал от $0{,}8 \times ОП_{крит.}$ до $ОП_{крит.}$, то результат анализа сомнительный. Рекомендуется повторить анализ такой сыворотки. При повторном сомнительном результате необходимо проанализировать сыворотку, полученную через 3–5 дней, параллельно с 1-м образцом сыворотки, на наличие IgM и IgG для выявления сероконверсии и подтверждения факта инфицирования вирусом К-КГЛ.

Если $ОП_{обр.} \leq 0{,}8 \times ОП_{крит.}$, то результат анализа считают отрицательным, IgM к вирусу К-КГЛ не определены. Но это не означает, что пациент не инфицирован вирусом К-КГЛ. Если кровь взята у больного в начале острой фазы заболевания, IgM в сыворотке крови могут отсутствовать, поэтому при подозрении на наличие инфекции (клинические проявления) рекомендуется исследовать сыворотку, взятую у пациента через 3–5 дней, на наличие IgM повторно.

10. УСЛОВИЯ ХРАНЕНИЯ И ПРИМЕНЕНИЯ НАБОРА

10.1. Транспортирование набора – при температуре от 2 до 8°С. Допускается транспортирование при температуре до 25°С не более 10 сут. Замораживание компонентов набора не допускается.

10.2. Хранение набора в упаковке предприятия-изготовителя – при температуре от 2 до 8°С. Замораживание компонентов набора не допускается.

10.3. Срок годности – 12 мес. Набор с истекшим сроком годности применению не подлежит.

По вопросам, касающимся качества набора «ВектоКрым-КГЛ-IgM»,

следует обращаться в АО «Вектор-БЕСТ»

по адресу:

630559, Новосибирская область,
Новосибирский район,
п. Кольцово, а/я 121,
тел. (383) 363-13-46,
E-mail: vbobtk@vector-best.ru

ДОПОЛНИТЕЛЬНАЯ ИНФОРМАЦИЯ ДЛЯ ПОТРЕБИТЕЛЕЙ

Набор предназначен для профессионального применения в клинической лабораторной диагностике обученным персоналом.

Требования безопасности к медицинским лабораториям приведены в ГОСТ Р 52905-2007.

Все реагенты наборов, содержащие в своем составе материалы человеческого происхождения, инактивированы.

Антитела к ВИЧ-1,2 и вирусу гепатита С и HBsAg отсутствуют.

Транспортирование должно проводиться всеми видами крытого транспорта в соответствии с правилами перевозок, действующими на транспорте данного вида.

При динамическом наблюдении пациента для получения результатов, адекватно отражающих изменение концентрации IgM к вирусу К-КГЛ в крови, необходимо использовать наборы реагентов одного наименования (одного предприятия-изготовителя).

1. Гарантийные обязательства

Производитель гарантирует соответствие выпускаемых изделий требованиям нормативной и технической документации.

Безопасность и качество изделия гарантируются в течение всего срока годности.

Производитель отвечает за недостатки изделия, за исключением дефектов, возникших

вследствие нарушения правил пользования, условий транспортирования и хранения, либо действия третьих лиц, либо непреодолимой силы.

Производитель обязуется за свой счет заменить изделие, технические и функциональные характеристики (потребительские свойства) которого не соответствуют нормативной и технической документации, если указанные недостатки явились следствием скрытого дефекта материалов или некачественного изготовления изделия производителем.

2. Обеспечение безопасности персонала

Обращение с материалами, контактирующими с исследуемыми образцами

Материалы, контактирующие с исследуемыми образцами, следует дезинфицировать МУ-287-113 «Методические указания по дезинфекции, предстерилизационной очистке и стерилизации изделий медицинского назначения» (утв. департаментом госсанэпиднадзора Минздрава РФ от 30.12.1998).

Порядок утилизации или уничтожения компонентов набора

При использовании набора образуются отходы классов А, Б и Г, которые классифицируются и уничтожаются (утилизируются) в соответствии с СанПиНом 2.1.7.2790-10 «Санитарно-эпидемиологические требования к обращению с ме-

дицинскими отходами». Дезинфекцию наборов реагентов следует проводить по МУ 287-113 «Методические указания по дезинфекции, предстерилизационной очистке и стерилизации изделий медицинского назначения».

3. Обеспечение получения правильных результатов анализа

Достоверность и воспроизводимость результатов анализа зависят от выполнения следующих основных правил:

– не проводите ИФА в присутствии паров кислот, щелочей, альдегидов или пыли, которые могут менять ферментативную активность конъюгатов;

– ферментативная реакция чувствительна к присутствию ионов металлов, поэтому не допускайте контактов каких-либо металлических предметов с конъюгатом и раствором ТМБ;

– избегайте загрязнения компонентов набора микроорганизмами и химическими примесями, для этого используйте в работе чистую посуду и чистые одноразовые наконечники для каждого реагента, контроля, образца;

– никогда не используйте одну и ту же емкость для конъюгата и раствора ТМБ;

– перед отбором раствора ТМБ из флакона необходимо протереть конус пипетки (внутреннюю и внешнюю поверхности) сначала дистиллированной водой, а затем 70% этиловым

спиртом, так как малейшее загрязнение пипеток конъюгатом может привести к контаминации всего содержимого флакона;

– если допущена ошибка при внесении анализируемых образцов, нельзя, опорожнив эту лунку, вносить в нее новый образец; такая лунка бракуется.

Качество промывки лунок планшета играет важную роль для получения правильных результатов анализа:

– Для аспирации анализируемых образцов и последующей промывки рекомендуется использовать автоматическое или ручное промывочное устройство.

– Для предотвращения засорения игл промывочного устройства в конце рабочего дня обязательно выполните процедуру ополаскивания системы подачи жидкости дистиллированной водой.

Ложноположительные результаты могут быть обусловлены:

а) получением неправильного рабочего разведения исследуемых сывороток (например, 1:70; 1:50);

б) контаминацией отрицательных сывороток в рабочем и вспомогательных планшетах положительными сыворотками крови из соседних лунок.

Для получения правильного рабочего разведения исследуемых сывороток необходимо:

а) при отборе 10 мкл сыворотки для предварительного разведения не погружать нако-

нечник глубоко в сыворотку, чтобы исключить налипание сыворотки на внешнюю поверхность наконечника;

б) тщательно перемешивать сыворотку при предварительном разведении 1:10.

Для исключения взаимной контаминации сывороток во вспомогательном планшете для предварительного разведения сывороток и в рабочем планшете необходима тщательная и аккуратная работа, без разбрызгивания растворов.

4. Оценка анализа по коэффициенту позитивности

Результаты анализа можно оценить по коэффициенту позитивности (КП), рассчитывая отношение ОП в лунке с образцом пациента относительно $ОП_{крит.}$ (п. 9.1).

Для расчета коэффициента позитивности образцов использовать формулу:

$$КП_{обр.} = \frac{ОП_{обр.}}{ОП_{крит.}}$$

Результат анализа **положительный**, если $КП_{обр.} \geq 1$, где $КП_{обр.}$ – коэффициент позитивности исследуемого образца.

Результат анализа **отрицательный**, если $КП_{обр.} \leq 0{,}8$.

Результат анализа **сомнительный**, если соответствующее ему значение $КП_{обр.}$ попадает в интервал от 0,8 до 1,0.

Расчет КП целесообразно проводить для оценки концентрации специфических антител класса М в исследуемых образцах и при наблюдении за изменением концентрации IgM к вирусу К-КГЛ в динамике в парных образцах сывороток.

5. Краткая схема проведения ИФА для набора реагентов «ВектоКрым-КГЛ-IgM»

Использовать только после тщательного ознакомления с инструкцией!

Внести:	по 100 мкл $К^+$, $К^-$; по 90 мкл РРС и по 10 мкл предварительно разведенных анализируемых образцов.
Инкубировать:	60 мин, 37°С.
Промыть:	промывочным раствором, 400 мкл, 5 раз.
Внести:	по 100 мкл конъюгата.
Инкубировать:	90 мин, 37°С.
Промыть:	промывочным раствором, 400 мкл, 5 раз.
Внести:	по 100 мкл раствора тетраметилбензидина.
Инкубировать:	25 мин, 18–25°С, в темноте.
Внести:	по 100 мкл стоп-реагента.
Измерить:	ОП при 450 нм / референсная длина волны 620–655 нм.

6. Графические символы

REF	Номер по каталогу	IVD	Медицинское изделие для диагностики *in vitro*
Σ n	Содержимого достаточно для проведения n-количества тестов	NON STERILE	Не стерильно
LOT	Код партии		Температурный диапазон
	Изготовитель		Дата изготовления
	Использовать до …		Обратитесь к инструкции по применению
	Осторожно! Обратитесь к Инструкции по применению	YYYY-MM-DD YYYY-MM	Дата в формате Год-Месяц-День Год-Месяц

Консультацию специалиста по работе с набором можно получить по тел.: (383) 363-57-95.

29.02.16.

ВЕКТОР

БЕСТ

ВектоКрым-КГЛ-антиген

Набор реагентов
для иммуноферментного выявления
антигена вируса Крымской-Конго
геморрагической лихорадки

ИНСТРУКЦИЯ ПО ПРИМЕНЕНИЮ

Утверждена 04.05.2010
Приказом Росздравнадзора № 3681-Пр/10

НАБОР РЕАГЕНТОВ
D-5056

1. НАЗНАЧЕНИЕ

1.1. Набор предназначен для выявления вируса Крымской-Конго геморрагической лихорадки в клещах и других вируссодержащих материалах (культуральной жидкости, секционном материале) методом иммуноферментного анализа и может быть использован в клинических и эпидемиологических исследованиях.

1.2. Набор реагентов не предназначен для исследования сыворотки и плазмы крови человека и животных.

1.3. Набор рассчитан на проведение 96 анализов, включая контроли. Для исследования небольшой партии проб возможны 12 независимых постановок ИФА по 8 анализов каждая, включая контрольные образцы.

2. ХАРАКТЕРИСТИКИ НАБОРА

2.1. Принцип метода

Метод определения основан на твердофазном иммуноферментном анализе.

Принцип метода заключается во взаимодействии антигена вируса К-КГЛ из исследуемого материала с антителами, иммобилизованными в лунках полистиролового планшета. Связавшийся антиген выявляют с помощью анти-К-КГЛ-антител, меченных пероксидазой хрена (конъюгат). По завершении постановки теста развивается окрашивание, свидетельствующее о присутствии антигена вируса К-КГЛ в образце (хромогеном

является тетраметилбензидин). Степень окраски пропорциональна концентрации антигена вируса К-КГЛ в анализируемых образцах.

Реакцию останавливают добавлением стоп-реагента. Результаты ИФА регистрируют с помощью спектрофотометра, измеряя оптическую плотность (ОП) в двухволновом режиме: основной фильтр – 450 нм, референс-фильтр – в диапазоне 620–650 нм. Допустима регистрация результатов только с фильтром 450 нм.

После измерения оптической плотности раствора в лунках на основании рассчитанного значения $ОП_{крит.}$ анализируемые образцы оцениваются как положительные или отрицательные.

2.2. Состав набора

В состав набора входят:

- планшет разборный с иммобилизованными поликлональными антителами к вирусу Крымской-Конго геморрагической лихорадки (К-КГЛ) – 1 шт.;
- положительный контрольный образец ($К^+$) – буферный раствор, содержащий инактивированный антиген вируса К-КГЛ – прозрачная красного цвета жидкость – 1 фл., 1,5 мл;
- отрицательный контрольный образец ($К^-$) – буферный раствор, не содержащий антиген вируса К-КГЛ – прозрачная бесцветная или бледно-желтого цвета жидкость – 1 фл., 3,0 мл;
- конъюгат – поликлональные антитела к К-КГЛ, меченные пероксидазой хрена, готовый для использования – жидкость синего цвета – 1 фл., 13 мл;

– 25-кратный концентрат фосфатно-солевого буферного раствора с твином (ФСБ-Т×25) – прозрачная или слегка опалесцирующая бесцветная жидкость, возможно выпадение осадка солей, растворяющегося при нагревании до (30–40)°С – 1 фл., 28 мл;
– раствор для разведения образцов (РРО) – прозрачная бесцветная или бледно-желтого цвета жидкость – 2 фл. по 25 мл;
– раствор тетраметилбензидина (раствор ТМБ), готовый для использования – прозрачная бесцветная или светло-желтого цвета жидкость – 1 фл., 13 мл;
– стоп-реагент – прозрачная бесцветная жидкость – 1 фл., 12 мл.

Набор дополнительно комплектуется:

– пленкой для заклеивания планшета – 2 шт.;
– ванночками для реагентов – 2 шт.;
– наконечниками для пипетки на 4–200 мкл – 16 шт.

3. АНАЛИТИЧЕСКИЕ И ДИАГНОСТИЧЕСКИЕ ХАРАКТЕРИСТИКИ

3.1. Чувствительность набора, определенная по образцам стандартной панели предприятия, содержащим антиген вируса К-КГЛ (СПП 05-2-231), составляет 100%.

3.2. Специфичность набора, определенная по образцам стандартной панели предприятия, не содержащим антиген вируса К-КГЛ (СПП 05-2-231), составляет 100%.

4. МЕРЫ ПРЕДОСТОРОЖНОСТИ

Потенциальный риск применения набора – класс 2а (ГОСТ Р 51609-2000, приказ Минздравсоцразвития России №735 от 30 октября 2006 г.).

Все компоненты набора являются нетоксичными. Стоп-реагент (0,5 М раствор серной кислоты) обладает раздражающим действием. Избегать разбрызгивания и попадания на кожу и слизистые. В случае попадания стоп-реагента на кожу и слизистые необходимо промыть пораженный участок большим количеством проточной воды.

Дробное использование набора может быть реализовано в течение всего годности набора.

Для получения надежных результатов необходимо строгое соблюдение инструкции по применению набора.

При постановке ИФА нельзя использовать компоненты из наборов разных серий или смешивать их при приготовлении растворов, кроме неспецифических компонентов (ФСБ-Т×25, РПРС, стоп-реагент), которые взаимозаменяемы во всех наборах АО «Вектор-Бест».

5. ОБОРУДОВАНИЕ И МАТЕРИАЛЫ, НЕОБХОДИМЫЕ ПРИ РАБОТЕ С НАБОРОМ:

– спектрофотометр, позволяющий проводить измерения оптической плотности растворов в лунках планшета при длине волны 450 нм и/или в двухволновом режиме при основной длине волны 450 нм и длине волны сравнения в диапазоне 620–650 нм;

– термостат, поддерживающий температуру 37±1°С;
– холодильник бытовой, поддерживающий температуру от 2 до 8°С;
– пипетки полуавтоматические одноканальные с переменным или фиксированным объемом со сменными наконечниками, позволяющие отбирать объемы жидкости от 5 до 1000 мкл;
– пипетка полуавтоматическая многоканальная со сменными наконечниками, позволяющая отбирать объемы жидкостей от 5 мкл до 350 мкл;
– промывочное устройство для планшета;
– перчатки резиновые хирургические;
– бумага фильтровальная лабораторная;
– цилиндр мерный 2-го класса точности вместимостью 1000 мл;
– вода дистиллированная.

6. АНАЛИЗИРУЕМЫЕ ОБРАЗЦЫ

6.1. Приготовление суспензий клещей:

– Клеща обмыть 70% раствором этилового спирта, затем физиологическим раствором с антибиотиками (1000 ед. пенициллина и 500 мг стрептомицина на 1мл среды). Поместить в пробирки типа «Эппендорф» по 1 экземпляру и плотно закрыть.

Допускается хранение клещей до 3 месяцев при температуре не выше –18°С.

– Пробирку с клещом поместить в емкость с жидким азотом и заморозить в течение 20 мин.

– Замороженного клеща осторожно и тщательно растереть в гомогенизаторе или в про-

бирке стеклянной или металлической палочкой (диаметр палочки должен строго соответствовать диаметру дна пробирки).

При многократном использовании палочек, их следует выдержать в течение 30 мин в 70% растворе этилового спирта, затем промыть дистиллированной водой и высушить.

– В гомогенизатор или в пробирку с растертым клещом добавить 200 мкл раствора для разведения образцов. Тщательно перемешать до получения однородной суспензии.

– Для напитавшихся клещей РРО добавляют 0,5 мл РРО на одного клеща.

– Суспензии центрифугировать в течение 5 мин при 2000 об/мин. Полученные супернатанты использовать для анализа.

Возможно хранение супернатанта не более суток при температуре 2–8°С.

6.2. Приготовление суспензии секционного материала:

– Кусочки секционного материала (головной мозг, печень, селезенка, лимфатические узлы) растереть в фарфоровой ступке пестиком и добавить небольшое количество стеклянного песка. Добавить 4 мл раствора для разведения образцов на 1 г секционного материала. Суспензии центрифугировать в течение 5 мин при 2000 об/мин. Полученные супернатанты использовать для анализа.

7. ПРОВЕДЕНИЕ ИММУНОФЕРМЕНТНОГО АНАЛИЗА

7.1. Внимание! *Тщательное соблюдение описанных ниже требований позволит избежать искажения результатов ИФА.*

– Для приготовления растворов и проведения ИФА следует использовать чистую мерную посуду и автоматические пипетки с погрешностью измерения объемов не более 5%.

– Сразу после постановки реакции неиспользованный планшет и плотно закрытые флаконы с исходными компонентами необходимо поместить в холодильник, поддерживающий температуру от 2 до 8°С.

– При промывке лунки (стрипа, планшета) заполнять полностью, не допуская переливания промывочного раствора через края лунок, и не касаясь лунок наконечником пипетки. Время между заполнением и опорожнением лунок должно быть не менее 30 с.

– При использовании автоматического или ручного промывателя необходимо следить за состоянием емкости для промывочного раствора и соединительных шлангов: в них не должно быть заметного роста микроорганизмов. Раз в неделю емкость для промывочного раствора и шланги следует промывать 70% спиртом.

– Не допускать высыхания лунок стрипов между отдельными операциями.

– При приготовлении растворов и проведении ИФА следует использовать одноразовые наконечники для дозаторов.

– В случае повторного использования посуду (ванночки) для раствора конъюгата промыть проточной водой и тщательно ополоснуть дистиллированной водой; посуду (ванночки) для раствора ТМБ сразу после работы промыть 50% раствором этилового спирта, а затем дистиллированной водой.

– Для дезинфекции посуды и материалов, контактирующих с исследуемыми и контрольными образцами, рекомендуем использовать дезинфицирующие средства, не оказывающие негативного воздействия на качество ИФА, не содержащие активный кислород и хлор, например, комбинированные средства на основе четвертичных аммониевых соединений, спиртов, третичных аминов.

– Пипетки и рабочие поверхности обрабатывать только 70% раствором этилового спирта. Не использовать во время проведения ИФА перекись водорода, хлорамин и т.д.

7.2. Приготовление реагентов

Перед работой извлечь набор из холодильника, вскрыть упаковку и выдержать все компоненты при температуре от 18 до 25°С в течение 60 мин.

7.2.1. Подготовка планшета

Непосредственно перед использованием вскрыть пакет с планшетом со стороны замка, отступив примерно 1 см. Оставить на рамке необходимое для проведения анализа количество

стрипов; остальные стрипы снять с рамки и немедленно поместить вновь в пакет, удалить из него воздух и плотно закрыть замок.

Неиспользованные стрипы после первого вскрытия пакета можно хранить при температуре от 2 до 8°С в течение всего срока годности набора.

7.2.2. Приготовление контрольных образцов ($К^+$, $К^-$)

Контрольные образцы готовы к использованию и не требуют дополнительного разведения.

7.2.3. Приготовление промывочного раствора

В соответствии с числом стрипов (см. таблицу расхода реагентов) в мерный цилиндр вместимостью 1000 мл внести необходимое количество концентрата ФСБ-Т×25 и довести до необходимого объема дистиллированной водой, тщательно перемешать до полного растворения. При выпадении осадка солей в концентрате необходимо прогреть его при температуре (30–40)°С до полного растворения осадка.

Приготовленный промывочный раствор можно хранить при температуре от 2 до 8°С до 5 суток .

7.2.4. Подготовка конъюгата

Конъюгат готов к использованию. В соответствии с числом используемых стрипов (см. таблицу

расхода компонентов) отобрать в чистый флакон или в пластиковую ванночку для реагента необходимое количество конъюгата.

Остатки конъюгата из флакона или ванночки утилизировать ***(не сливать во флакон с исходным конъюгатом).***

7.2.5. Подготовка раствора тетраметилбензидина

Раствор ТМБ готов к использованию. Исключить воздействие прямого света на раствор тетраметилбензидина. Непосредственно перед внесением и в соответствии с числом используемых стрипов (см. таблицу расхода компонентов) отобрать необходимое количество раствора ТМБ в чистый флакон или в пластиковую ванночку для реагента.

Утилизировать раствор тетраметилбензидина, оставшийся в ванночке или флаконе после проведения ИФА ***(не сливать во флакон с исходным раствором).***

Внимание! *Для работы с раствором ТМБ необходимо использовать только одноразовые наконечники. Посуду, предназначенную для раствора ТМБ, нельзя отмывать с применением синтетических моющих средств, поскольку даже их следы ведут к неконтролируемому окислению ТМБ в ходе реакции. После работы посуду ополоснуть водой, промыть 70% этиловым спиртом и тщательно отмыть дистиллированной водой.*

Таблица расхода компонентов набора реагентов

Кол-во используемых стрипов	Конъюгат, мл	Раствор ТМБ, мл	Промывочный раствор	
			ФСБ-Т, концентрат мл	Дистил. вода мл
1	1,0	1,0	2,0	до 50
2	2,0	2,0	4,0	до 100
3	3,0	3,0	6,0	до 150
4	4,0	4,0	8,0	до 200
5	5,0	5,0	10,0	до 250
6	6,0	6,0	12,0	до 300
7	7,0	7,0	14,0	до 350
8	8,0	8,0	16,0	до 400
9	9,0	9,0	18,0	до 450
10	10,0	10,0	20,0	до 500
11	11,0	11,0	22,0	до 550
12	12,0	12,0	24,0	до 600

7.3. Проведение анализа

7.3.1. Внести в две лунки, например, А-1 и В-1, по 100 мкл отрицательного контрольного образца ($К^-$) и в одну лунку, например, С-1, – 100 мкл положительного контрольного образца ($К^+$).

В остальные лунки внести по 100 мкл исследуемых образцов, тщательно перемешать.

Время внесения образцов не должно превышать 10 мин при использовании всех лунок планшета.

7.3.2. Планшет заклеить пленкой и инкубировать 60 мин при температуре (37±1)°С.

7.3.3. По окончании инкубации снять липкую пленку и поместить ее в сосуд с дезинфицирующим раствором. С помощью промывочного устройства промыть лунки планшета 5 раз промывочным раствором (п. 7.2.3), чередуя аспирацию и немедленное заполнение лунок каждого стрипа. В каждую лунку вносить не менее 400 мкл жидкости в процессе каждого цикла промывки. Время между заполнением и опорожнением лунок должно быть не менее 30 сек. *Необходимо добиваться полного опорожнения лунок после каждого их заполнения.* По окончании промывки остатки влаги из лунок тщательно удалить, постукивая перевернутым планшетом по фильтровальной бумаге.

7.3.4. В лунки планшета внести по 100 мкл конъюгата (п. 7.2.4).

Для внесения раствора конъюгата использовать ванночку для реагентов и одноразовые наконечники, входящие в комплектацию.

Планшет заклеить пленкой и инкубировать 60 мин при температуре (37±1)°С.

7.3.5. По окончании инкубации содержимое лунок собрать в сосуд с дезинфицирующим раствором и промыть 5 раз промывочным раствором так, как указано в п. 7.3.3.

7.3.6. Внести во все лунки по 100 мкл раствора тетраметилбензидина (см. п. 7.2.5).

Для внесения раствора тетраметилбензидина использовать пластиковую ванночку и одноразовые наконечники, входящие в состав набора.

Планшет выдержать в защищенном от света месте в течение 25 мин при температуре от 18 до 25°С.

7.3.7. Внести во все лунки с той же скоростью и в той же последовательности, как и раствор тетраметилбензидина, по 100 мкл стоп-реагента.

В случае попадания на кожу раствора тетраметилбензидина или стоп-реагента необходимо немедленно смыть их водой с мылом.

8. РЕГИСТРАЦИЯ РЕЗУЛЬТАТОВ

Результаты ИФА регистрировать с помощью спектрофотометра, измеряя оптическую плотность (ОП) в двухволновом режиме: основной фильтр – 450 нм, референс-фильтр в диапазоне 620–650 нм. Допускается регистрация результатов только с фильтром 450 нм (выведение спектрофотометра на нулевой уровень (бланк) осуществлять по воздуху).

Время между остановкой реакции и измерением оптической плотности не должно превышать 5 мин.

9. УЧЕТ РЕЗУЛЬТАТОВ АНАЛИЗА

9.1. Рассчитать среднее арифметическое значение оптической плотности в лунках с отрицательным контрольным образцом.

На основании полученных данных вычислить критическое значение оптической плотности ($ОП_{крит.}$) по формуле:

$$ОП_{крит.} = ОП_{ср.}(К^{-}) + 0,2$$

где: $ОП_{ср.}(К^{-})$ – среднее арифметическое значение оптической плотности в лунках с отрицательным контрольным образцом.

9.2. Среднее значение оптической плотности в лунках с отрицательным контрольным образцом $ОП_{ср.}(К^{-})$ не должно превышать 0,25 ед. опт. плотн.

Значение оптической плотности в лунке с положительным контрольным образцом должно быть не менее 1,0 ед. опт. плотн.

9.3. Результат анализа считают **положительным**, если $ОП_{обр.} \geq ОП_{крит.}$;

Результат анализа считают **сомнительным**, если $0,8 \times ОП_{крит.} < ОП_{обр.} < ОП_{крит.}$;

Результат анализа считают **отрицательным**, если $ОП_{обр.} \leq 0,8 \times ОП_{крит.}$;

где $ОП_{обр.}$ – оптическая плотность в лунке с исследуемым образцом.

Если результат анализа сомнительный, рекомендуется повторить анализ такого образца.

10. УСЛОВИЯ ХРАНЕНИЯ И ПРИМЕНЕНИЯ НАБОРА

10.1. Транспортирование набора – при температуре от 2 до 8°С. Допускается транспортирование при температуре до 25°С не более 10 сут. Замораживание не допускается.

10.2. Хранение набора в упаковке предприятия-изготовителя – при температуре от 2 до 8°С. Замораживание компонентов набора не допускается.

10.3. Срок годности – 12 мес . Набор с истекшим сроком годности применению не подлежит.

По вопросам, касающимся качества набора «ВектоКрым-КГЛ-антиген»,
следует обращаться в АО «Вектор-БЕСТ»
по адресу:
630559, Новосибирская область,
Новосибирский район,
п. Кольцово, а/я 121,
тел. (383) 363-13-46,
E-mail: vbobtk@vector-best.ru

ДОПОЛНИТЕЛЬНАЯ ИНФОРМАЦИЯ ДЛЯ ПОТРЕБИТЕЛЕЙ

Набор предназначен для профессионального применения в клинической лабораторной диагностике обученным персоналом.

Требования безопасности к медицинским лабораториям приведены в ГОСТ Р 52905-2007.

Все реагенты наборов, содержащие в своем составе материалы человеческого происхождения, инактивированы.

Антитела к ВИЧ-1,2 и вирусу гепатита С и HBsAg отсутствуют.

Транспортирование должно проводиться всеми видами крытого транспорта в соответствии с правилами перевозок, действующими на транспорте данного вида.

1. Гарантийные обязательства

Производитель гарантирует соответствие выпускаемых изделий требованиям нормативной и технической документации.

Безопасность и качество изделия гарантируются в течение всего срока годности.

Производитель отвечает за недостатки изделия, за исключением дефектов, возникших вследствие нарушения правил пользования, условий транспортирования и хранения, либо действия третьих лиц, либо непреодолимой силы.

Производитель обязуется за свой счет заменить изделие, технические и функциональные

характеристики (потребительские свойства) которого не соответствуют нормативной и технической документации, если указанные недостатки явились следствием скрытого дефекта материалов или некачественного изготовления изделия производителем.

2. Обеспечение безопасности персонала

Обращение с материалами, контактирующими с исследуемыми образцами

Материалы, контактирующие с исследуемыми образцами, следует дезинфицировать МУ-287-113 «Методические указания по дезинфекции, предстерилизационной очистке и стерилизации изделий медицинского назначения» (утв. департаментом госсанэпиднадзора Минздрава РФ от 30.12.1998).

Порядок утилизации или уничтожения компонентов набора

При использовании набора образуются отходы классов А, Б и Г, которые классифицируются и уничтожаются (утилизируются) в соответствии с СанПиНом 2.1.7.2790-10 «Санитарно-эпидемиологические требования к обращению с медицинскими отходами». Дезинфекцию наборов реагентов следует проводить по МУ 287-113 «Методические указания по дезинфекции, предстерилизационной очистке и стерилизации изделий медицинского назначения».

3. Обеспечение получения правильных результатов анализа

Достоверность и воспроизводимость результатов анализа зависят от выполнения следующих основных правил:

– не проводите ИФА в присутствии паров кислот, щелочей, альдегидов или пыли, которые могут менять ферментативную активность конъюгатов;

– ферментативная реакция чувствительна к присутствию ионов металлов, поэтому не допускайте контактов каких-либо металлических предметов с конъюгатом и раствором ТМБ;

– избегайте загрязнения компонентов набора микроорганизмами и химическими примесями, для этого используйте в работе чистую посуду и чистые одноразовые наконечники для каждого реагента, контроля, образца;

– никогда не используйте одну и ту же емкость для конъюгата и раствора ТМБ;

– перед отбором раствора ТМБ из флакона необходимо протереть конус пипетки (внутреннюю и внешнюю поверхности) сначала дистиллированной водой, а затем 70% этиловым спиртом, так как малейшее загрязнение пипеток конъюгатом может привести к контаминации всего содержимого флакона;

– если допущена ошибка при внесении анализируемых образцов, нельзя, опорожнив эту лунку, вносить в нее новый образец; такая лунка бракуется.

Качество промывки лунок планшета играет важную роль для получения правильных результатов анализа:

– Для аспирации анализируемых образцов и последующей промывки рекомендуется использовать автоматическое или ручное промывочное устройство.

– Для предотвращения засорения игл промывочного устройства в конце рабочего дня обязательно выполните процедуру ополаскивания системы подачи жидкости дистиллированной водой.

4. Краткая схема проведения ИФА для набора реагентов «ВектоКрым-КГЛ-антиген»

Использовать только после внимательного ознакомления с инструкцией!

Внести:	по 100 мкл $К^+$, $К^-$; по 100 мкл анализируемых образцов.
Инкубировать:	60 мин, 37°С.
Промыть:	промывочным раствором, 400 мкл, 5 раз.
Внести:	по 100 мкл конъюгата.
Инкубировать:	60 мин, 37°С.
Промыть:	промывочным раствором, 400 мкл, 5 раз.
Внести:	по 100 мкл раствора тетраметилбензидина.
Инкубировать:	25 мин, 18–25°С, в темноте.
Внести:	по 100 мкл стоп-реагента.
Измерить:	ОП при 450 нм / референсная длина волны 620–655 нм.

5. Графические символы

REF	Номер по каталогу	IVD	Медицинское изделие для диагностики *in vitro*
Σ n	Содержимого достаточно для проведения n-количества тестов	NON STERILE	Не стерильно
LOT	Код партии		Температурный диапазон
	Изготовитель		Дата изготовления
	Использовать до …		Обратитесь к инструкции по применению
	Осторожно! Обратитесь к Инструкции по применению	YYYY-MM-DD YYYY-MM	Дата в формате Год-Месяц-День Год-Месяц

Консультацию специалиста по работе с набором можно получить по тел.: (383) 363-57-95.

29.02.16.

ВЕКТОР

БЕСТ

ВектоВКЭ-IgM

Набор реагентов
для иммуноферментного выявления
иммуноглобулинов класса М
к вирусу клещевого энцефалита

Инструкция утверждена 15.09.16

НАБОР РЕАГЕНТОВ
D-1152

1. НАЗНАЧЕНИЕ

1.1. Набор реагентов для иммуноферментного выявления иммуноглобулинов класса М к вирусу клещевого энцефалита «ВектоВКЭ-IgM» (далее по тексту – набор) предназначен для выявления иммуноглобулинов класса М (IgM) к вирусу клещевого энцефалита (ВКЭ) в сыворотке (плазме) крови человека методом твердофазного иммуноферментного анализа.

1.2. Набор рассчитан на проведение 96 анализов, включая контроли. Для исследования небольшой партии проб возможны 12 независимых постановок ИФА по 8 анализов каждая, включая контроли.

2. ХАРАКТЕРИСТИКА НАБОРА

2.1. Принцип действия

Определение IgM к ВКЭ основано на методе «захвата» твердофазного иммуноферментного анализа (“capture”-метод) с использованием моноклональных антител к IgM человека.

Во время первой инкубации происходит связывание содержащихся в анализируемом образце иммуноглобулинов класса М с моноклональными антителами к IgM человека, иммобилизованными на внутренней поверхности лунок планшета.

Во время второй инкубации связавшиеся специфические IgM взаимодействуют с антигеном ВКЭ, находящемся в комплексе с пероксидазным конъюгатом.

Количество связавшегося конъюгата определяют цветной реакцией с использованием субстрата пероксидазы – перекиси водорода и хромогена – тетраметилбензидина. Интенсивность окрашивания пропорциональна концентрации IgM к ВКЭ в анализируемом образце.

2.2. Состав набора

В состав набора входят:

- планшет разборный (12 восьмилуночных стрипов) с иммобилизованными на внутренней поверхности лунок моноклональными антителами к IgM человека, готовый для использования – 1 шт.;
- положительный контрольный образец ($К^+$) на основе инактивированной сыворотки крови человека, содержащий IgM к ВКЭ, готовый для использования – 1 флакон (1,5 мл);
- отрицательный контрольный образец ($К^-$) на основе инактивированной сыворотки крови человека, не содержащий IgM к ВКЭ, готовый для использования – 1 флакон (2,5 мл);
- конъюгат моноклональных антител к вирусу клещевого энцефалита с пероксидазой хрена, готовый для использования – 1 флакон (13 мл);
- антиген ВКЭ – 1 флакон (1,5 мл);
- раствор для предварительного разведения сывороток (РПРС) – 1 флакон (10 мл);
- раствор для разведения сывороток (РРС) – 1 флакон (12 мл);
- концентрат фосфатно-солевого буферного раствора с твином (ФСБ-Т×25) – 1 флакон (28 мл);

– раствор тетраметилбензидина (раствор ТМБ), готовый для использования – 1 флакон (13 мл);
– стоп-реагент, готовый для использования – 1 флакон (12 мл).

Принадлежности:

– пленки для заклеивания планшета – 2 шт.;
– ванночки для реагентов – 2 шт.;
– наконечники для дозаторов на 2–200 мкл – 16 шт.;
– планшет для предварительного разведения образцов – 1 шт.

3. АНАЛИТИЧЕСКИЕ И ДИАГНОСТИЧЕСКИЕ ХАРАКТЕРИСТИКИ

3.1. Чувствительность выявления иммуноглобулинов класса М к ВКЭ – соответствие результатов определения набором IgM к ВКЭ требованиям стандартной панели предприятия (рег. № 05-2-582), аттестованной ОБТК АО «Вектор-Бест» – составляет 100%: среднее арифметическое значение оптической плотности в лунках с положительными сыворотками $ОП_{№1–№8} \geq ОП_{крит.}$.

3.2. Специфичность выявления иммуноглобулинов класса М к ВКЭ – соответствие результатов определения набором IgM к ВКЭ требованиям стандартной панели предприятия (рег. № 05-2-171), аттестованной ОБТК АО «Вектор-Бест» – составляет 100%: среднее арифметическое значение оптической плотности в лунках с отрицательными сыворотками $ОП_{№1–№16} < ОП_{крит.}$.

3.3. Диагностическая чувствительность выявления иммуноглобулинов класса М к вирусу

клещевого энцефалита: клинические испытания, проведенные на 175 положительных образцах сыворотки и плазмы крови, полученных от 100 пациентов, показали 100% чувствительность (интервал 97–100%, с доверительной вероятностью 90%);

3.4. Диагностическая специфичность выявления иммуноглобулинов класса М к вирусу клещевого энцефалита: клинические испытания, проведенные на 175 отрицательных образцах сыворотки и плазмы крови, полученных от 100 пациентов, показали 100% специфичность (интервал 97–100%, с доверительной вероятностью 90%).

4. МЕРЫ ПРЕДОСТОРОЖНОСТИ

4.1. Потенциальный риск применения набора – класс 2б (Приказ МЗ РФ от 06.06.2012 № 4н).

4.2. Все компоненты набора являются нетоксичными. Стоп-реагент обладает раздражающим действием. Избегать разбрызгивания и попадания на кожу и слизистые. В случае попадания стоп-реагента на кожу и слизистые необходимо промыть пораженный участок большим количеством проточной воды.

4.3. При работе с исследуемыми образцами следует соблюдать меры предосторожности, принятые при работе с потенциально инфекционным материалом. Основные правила работы изложены в «Инструкции по мерам профилак-

тики распространения инфекционных заболеваний при работе в клинико-диагностических лабораториях лечебно-профилактических учреждений», утвержденной Минздравом СССР 17 января 1991 г. и в методических указаниях МУ 287-113 («Методические указания по дезинфекции, предстерилизационной очистке и стерилизации изделий медицинского назначения», утв. департаментом госсанэпиднадзора Минздрава РФ от 30.12.1998 г).

4.4. При работе с набором следует надевать одноразовые резиновые или пластиковые перчатки, так как образцы сыворотки крови человека следует рассматривать как потенциально инфекционные, способные длительное время сохранять и передавать ВИЧ, вирусы гепатита или возбудителей других инфекций.

4.5. Химическая посуда и оборудование, которые используются в работе с набором, должны быть соответствующим образом промаркированы и храниться отдельно.

4.6. Запрещается прием пищи, использование косметических средств и курение в помещениях, предназначенных для работы с наборами.

4.7. Для дезинфекции посуды и материалов, контактировавших с исследуемыми и контрольными образцами, рекомендуем использовать дезинфицирующие средства, не оказывающие негативного воздействия на качество ИФА, не содержащие активный кислород и хлор, напри-

мер, комбинированные средства на основе ЧАС, спиртов, третичных аминов. Использование дезинфицирующих средств, содержащих активный кислород и хлор (H_2O_2, деохлор, хлорамин), приводит к серьезному искажению результатов.

4.8. При использовании набора образуются отходы классов А, Б и Г, которые классифицируются и уничтожаются (утилизируются) в соответствии с СанПиН 2.1.7.2790-10 «Санитарно-эпидемиологические требования к обращению с медицинскими отходами». Дезинфекцию наборов реагентов следует проводить по МУ 287-113 «Методические указания по дезинфекции, предстерилизационной очистке и стерилизации изделий медицинского назначения».

5. ОБОРУДОВАНИЕ И МАТЕРИАЛЫ, НЕОБХОДИМЫЕ ПРИ РАБОТЕ С НАБОРОМ:

- спектрофотометр вертикального сканирования, позволяющий проводить измерения оптической плотности растворов в лунках стрипов при основной длине волны 450 нм и длине волны сравнения в диапазоне 620–655 нм; допускается измерение только при длине волны 450 нм;
- термостат, поддерживающий температуру (37±1)°С;
- холодильник бытовой;
- бумага фильтровальная лабораторная;
- дозаторы полуавтоматические одноканальные со сменными наконечниками, позволяющие отбирать объемы жидкости 5–5000 мкл (погрешность не более 5%);

– промывочное устройство для планшетов;
– перчатки медицинские диагностические одноразовые;
– цилиндр мерный 2-го класса точности вместимостью 1000 мл;
– вода дистиллированная;
– дезинфицирующий раствор.

6. АНАЛИЗИРУЕМЫЕ ОБРАЗЦЫ

6.1. Для проведения анализа не следует использовать мутную, гемолизованную сыворотку крови.

6.2. Для проведения анализа можно использовать образцы плазмы, полученной с использованием в качестве антикоагулянта цитрата натрия, гепарина или ЭДТА.

6.3. Образцы сыворотки (плазмы) крови можно хранить при температуре от 2 до 8°С не более 5 суток при условии отсутствия микробной контаминации или при температуре минус 20°С (и ниже) не более 6 мес.

Следует избегать многократного замораживания/оттаивания, так как это может привести к получению неправильных результатов. После размораживания образцы следует тщательно перемешать.

6.4. Образцы сывороток (плазмы) крови, содержащие осадок, необходимо очистить центрифугированием при 5000–10000 об/мин в течение 5 мин при температуре от 18 до 25°С.

7. ПРОВЕДЕНИЕ АНАЛИЗА

ПОДГОТОВКА РЕАГЕНТОВ

7.1. Перед работой извлечь набор из холодильника, вскрыть упаковку и выдержать все компоненты при температуре от 18 до 25°С не менее 1 ч.

7.2. Подготовка планшета

Вскрыть пакет выше замка и установить на рамку необходимое для проведения анализа количество стрипов. Оставшиеся неиспользованные стрипы немедленно поместить вновь в пакет с влагопоглотителем, удалить из него воздух, плотно закрыть замок и поместить в холодильник.

Хранить при температуре от 2 до 8°С в течение всего срока годности.

7.3. Приготовление промывочного раствора

В соответствии с числом используемых стрипов (см. таблицу расхода компонентов) внести в мерный цилиндр необходимое количество ФСБ-Т×25 и довести до соответствующего объема дистиллированной водой. При выпадении осадка солей в концентрате необходимо прогреть его при температуре от 30 до 40°С до полного растворения осадка.

Приготовленный промывочный раствор можно хранить при температуре от 2 до 8°С не более 5 суток.

Таблица расхода компонентов набора реагентов

Кол-во используемых стрипов	Промывочный раствор		Смесь коньюгата и антигена ВКЭ		Раствор ТМБ, мл
	ФСБ-Т×25, мл	Дистил. вода, мл	Конъюгат, мл	Антиген ВКЭ, мл	
1	2,0	До 50	1,0	0,1	1,0
2	4,0	до 100	2,0	0,2	2,0
3	6,0	до 150	3,0	0,3	3,0
4	8,0	до 200	4,0	0,4	4,0
5	10,0	до 250	5,0	0,5	5,0
6	12,0	до 300	6,0	0,6	6,0
7	14,0	до 350	7,0	0,7	7,0
8	16,0	до 400	8,0	0,8	8,0
9	18,0	до 450	9,0	0,9	9,0
10	20,0	до 500	10,0	1,0	10,0
11	22,0	до 550	11,0	1,1	11,0
12	24,0	до 600	12,0	1,2	12,0

7.4. Подготовка исследуемых образцов

Исследуемые образцы развести в 10 раз раствором для предварительного разведения сывороток. Для этого внести в лунки вспомогательного планшета по 90 мкл РПРС и добавить по 10 мкл цельного образца сыворотки (плазмы), тщательно перемешать. При разведении сыворотки крас-

ный цвет должен измениться на желтый. Если изменения цвета не произошло, анализ образца сыворотки может дать неправильный результат. При разведении плазмы цвет раствора в лунке меняется незначительно.

Хранить при температуре от 18 до 25°С в течение 3 часов.

7.5. Подготовка положительного и отрицательного контрольных образцов

Положительный и отрицательный контрольные образцы готовы к использованию и не требуют дополнительного разведения.

Контрольные образцы после первого вскрытия флаконов можно хранить при температуре от 2 до 8°С в течение всего срока годности.

7.6. Подготовка смеси конъюгата и антигена ВКЭ

В соответствии с числом используемых стрипов (см. таблицу расхода компонентов) в отдельный чистый флакон или в пластиковую ванночку для реагента внести необходимое количество конъюгата, добавить соответствующее количество антигена ВКЭ, тщательно перемешать.

Хранить при температуре от 18 до 25°С в течение 3 часов.

Конъюгат после первого вскрытия флакона можно хранить при температуре от 2 до 8°С в течение всего срока годности.

Антиген после первого вскрытия флакона можно хранить при температуре от 2 до 8°С в течение всего срока годности.

7.7. Подготовка раствора тетраметилбензидина

Раствор ТМБ готов к использованию.

Необходимо исключить воздействие прямого света на раствор ТМБ.

В соответствии с числом используемых стрипов (см. таблицу расхода компонентов) отобрать в чистый флакон или в пластиковую ванночку для реагента необходимое количество раствора ТМБ.

Остатки раствора ТМБ из ванночки утилизировать ***(не сливать во флакон с исходным раствором ТМБ).***

Раствор ТМБ после первого вскрытия флакона можно хранить при температуре от 2 до 8°С в течение всего срока годности.

Внимание! *В набор вложены ванночка для реагентов и наконечники для пипетки, которые следует использовать только для работы с раствором тетраметилбензидина. Посуду и пипетки (наконечники), контактирующие с раствором тетраметилбензидина, нельзя отмывать с применением синтетических моющих средств, поскольку даже их следовые количества ведут к неконтролируемому разложению тетраметилбензидина в ходе реакции. После работы посуду и наконечники*

ополоснуть водой, промыть 70% раствором этилового спирта и тщательно отмыть дистиллированной водой.

7.8. Стоп-реагент готов к использованию.

Стоп-реагент после первого вскрытия флакона можно хранить при температуре от 2 до 8°С в течение всего срока годности.

ПРОВЕДЕНИЕ ИФА

7.9. Внесение образцов.

В лунки А-1, В-1 внести по 100 мкл отрицательного контрольного образца ($К^-$).

В лунку С-1 внести 100 мкл положительного контрольного образца ($К^+$).

В остальные лунки внести по 90 мкл РРС и по 10 мкл предварительно подготовленных исследуемых образцов, тщательно перемешать. Таким образом, исследуемый образец в лунке разбавляется в 100 раз.

Время внесения образцов не должно превышать 10 мин при использовании всех лунок планшета.

7.10. Планшет заклеить пленкой и инкубировать в термостате в течение 60 мин при температуре (37±1)°С.

7.11. По окончании инкубации снять липкую пленку и поместить ее в сосуд с дезинфицирующим раствором. С помощью промывочного устройства промыть лунки планшета 5 раз промывочным

раствором (п. 7.3), чередуя аспирацию и немедленное заполнение лунок каждого стрипа. В каждую лунку вносить не менее 400 мкл жидкости в процессе каждого цикла промывки. Время между заполнением и опорожнением лунок должно быть не менее 30 сек. *Необходимо добиваться полного опорожнения лунок после каждого их заполнения.* По окончании промывки остатки влаги из лунок тщательно удалить, постукивая перевернутым планшетом по фильтровальной бумаге.

7.12. Внести во все лунки по 100 мкл смеси конъюгата и антигена ВКЭ (п. 7.6).

7.13. Планшет заклеить пленкой и инкубировать в термостате в течение 60 мин при температуре (37±1)°С.

Для внесения смеси конъюгата и антигена ВКЭ использовать ванночку для реагента и одноразовые наконечники, входящие в состав набора.

7.14. По окончании инкубации промыть планшет как описано в п. 7.11.

7.15. Внести во все лунки по 100 мкл раствора тетраметилбензидина.

Планшет выдержать в защищенном от света месте в течение 25 мин при температуре от 18 до 25°С.

Для внесения раствора тетраметилбензидина использовать ванночку для реагента и одноразовые наконечники, входящие в состав набора.

7.16. Внести во все лунки по 100 мкл стоп-реагента.

8. РЕГИСТРАЦИЯ РЕЗУЛЬТАТОВ

8.1. Измерить оптическую плотность с помощью спектрофотометра в режиме: основной фильтр – 450 нм, референс-фильтр – в диапазоне 620–655 нм. Допускается измерение без использования референс-фильтра.

Время между остановкой реакции и измерением оптической плотности не должно превышать 5 мин.

9. УЧЕТ РЕЗУЛЬТАТОВ РЕАКЦИИ

9.1. Рассчитать среднее арифметическое значение оптической плотности в лунках с отрицательным контрольным образцом.

9.2. На основании полученных данных вычислить критическое значение оптической плотности ($ОП_{крит.}$) по формуле:

$$ОП_{крит.} = ОП_{ср.}\,К^{-} + 0{,}2$$

где $ОП_{ср.}\,К^{-}$ – среднее арифметическое значение оптической плотности в лунках с отрицательным контрольным образцом, измеренное в двухволновом режиме 450/620–655 нм (или только с фильтром 450 нм).

9.3. Оценка результатов:

– среднее арифметическое значение оптической плотности в лунках с отрицательным контрольным образцом должно быть не более 0,25 ед. опт. плотн.

– значение оптической плотности в лунке с положительным контрольным образцом должно быть не менее 1,80 ед. опт. плотн.

9.4. Только при соблюдении положений п. 9.3 можно учитывать результаты, полученные для анализируемых образцов сыворотки (плазмы) крови.

Результат анализа считать **положительным**, если $ОП_{обр.} \geq ОП_{крит.}$.

Результат анализа считать **отрицательным**, если $ОП_{обр.} < ОП_{крит.}$.

9.5. Результаты анализа можно оценивать по коэффициенту позитивности (КП).

$$КП = \frac{ОП_{обр.}}{ОП_{крит.}}$$

Исследуемый образец расценивают как **положительный**, если соответствующее ему значение КП превышает или равно 1 ($КП \geq 1$).

Исследуемый образец расценивают как **отрицательный**, если соответствующее ему значение КП меньше 1 ($КП<1$).

10. УСЛОВИЯ ТРАНСПОРТИРОВАНИЯ, ХРАНЕНИЯ И ПРИМЕНЕНИЯ НАБОРА

10.1. Транспортировать изделия следует транспортом всех видов в крытых транспортных средствах в соответствии с правилами перевозок, действующими на транспорте данного вида, при температуре от 2 до 8°С. Допускается транспортирование при температуре до 25°С не более 10 суток.

10.2. Хранение набора в упаковке предприятия-изготовителя должно осуществляться при температуре от 2 до 8°С в течение всего срока годности в холодильных камерах или холодильниках, обеспечивающих регламентированный температурный режим с ежедневной регистрацией температуры.

10.3. Срок годности набора – 12 месяцев со дня выпуска. Не допускается применение наборов по истечении срока их годности.

10.4. Дробное использование набора может быть реализовано в течение всего срока годности. В случае дробного использования набора:

– неиспользованные стрипы можно хранить в плотно закрытом пакете при температуре от 2 до 8°С в течение всего срока годности;
– концентрат фосфатно-солевого буферного раствора с твином; раствор для предварительного разведения сывороток; раствор для разведения сывороток; раствор тетраметилбензидина; контрольные образцы; антиген ВКЭ, конъюгат и стоп-реагент после

вскрытия флаконов можно хранить в плотно закрытых флаконах при температуре от 2 до 8°С в течение всего срока годности;

– промывочный раствор можно хранить при температуре от 2 до 8°С не более 5 сут.

10.5. Для получения надежных результатов необходимо строгое соблюдение инструкции по применению набора.

10.6. При постановке ИФА нельзя использовать компоненты из наборов разных серий или смешивать их при приготовлении растворов, кроме неспецифических компонентов (РПРС, ФСБ-Т×25, раствор ТМБ, стоп-реагент), которые взаимозаменяемы в наборах АО «Вектор-Бест».

11. ГАРАНТИЙНЫЕ ОБЯЗАТЕЛЬСТВА

11.1. Производитель гарантирует соответствие выпускаемых изделий требованиям нормативной и технической документации.

Безопасность и качество изделия гарантируются в течение всего срока годности.

11.2. Производитель отвечает за недостатки изделия, за исключением дефектов, возникших вследствие нарушения правил пользования, условий транспортирования и хранения, либо действия третьих лиц, либо непреодолимой силы.

11.3. Производитель обязуется за свой счет заменить изделие, технические и функциональные характеристики (потребительские свойства) которого не соответствуют нормативной и технической документации, если указанные недостатки явились следствием скрытого дефекта материалов или некачественного изготовления изделия производителем.

По вопросам, касающимся качества набора «ВектоВКЭ-IgM»,
следует обращаться в АО «Вектор-Бест»
по адресу:
630559, Новосибирская область,
Новосибирский район,
п. Кольцово, а/я 121,
тел. (383) 227-67-64,
E-mail: vbobtk@vector-best.ru

ДОПОЛНИТЕЛЬНАЯ ИНФОРМАЦИЯ ДЛЯ ПОТРЕБИТЕЛЕЙ

Набор предназначен для профессионального применения в клинической лабораторной диагностике обученным персоналом.

Требования безопасности к медицинским лабораториям приведены в ГОСТ Р 52905-2007.

Все реагенты наборов, содержащие в своем составе материалы человеческого происхождения, инактивированы и не содержат HBsAg, антител к ВГС, ВИЧ-1, ВИЧ-2, *Treponema pallidum*, антигена p24 ВИЧ-1.

Не применять набор реагентов по назначению после окончания срока годности.

При динамическом наблюдении пациента для получения результатов, адекватно отражающих изменение концентрации IgM к ВКЭ в крови, необходимо использовать наборы реагентов одного наименования (одного предприятия-изготовителя).

1. Обеспечение безопасности персонала

Обращение с материалами, контактирующими с исследуемыми образцами

Материалы, контактирующие с исследуемыми образцами, следует дезинфицировать согласно МУ-287-113 «Методические указания по дезинфекции, предстерилизационной очистке и стерилизации изделий медицинского назначения» (утв. департаментом госсанэпиднадзора Минздрава РФ от 30.12.1998).

2. Обеспечение получения правильных результатов анализа

Достоверность и воспроизводимость результатов анализа зависят от выполнения следующих основных правил:

– нельзя использовать реагенты из наборов других фирм-производителей;

– не проводите ИФА в присутствии паров кислот, щелочей, альдегидов или пыли, которые могут менять ферментативную активность конъюгатов;

– ферментативная реакция чувствительна к присутствию ионов металлов, поэтому не допускайте контактов каких-либо металлических предметов с конъюгатом и раствором ТМБ;

– избегайте загрязнения компонентов набора микроорганизмами и химическими примесями, для этого используйте в работе чистую посуду и чистые одноразовые наконечники для каждого реагента, контроля, образца;

– рабочие поверхности столов, оборудования следует обрабатывать 70% этиловым спиртом (не допускается использование перекиси водорода, хлорсодержащих растворов);

– никогда не используйте одну и ту же емкость для конъюгата и раствора ТМБ;

– перед отбором раствора ТМБ из флакона необходимо протереть конус пипетки (внутреннюю и внешнюю поверхности) 70% этиловым спиртом, так как малейшее загрязнение пипе-

ток конъюгатом может привести к контаминации всего содержимого флакона;

– если допущена ошибка при внесении анализируемых образцов, нельзя, опорожнив эту лунку, вносить в нее новый образец; такая лунка бракуется.

Качество промывки лунок планшета играет важную роль для получения правильных результатов анализа:

– Для аспирации анализируемых образцов и последующей промывки рекомендуется использовать автоматическое или ручное промывочное устройство.

– Не допускайте высыхания лунок планшета в перерыве между завершением промывки и внесением реагентов.

– Добивайтесь полного заполнения и опорожнения всех лунок планшета в процессе промывки. Недостаточная аспирация жидкости в процессе промывки может привести к понижению чувствительности и специфичности анализа.

– Следите за состоянием промывочного устройства – регулярно (1 раз в неделю) обрабатывайте шланги и емкости 70% этиловым спиртом.

– Для предотвращения засорения игл промывочного устройства в конце рабочего дня обязательно выполните процедуру ополаскивания системы подачи жидкости дистиллированной водой.

3. Проведение проверочного теста

Для подтверждения специфичности выявления IgM к ВКЭ рекомендуется провести проверочный тест. Выявленные положительные образцы вносятся в дублях в лунки стрипа – одна лунка используется для анализа со смесью антиген ВКЭ – конъюгат, вторая – только с конъюгатом (без антигена). В результате проведенного таким образом ИФА в лунках с образцами, содержащими IgM к ВКЭ, развивается окрашивание только в присутствии антигена ВКЭ. Образцы, неспецифически реагирующие в наборе, дают положительную реакцию в лунках с антигеном ВКЭ и без него.

3.1. Проведение анализа

3.1.1. Внесение образцов.

Стрипы следует использовать попарно: один – для проведения реакции со смесью антиген ВКЭ-конъюгат, другой – с конъюгатом без антигена.

Внести контрольные образцы: в **2 лунки** – по 100 мкл $К^+$ и в **4 лунки** – по 100 мкл $К^-$.

Например, в лунки А-1 и А-2, В-1 и В-2 внести по 100 мкл $К^-$, в лунки С-1, С-2 – по 100 мкл $К^+$.

В остальные лунки внести по 90 мкл РРС и попарно в лунки двух стрипов по 10 мкл предварительно подготовленных исследуемых образцов (п. 7.4), тщательно перемешать. Таким образом, исследуемый образец в лунке разбавляется в 100 раз.

Планшет заклеить пленкой и инкубировать в термостате в течение 60 мин при температуре 37°С.

3.1.2. По окончании инкубации снять липкую пленку и поместить ее в сосуд с дезинфицирующим раствором. С помощью промывочного устройства промыть лунки планшета 5 раз промывочным раствором (п. 7.3), чередуя аспирацию и немедленное заполнение лунок каждого стрипа. В каждую лунку вносить не менее 400 мкл жидкости в процессе каждого цикла промывки. Время между заполнением и опорожнением лунок должно быть не менее 30 сек. *Необходимо добиваться полного опорожнения лунок после каждого их заполнения.* По окончании промывки остатки влаги из лунок тщательно удалить, постукивая перевернутым планшетом по фильтровальной бумаге.

3.1.3. В лунки первого стрипа внести по 100 мкл смеси антиген ВКЭ-конъюгат (п. 7.6), в лунки второго стрипа – по 100 мкл конъюгата.

Планшет заклеить пленкой и инкубировать в термостате в течение 60 мин при температуре 37°С.

3.1.4. По окончании инкубации промыть планшет 5 раз как описано выше.

3.1.5. Внести во все лунки по 100 мкл раствора тетраметилбензидина (п. 7.7).

Планшет выдержать в защищенном от света месте в течение 25 мин при температуре 18–25°С.

3.1.6. Внести во все лунки по 100 мкл стоп-реагента.

3.2. Регистрация результатов

Измерить оптическую плотность с помощью спектрофотометра в двухволновом режиме: основной фильтр – 450 нм, референс-фильтр – в диапазоне 620–655 нм. Допускается измерение только с фильтром 450 нм.

Время между остановкой реакции и измерением оптической плотности не должно превышать 5 мин.

3.3. Условия правильности работы набора (при постановке проверочного теста)

Результаты анализа исследуемых образцов учитывать, если будут выполнены следующие условия:

В лунках стрипа со смесью антиген ВКЭ–конъюгат:

– среднее значение оптической плотности в лунках с отрицательным контрольным образцом не более 0,25 о.е.;

– значение оптической плотности в лунке с положительным контрольным образцом не менее 1,80 о.е.

В лунках стрипа с конъюгатом без антигена среднее значение оптической плотности в лунках с контрольными образцами не более 0,25 о.е.

3.4. Расчет результатов проверочного теста

Вычислить коэффициент соотношения сигналов (k) при постановке ИФА в присутствии антигена ВКЭ и без него по формуле:

$$k = \frac{ОП_{конъюгат}}{ОП_{смесь}} \times 100$$

где: $ОП_{конъюгат}$ – оптическая плотность в лунке с анализируемым образцом при инкубации с конъюгатом без антигена,

$ОП_{смесь}$ – оптическая плотность в лунке с анализируемым образцом при инкубации со смесью антиген ВКЭ–конъюгат.

Если $k \leq 50$, то исследуемый образец признают **положительным**, содержащим IgM к ВКЭ.

Если $k > 50$, то это указывает на неспецифическую реакцию анализируемого образца, и образец признают **отрицательным**, не содержащим IgM к ВКЭ.

4. Краткая схема проведения ИФА для набора реагентов «ВектоВКЭ-IgM»

Использовать только после внимательного ознакомления с инструкцией!

Внести:	100 мкл $К^+$, $К^-$; по 90 мкл РРС и по 10 мкл предварительно разведенных анализируемых образцов.
Инкубировать:	60 мин, 37°С.
Промыть:	промывочным раствором, 400 мкл, 5 раз.
Внести:	по 100 мкл смеси конъюгата и антигена ВКЭ.
Инкубировать:	60 мин, 37°С.
Промыть:	промывочным раствором, 400 мкл, 5 раз.
Внести:	по 100 мкл раствора тетраметилбензидина.
Инкубировать:	25 мин, 18–25°С в темноте.
Внести:	по 100 мкл стоп-реагента.
Измерить:	ОП при 450 нм / референсная длина волны 620–655 нм.

5. Графические символы

REF	Номер по каталогу	IVD	Медицинское изделие для диагностики *in vitro*
Σ n	Содержимого достаточно для проведения n-количества тестов	NON STERILE	Не стерильно
LOT	Код партии		Температурный диапазон
	Изготовитель		Дата изготовления
	Использовать до …		Обратитесь к инструкции по применению
	Осторожно! Обратитесь к Инструкции по применению	YYYY-MM-DD YYYY-MM	Дата в формате Год-Месяц-День Год-Месяц

Консультацию специалиста по работе с набором можно получить по тел.: (383) 363-57-86.

27.12.16.

АКЦИОНЕРНОЕ ОБЩЕСТВО
«ВЕКТОР-БЕСТ»

Международный сертификат ISO 13485

Наш адрес: 630117, Новосибирск-117, а/я 492
Тел./факс: (383) 227-73-60 (многоканальный)
Тел.: (383) 332-37-10, 332-37-58, 332-36-34,
332-67-49, 332-67-52
E-mail: vbmarket@vector-best.ru
Internet: www.vector-best.ru

ВЕКТОР БЕСТ

ВектоВКЭ-антиген

Набор реагентов
для иммуноферментного выявления
антигена вируса клещевого энцефалита

ИНСТРУКЦИЯ ПО ПРИМЕНЕНИЮ

Утверждена 05.12.2016

НАБОР РЕАГЕНТОВ
D-1154

1. НАЗНАЧЕНИЕ

1.1. Набор реагентов для иммуноферментного выявления антигена вируса клещевого энцефалита «ВектоВКЭ-антиген» (далее по тексту – набор) предназначен для выявления антигена вируса клещевого энцефалита (ВКЭ) в клещах и ликворе человека методом твердофазного иммуноферментного анализа (ИФА).

1.2. Набор рассчитан на проведение 96 анализов, включая контроли. Для исследования небольшой партии проб возможны 12 независимых постановок ИФА по 8 анализов каждая, включая контроли.

2. ХАРАКТЕРИСТИКА НАБОРА

2.1. Принцип действия

Метод определения основан на двухстадийном твердофазном иммуноферментном анализе с использованием моноклональных антител к ВКЭ.

Во время первой инкубации происходит связывание содержащегося в анализируемом образце антигена ВКЭ с моноклональными антителами к ВКЭ, иммобилизованными на внутренней поверхности лунок планшета,

Во время второй инкубации связавшийся антиген ВКЭ взаимодействует с конъюгатом моноклональных антител к ВКЭ с пероксидазой хрена.

Количество связавшегося конъюгата определяют цветной реакцией с использованием субстрата пероксидазы – перекиси водорода и хромогена –

тетраметилбензидина. Интенсивность окрашивания пропорциональна концентрации антигена ВКЭ в анализируемом образце.

2.2. Состав набора

В состав набора входят следующие реагенты:

- планшет разборный (12 восьмилуночных стрипов, «ломающихся» по 1 лунке) с иммобилизованными на внутренней поверхности лунок моноклональными антителами к ВКЭ, готовый для использования – 1 шт.;
- положительный контрольный образец ($К^+$) – буферный раствор, содержащий антиген ВКЭ, готовый для использования –1 флакон (1,5 мл);
- отрицательный контрольный образец ($К^-$) – буферный раствор, не содержащий антиген ВКЭ, готовый для использования – 1 флакон (2,5 мл);
- конъюгат – моноклональные антитела к ВКЭ, конъюгированные с пероксидазой хрена, готовый для использования – 1 флакон (13 мл);
- раствор для разведения образцов (РРО) – 3 флакона (по 12 мл);
- концентрат фосфатно-солевого буферного раствора с твином (ФСБ-Т×25) – 1 флакон (28 мл);
- раствор тетраметилбензидина (раствор ТМБ), готовый для использования – 1 флакон (13 мл);
- стоп-реагент – 1 флакон (12 мл).

Принадлежности:

- пленки для заклеивания планшета – 2 шт.;
- ванночки для реагентов – 2 шт.;
- наконечники для дозаторов на 2–200 мкл – 16 шт.

3. АНАЛИТИЧЕСКИЕ И ДИАГНОСТИЧЕСКИЕ ХАРАКТЕРИСТИКИ

3.1. Чувствительность выявления антигена ВКЭ – соответствие результатов определения набором антигена ВКЭ требованиям стандартного образца предприятия (СОП$^+$) (рег. № 05-2-84), аттестованного ОБТК АО «Вектор-Бест». СОП$^+$ имеет титр не менее 1:32. Титр СОП$^+$ – наибольшее разведение, при котором среднее арифметическое значение оптической плотности в лунках с СОП$^+$ больше либо равно величине критического значения оптической плотности $ОП_{крит.}$.

3.2. Специфичность выявления антигена ВКЭ – соответствие результатов определения набором антигена ВКЭ требованиям стандартного образца предприятия (рег. № 05-2-83), аттестованного ОБТК АО «Вектор-Бест»: среднее арифметическое значение оптической плотности в лунках с СОП– меньше $ОП_{крит.}$.

3.3. Диагностическая чувствительность выявления антигена вируса клещевого энцефалита: клинические испытания, проведенные на 136 положительных образцах, показали 100% чувствительность (интервал 97,8–100%, с доверительной вероятностью 90%);

3.4. Диагностическая специфичность выявления антигена вируса клещевого энцефалита: клинические испытания, проведенные на 1238 отрицательных образцах, показали 100% специфичность (интервал 99,8–100%, с доверительной вероятностью 90%).

4. МЕРЫ ПРЕДОСТОРОЖНОСТИ

4.1. Потенциальный риск применения набора – класс 2б (Приказ МЗ РФ от 06.06.2012 № 4н).

4.2. Все компоненты набора являются нетоксичными. Стоп-реагент обладает раздражающим действием. Избегать разбрызгивания и попадания на кожу и слизистые. В случае попадания стоп-реагента на кожу и слизистые необходимо промыть пораженный участок большим количеством проточной воды.

4.3. При работе с исследуемыми образцами следует соблюдать меры предосторожности, принятые при работе с потенциально инфекционным материалом. Основные правила работы изложены в «Инструкции по мерам профилактики распространения инфекционных заболеваний при работе в клинико-диагностических лабораториях лечебно-профилактических учреждений», утвержденной Минздравом СССР 17 января 1991 г. и в методических указаниях МУ 287-113 («Методические указания по дезинфекции, предстерилизационной очистке и стерилизации изделий медицинского назначения», утв. департаментом госсанэпиднадзора Минздрава РФ от 30.12.1998 г.).

4.4. При работе с набором следует надевать одноразовые резиновые или пластиковые перчатки, так как образцы клещей и ликвор человека следует рассматривать как потенциально инфекционные, способные длительное время сохранять и передавать вирус клещевого энцефалита, возбудителей других инфекций.

4.5. Химическая посуда и оборудование, которые используются в работе с набором, должны быть соответствующим образом промаркированы и храниться отдельно.

4.6. Запрещается прием пищи, использование косметических средств и курение в помещениях, предназначенных для работы с наборами.

4.7. Для дезинфекции посуды и материалов, контактировавших с исследуемыми и контрольными образцами, рекомендуем использовать дезинфицирующие средства, не оказывающие негативного воздействия на качество ИФА, не содержащие активный кислород и хлор, например, комбинированные средства на основе ЧАС, спиртов, третичных аминов. Использование дезинфицирующих средств, содержащих активный кислород и хлор (H_2O_2, деохлор, хлорамин), приводит к серьезному искажению результатов.

4.8. При использовании набора образуются отходы классов А, Б и Г, которые классифицируются и уничтожаются (утилизируются) в соответствии с СанПиН 2.1.7.2790-10 «Санитарно-эпидемиологические требования к обращению с медицинскими отходами». Дезинфекцию наборов реагентов следует проводить по МУ 287-113 «Методические указания по дезинфекции, предстерилизационной очистке и стерилизации изделий медицинского назначения».

5. ОБОРУДОВАНИЕ И МАТЕРИАЛЫ, НЕОБХОДИМЫЕ ПРИ РАБОТЕ С НАБОРОМ:

– спектрофотометр вертикального сканирования, позволяющий проводить измерения оптической плотности растворов в лунках стрипов при основной длине волны 450 нм и длине волны сравнения в диапазоне 620–655 нм; допускается измерение только при длине волны 450 нм;
– термостатируемый шейкер орбитального типа на 400 об/мин, поддерживающий температуру (24–26)°С;
– холодильник бытовой;
– бумага фильтровальная лабораторная;
– дозаторы полуавтоматические одноканальные со сменными наконечниками, позволяющие отбирать объемы жидкости 5–5000 мкл (погрешность не более 5%);
– промывочное устройство для планшетов;
– перчатки медицинские диагностические одноразовые;
– цилиндр мерный 2-го класса точности вместимостью 1000 мл;
– вода дистиллированная;
– дезинфицирующий раствор.

Для подготовки суспензий клещей:

– микропробирки вместимостью 1,5–2,0 мл;
– штативы для микропробирок;
– металлические пестики для измельчения клещей и жидкий азот либо гомогенизатор для измельчения биологических материалов;
– центрифуга для микропробирок.

6. АНАЛИЗИРУЕМЫЕ ОБРАЗЦЫ

6.1. Требования к образцам

Для проведения анализа следует использовать целые экземпляры клещей, так как результаты анализа фрагментов могут быть недостоверны.

Допускается использование для анализа ликвора и клещей, хранившихся при температуре (2–8)°С не более 5 суток либо при температуре минус (18–60)°С не более 3 месяцев.

6.2. Подготовка образцов

Ликвор использовать для анализа без дополнительной подготовки.

Для очищения клещей от загрязнений веществами, использованными для удаления присосавшихся особей (масло, бензин и т.п.), перед приготовлением суспензии следует провести их промывку 70%-ным раствором этилового спирта, после чего высушить при помощи фильтровальной бумаги. В случае если клещ не загрязнен, можно сразу приступить к приготовлению суспензии.

Клеща поместить в пронумерованную пробирку типа «Эппендорф» объемом 1,5 мл и плотно закрыть.

При использовании для измельчения клещей гомогенизатора, работу по подготовке проб проводить в соответствии с инструкцией по применению набора.

Для гомогенизации клеща ручным способом пробирку с клещом поместить в емкость с

жидким азотом и заморозить в течение 20 мин. Достать замороженную пробирку с клещом и сразу тщательно измельчить клеща с помощью пестика для гомогенизации клещей, совмещая вращательные движения с надавливанием. Не вынимая пестика, поставить пробирку с измельченным клещом в штатив.

Добавить в пробирку с измельченным клещом 220 мкл РРО. Аккуратно ополоснув пестик (в случае гомогенизации ручным способом) в содержимом пробирки, вынуть его и поместить в дезинфицирующий раствор. Для напитавшихся клещей, а также клещей больших размеров РРО вносить в количестве, пропорциональном их объему, но не менее 400 мкл. Перемешать содержимое пробирки на шейкере (5–10 секунд).

Центрифугировать в течение 5 мин при 3000 об/мин. Для анализа использовать супернатант.

Для каждого образца клеща следует использовать отдельный пестик во избежание контаминации. Для повторного использования пестик необходимо инактивировать: выдержать в 70%-ном растворе этилового спирта в течение 30 мин, затем промыть дистиллированной водой и высушить.

Допускается хранение подготовленных для анализа образцов клещей в течение суток при температуре от 2 до 8°С или в течение 3-х месяцев при температуре минус (18–60)°С. Транспортировка клещей и образцов суспензий

клещей должна осуществляться в специальных термоконтейнерах с хладоэлементом, термосах с термопакетами, льдом.

Не допускается использовать для гомогенизации клеща пористые материалы, такие как керамическая ступка с пестиком.

7. ПРОВЕДЕНИЕ АНАЛИЗА

ПОДГОТОВКА РЕАГЕНТОВ

7.1. Перед работой извлечь набор из холодильника, вскрыть упаковку и выдержать все компоненты при температуре от 18 до 25°С не менее 1 ч.

7.2. Подготовка планшета

Вскрыть пакет выше замка и установить на рамку необходимое для проведения анализа количество стрипов. Оставшиеся неиспользованные стрипы немедленно поместить вновь в пакет с влагопоглотителем, удалить из него воздух, плотно закрыть замок и поместить в холодильник.

Хранить при температуре от 2 до 8°С в течение всего срока годности набора.

7.3. Приготовление промывочного раствора

В соответствии с числом используемых стрипов (см. таблицу расхода компонентов) внести в мерный цилиндр необходимое количество ФСБ-Т×25 и довести до соответствующего объема дистиллированной водой. При выпадении осадка солей в концентрате необходимо про-

греть его при температуре от 30 до 40°С до полного растворения осадка.

Приготовленный промывочный раствор можно хранить при температуре от 2 до 8°С не более 5 суток.

7.4. Подготовка положительного и отрицательного контрольных образцов

Положительный и отрицательный контрольные образцы готовы к использованию и не требуют дополнительного разведения.

Контрольные образцы после первого вскрытия флаконов можно хранить при температуре от 2 до 8°С в течение всего срока годности набора.

7.5. Подготовка конъюгата

Конъюгат готов к использованию.

В соответствии с числом используемых стрипов (см. таблицу расхода компонентов) отобрать в чистый флакон или в пластиковую ванночку для реагента необходимое количество конъюгата.

Остатки конъюгата из флакона или ванночки утилизировать ***(не сливать во флакон с исходным конъюгатом).***

Внимание! *Посуду (ванночки) для конъюгата в случае повторного использования необходимо промыть проточной водой и тщательно ополоснуть дистиллированной водой. Не обрабатывайте посуду, предназначенную для конъюгата, дезинфицирующими растворами и моющими средствами.*

Таблица расхода компонентов набора реагентов

Кол-во используемых стрипов	Промывочный раствор		Конъюгат, мл	Раствор ТМБ, мл
	ФСБ-Т×25, мл	Дистил. вода, мл		
1	2,0	до 50	1,0	1,0
2	4,0	до 100	2,0	2,0
3	6,0	до 150	3,0	3,0
4	8,0	до 200	4,0	4,0
5	10,0	до 250	5,0	5,0
6	12,0	до 300	6,0	6,0
7	14,0	до 350	7,0	7,0
8	16,0	до 400	8,0	8,0
9	18,0	до 450	9,0	9,0
10	20,0	до 500	10,0	10,0
11	22,0	до 550	11,0	11,0
12	24,0	до 600	12,0	12,0

Конъюгат после первого вскрытия флакона можно хранить при температуре от 2 до 8°С в течение всего срока годности набора.

7.6. Подготовка раствора тетраметилбензидина

Раствор ТМБ готов к использованию.

Необходимо исключить воздействие прямого света на раствор ТМБ.

В соответствии с числом используемых стрипов (см. таблицу расхода компонентов) отобрать в

чистый флакон или в пластиковую ванночку для реагента необходимое количество раствора ТМБ.

Остатки раствора ТМБ из флакона или ванночки утилизировать ***(не сливать во флакон с исходным ТМБ).***

Внимание! *Для работы с раствором ТМБ необходимо использовать только одноразовые наконечники. Посуду, предназначенную для раствора ТМБ, нельзя отмывать с применением синтетических моющих средств, поскольку даже их следы ведут к неконтролируемому окислению ТМБ в ходе реакции. После работы посуду ополоснуть водой, промыть 70% этиловым спиртом и тщательно отмыть дистиллированной водой.*

Раствор ТМБ плюс после первого вскрытия флаконов можно хранить при температуре от 2 до 8°С в течение всего срока годности набора.

7.7. Стоп-реагент готов к использованию.

Стоп-реагент после первого вскрытия флакона можно хранить при температуре от 2 до 8°С в течение всего срока годности набора.

ПРОВЕДЕНИЕ ИФА

7.8. В лунки А-1, В-1 внести по 100 мкл отрицательного контрольного образца ($К^-$).

В лунку С-1 внести 100 мкл положительного контрольного образца ($К^+$).

В остальные лунки внести по 100 мкл исследуемых образцов. *Время внесения образцов*

не должно превышать 20 мин при использовании всех лунок планшета.

7.9. Планшет заклеить пленкой и инкубировать 1 час при температуре (24–26)°С в термошейкере с интенсивностью перемешивания 400 об/мин.

7.10. По окончании инкубации снять липкую пленку и поместить ее в сосуд с дезинфицирующим раствором. Для предотвращения засорения игл промывочного устройства рекомендуется по окончании инкубации отобрать при помощи дозатора содержимое лунок с пробами клещей, а затем промыть лунки планшета 5 раз промывочным раствором (п. 7.3) с использованием промывочного устройства, чередуя аспирацию и немедленное заполнение лунок каждого стрипа. В каждую лунку вносить не менее 400 мкл жидкости в процессе каждого цикла промывки. Время между заполнением и опорожнением лунок должно быть не менее 30 сек. Необходимо добиваться полного опорожнения лунок после каждого их заполнения.

7.11. Внести во все лунки по 100 мкл конъюгата.

Для внесения конъюгата использовать ванночку для реагента и одноразовые наконечники, входящие в состав набора.

7.12. Планшет заклеить пленкой и инкубировать 1 час при температуре (24–26)°С в термошейкере с интенсивностью перемешивания 400 об/мин.

7.13. По окончании инкубации промыть планшет как описано в п. 7.10.

7.14. Внести во все лунки по 100 мкл раствора тетраметилбензидина.

Для внесения раствора тетраметилбензидина использовать ванночку для реагента и одноразовые наконечники, входящие в состав набора.

Планшет выдержать в защищенном от света месте в течение 25 мин при температуре от 18 до 25°С.

7.15. Внести во все лунки по 100 мкл стоп-реагента.

8. РЕГИСТРАЦИЯ РЕЗУЛЬТАТОВ

8.1. Измерить оптическую плотность с помощью спектрофотометра в режиме: основной фильтр – 450 нм, референс-фильтр – в диапазоне 620–655 нм. Допускается измерение без использования референс-фильтра.

Время между остановкой реакции и измерением оптической плотности не должно превышать 5 мин.

9. УЧЕТ РЕЗУЛЬТАТОВ РЕАКЦИИ

9.1. Рассчитать среднее арифметическое значение оптической плотности в лунках с отрицательным контрольным образцом.

9.2. На основании полученных данных вычислить критическое значение оптической плотности ($ОП_{крит.}$) по формуле:

$$ОП_{крит.} = ОП_{ср.}К^{-} + 0{,}25$$

где $ОП_{ср.}К^{-}$ – среднее арифметическое значение оптической плотности в лунках с отрицательным контрольным образцом, измеренное в двухволновом режиме 450/620–655 нм (или только с фильтром 450 нм).

9.3. Оценка результатов:

– среднее арифметическое значение оптической плотности в лунках с отрицательным контрольным образцом должно быть не более 0,2 ед. опт. плотн.

– значение оптической плотности в лунке с положительным контрольным образцом должно быть не менее 1,8 ед. опт. плотн.

9.4. Только при соблюдении положений п. 9.3 можно учитывать результаты, полученные для анализируемых образцов сыворотки (плазмы) крови.

Результат анализа считать **положительным**, если $ОП_{обр.} \geq ОП_{крит.}$.

Результат анализа считать **отрицательным**, если $ОП_{обр.} < ОП_{крит.}$.

10. УСЛОВИЯ ТРАНСПОРТИРОВАНИЯ, ХРАНЕНИЯ И ПРИМЕНЕНИЯ НАБОРА

10.1. Транспортировать изделия следует транспортом всех видов в крытых транспортных средствах в соответствии с правилами перевозок, действующими на транспорте данного вида, при температуре от 2 до 8°С. Допускается транспортирование при температуре до 25°С не более 10 суток.

10.2. Хранение набора в упаковке предприятия-изготовителя должно осуществляться при

температуре от 2 до 8°С в течение всего срока годности в холодильных камерах или холодильниках, обеспечивающих регламентированный температурный режим с ежедневной регистрацией температуры.

10.3. Срок годности набора – 12 месяцев со дня выпуска. Не допускается применение наборов по истечении срока их годности.

10.4. Дробное использование набора может быть реализовано в течение всего срока годности набора. В случае дробного использования набора:

- неиспользованные стрипы можно хранить в плотно закрытом пакете при температуре от 2 до 8°С в течение всего срока годности;
- концентрат фосфатно-солевого буферного раствора с твином, раствор для разведения образцов, раствор тетраметилбензидина, контрольные образцы, конъюгат и стоп-реагент после вскрытия флаконов можно хранить в плотно закрытых флаконах при температуре от 2 до 8°С в течение всего срока годности;
- промывочный раствор можно хранить при температуре от 2 до 8°С не более 5 сут.

10.5. Для получения надежных результатов необходимо строгое соблюдение инструкции по применению набора.

10.6. При постановке ИФА нельзя использовать компоненты из наборов разных серий или смешивать их при приготовлении растворов, кроме неспецифических компонентов (ФСБ-Т×25, раствор ТМБ, стоп-реагент), которые взаимозаменяемы в наборах АО «Вектор-Бест».

11. ГАРАНТИЙНЫЕ ОБЯЗАТЕЛЬСТВА

11.1. Производитель гарантирует соответствие выпускаемых изделий требованиям нормативной и технической документации.

Безопасность и качество изделия гарантируются в течение всего срока годности.

11.2. Производитель отвечает за недостатки изделия, за исключением дефектов, возникших вследствие нарушения правил пользования, условий транспортирования и хранения, либо действия третьих лиц, либо непреодолимой силы.

11.3. Производитель обязуется за свой счет заменить изделие, технические и функциональные характеристики (потребительские свойства) которого не соответствуют нормативной и технической документации, если указанные недостатки явились следствием скрытого дефекта материалов или некачественного изготовления изделия производителем.

По вопросам, касающимся качества набора «ВектоВКЭ-антиген»,

следует обращаться в АО «Вектор-Бест»
по адресу:

630559, Новосибирская область,
Новосибирский район,
р. п. Кольцово, а/я 121,
тел. (383) 227-67-64.
E-mail: vbobtk@vector-best.ru

ДОПОЛНИТЕЛЬНАЯ ИНФОРМАЦИЯ ДЛЯ ПОТРЕБИТЕЛЕЙ

Набор предназначен для профессионального применения в клинической лабораторной диагностике обученным персоналом.

Требования безопасности к медицинским лабораториям приведены в ГОСТ Р 52905-2007.

Все реагенты наборов, содержащие в своем составе материалы человеческого происхождения, инактивированы.

1. Обеспечение получения правильных результатов анализа

Достоверность и воспроизводимость результатов анализа зависят от выполнения следующих основных правил:

– не проводите ИФА в присутствии паров кислот, щелочей, альдегидов или пыли, которые могут менять ферментативную активность конъюгатов;

– ферментативная реакция чувствительна к присутствию ионов металлов, поэтому не допускайте контактов каких-либо металлических предметов с конъюгатом и раствором ТМБ;

– избегайте загрязнения компонентов набора микроорганизмами и химическими примесями, для этого используйте в работе чистую посуду и чистые одноразовые наконечники для каждого реагента, контроля, образца;

– рабочие поверхности столов, оборудования следует обрабатывать 70% этиловым спир-

том (не допускается использование перекиси водорода, хлорсодержащих растворов);

– никогда не используйте одну и ту же емкость для конъюгата и раствора ТМБ;

– перед отбором раствора ТМБ из флакона необходимо протереть конус пипетки (внутреннюю и внешнюю поверхности) сначала дистиллированной водой, а затем 70% этиловым спиртом, так как малейшее загрязнение пипеток конъюгатом может привести к контаминации всего содержимого флакона;

– если допущена ошибка при внесении анализируемых образцов, нельзя, опорожнив эту лунку, вносить в нее новый образец; такая лунка бракуется.

Качество промывки лунок планшета играет важную роль для получения правильных результатов анализа:

– Для аспирации анализируемых образцов и последующей промывки рекомендуется использовать автоматическое или ручное промывочное устройство.

– Не допускайте высыхания лунок планшета в перерыве между завершением промывки и внесением реагентов.

– Добивайтесь полного заполнения и опорожнения всех лунок планшета в процессе промывки. Недостаточная аспирация жидкости в процессе промывки может привести к понижению чувствительности и специфичности анализа.

– Следите за состоянием промывочного устройства – регулярно (1 раз в неделю) обрабатывайте шланги и емкости 70% этиловым спиртом.

– Для предотвращения засорения игл промывочного устройства в конце рабочего дня обязательно выполните процедуру ополаскивания системы подачи жидкости дистиллированной водой.

2. Краткая схема проведения ИФА для набора реагентов «ВектоВКЭ-антиген»

Использовать только после внимательного ознакомления с инструкцией!

Внести:	по 100 мкл контрольных и исследуемых образцов.
Инкубировать:	1 час, 24–26°С, 400 об/мин.
Промыть:	промывочным раствором, 400 мкл, 5 раз.
Внести:	по 100 мкл конъюгата.
Инкубировать:	1 час, 24–26°С, 400 об/мин.
Промыть:	промывочным раствором, 400 мкл, 5 раз.
Внести:	по 100 мкл раствора тетраметилбензидина.
Инкубировать:	25 мин, 18–25°С, в темноте.
Внести:	100 мкл стоп-реагента.
Измерить:	ОП при 450 нм / референсная длина волны 620–655 нм.

3. Графические символы

REF	Номер по каталогу	IVD	Медицинское изделие для диагностики *in vitro*
Σ n	Содержимого достаточно для проведения n-количества тестов	NON STERILE	Не стерильно
LOT	Код партии		Температурный диапазон
	Изготовитель		Дата изготовления
	Использовать до …		Обратитесь к инструкции по применению
	Осторожно! Обратитесь к Инструкции по применению	YYYY-MM-DD YYYY-MM	Дата в формате Год-Месяц-День Год-Месяц

Консультацию специалиста по работе с набором можно получить по тел.: (383) 363-57-86.

13.02.17.

Certificate

TÜVRheinland®

Quality Management System
EN ISO 13485:2016

Registration No.: SX 1483000-1

Organization:
EUROIMMUN
Medizinische Labordiagnostika AG
Seekamp 31
23560 Lübeck
Germany

Scope: Design and development, manufacture, installation, service and distribution of immunobiochemical test systems, immunofluorescence test systems, molecular diagnostic / genetic test systems, test systems for the determination of infectious agents, and instruments / software for in vitro diagnostics

The Certification Body of TÜV Rheinland LGA Products GmbH certifies that the organization has established and applies a quality management system for medical devices.
Proof has been furnished that the requirements specified in the abovementioned standard are fulfilled. The quality management system is subject to yearly surveillance.

Report No.: 3313978-90

Effective date: 2020-05-19

Expiry date: 2023-05-18

Issue date: 2020-05-14

TÜV Rheinland LGA Products GmbH · TÜVRheinland · Zertifizierungsstelle

D. Wiedemuth

Dipl.-Ing. (FH) D. Wiedemuth
TÜV Rheinland LGA Products GmbH
Tillystraße 2 · 90431 Nürnberg · Germany

DAkkS
Deutsche
Akkreditierungsstelle
D-ZM-14169-01-02

Certificate

TÜVRheinland®

Quality Management System
EN ISO 13485:2016

Registration No.: SX 1483000-1

Organization:
EUROIMMUN
Medizinische Labordiagnostika AG
Seekamp 31
23560 Lübeck
Germany

No.	Facility	Scope
/01	EUROIMMUN Medizinische Labordiagnostika AG Seekamp 31 23560 Lübeck Germany	Design and development and manufacture of immunobiochemical test systems, immunofluorescence test systems, molecular diagnostic / genetic test systems, test systems for the determination of infectious agents, and instruments / software for instruments for in vitro diagnostics
/02	EUROIMMUN Medizinische Labordiagnostika AG Werkstr. 1 23942 Dassow Germany	Design, development, manufacture and distribution of immunobiochemical test systems, immunofluorescence test systems, molecular diagnostic / genetic test systems, test systems for the determination of infectious agents and instruments / software for in vitro diagnostics
/03	EUROIMMUN Medizinische Labordiagnostika AG An der Trave 1 23923 Selmsdorf Germany	Design, development, manufacture, service and distribution of immunofluorescence test systems, molecular diagnostic / genetic test systems, test systems for the determination of infectious agents and instruments / software for in vitro diagnostics

Report No.: 3313978-90

Effective date: 2020-05-19

Expiry date: 2023-05-18

Issue date: 2020-05-14

TÜV Rheinland LGA Products GmbH Zertifizierungsstelle TÜVRheinland

D. Wiedemuth

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DAkkS
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D-ZM-14169-01-02

Certificate

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Quality Management System
EN ISO 13485:2016

Registration No.: SX 1483000-1

Organization:
EUROIMMUN
Medizinische Labordiagnostika AG
Seekamp 31
23560 Lübeck
Germany

/04	EUROIMMUN Medizinische Labordiagnostika AG Am Sonnenberg 9 23627 Groß Grönau Germany	Design, development and manufacture of immunofluorescence test systems, molecular diagnostic / genetic test systems and, test systems for the determination of infectious agents for in vitro diagnostics
/05	EUROIMMUN Medizinische Labordiagnostika AG Am Born 24 23627 Groß Grönau Germany	Design and development of software for in vitro diagnostics
/06	EUROIMMUN Medizinische Labordiagnostika AG Im Kreppel 1 02747 Herrnhut Germany	Manufacture of immunobiochemical test systems, immunofluorescence test systems, molecular diagnostic / genetic test systems and test systems for the determination of infectious agents for in vitro diagnostics
/07	EUROIMMUN Medizinische Labordiagnostika AG Am Pließnitztal 1 02748 Bernstadt Germany	Manufacture of immunobiochemical test systems, test systems for the determination of infectious agents and instruments for in vitro diagnostics

Report No.: 3313978-90

Effective date: 2020-05-19

Expiry date: 2023-05-18

Issue date: 2020-05-14

TÜV Rheinland LGA Products GmbH Zertifizierungsstelle

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DAkkS
Deutsche
Akkreditierungsstelle
D-ZM-14169-01-02

Certificate

TÜVRheinland®

Quality Management System
EN ISO 13485:2016

Registration No.: SX 1483000-1

Organization:
EUROIMMUN
Medizinische Labordiagnostika AG
Seekamp 31
23560 Lübeck
Germany

/08	EUROIMMUN Medizinische Labordiagnostika AG Schloßstr. 11 91257 Pegnitz Germany	Manufacture of immunofluorescence test systems, installation and service of instruments / software for in vitro diagnostic
/09	EUROIMMUN Medizinische Labordiagnostika AG Am Flugplatz 4 23560 Lübeck Germany	Installation, service and distribution of immunobiochemical test systems, immunofluorescence test systems, molecular diagnostic / genetic test systems, test systems for the determination of infectious agents, and instruments / software for in vitro diagnostics
/10	EUROIMMUN Medizinische Labordiagnostika AG Gewerbestr. 19 23942 Dassow Germany	Manufacture of sheet metal and other components for instruments for in vitro diagnostics

Report No.: 3313978-90

Effective date: 2020-05-19

Expiry date: 2023-05-18

Issue date: 2020-05-14

TÜV Rheinland LGA Products GmbH Zertifizierungsstelle TÜVRheinland

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DAkkS
Deutsche
Akkreditierungsstelle
D-ZM-14169-01-02

Certificate

Standard **ISO 9001:2015**

Certificate Registr. No. **01 100 1810000**

Certificate Holder: **EUROIMMUN**
Medizinische Labordiagnostika AG
Seekamp 31
23560 Lübeck
Germany

including the locations according to annex

Scope: Design, development, manufacture, installation, service and sales of immunobiochemical test systems, immunofluorescence test systems, molecular diagnostic / genetic test systems, test systems for the determination of infectious agents, and instruments / software for in vitro diagnostics in humans and animals; trainings

Proof has been furnished by means of an audit that the requirements of ISO 9001:2015 are met.

Validity: The certificate is valid from 2020-05-19 until 2023-05-18.
First certification 2018

2021-10-12 (Change)

TÜV Rheinland Cert GmbH
Am Grauen Stein · 51105 Köln

www.tuv.com

DAkkS Deutsche Akkreditierungsstelle D-ZM-16031-01-00

TÜVRheinland®
Precisely Right.

Annex to certificate

Standard **ISO 9001:2015**

Certificate Registr. No. **01 100 1810000**

No.	Location	Scope
/01	c/o EUROIMMUN Medizinische Labordiagnostika AG Seekamp 31 23560 Lübeck Germany	Design, development, manufacture, installation, service and sales of immunobiochemical test systems, immunofluorescence test systems, molecular diagnostic / genetic test systems, test systems for the determination of infectious agents, and instruments / software for in vitro diagnostics in humans and animals; trainings
/02	c/o EUROIMMUN Medizinische Labordiagnostika AG Werkstr. 1 23942 Dassow Germany	Design, development, manufacture and sales of immunobiochemical test systems, immunofluorescence test systems, molecular diagnostic / genetic test systems, test systems for the determination of infectious agents and instruments / software for in vitro diagnostics in humans and animals
/03	c/o EUROIMMUN Medizinische Labordiagnostika AG An der Trave 1 23923 Selmsdorf Germany	Design, development, manufacture, service and sales of immunobiochemical test systems, immunofluorescence test systems and instruments / software for in vitro diagnostics for humans
/04	c/o EUROIMMUN Medizinische Labordiagnostika AG Am Sonnenberg 9 23627 Groß Grönau Germany	Design, development and manufacture of immunofluorescence test systems for in vitro diagnostics for humans and animals
/05	c/o EUROIMMUN Medizinische Labordiagnostika AG Am Born 24 23627 Groß Grönau Germany	Design and development of software for in vitro diagnostics for humans and animals

Annex to certificate

Standard **ISO 9001:2015**

Certificate Registr. No. **01 100 1810000**

/06	c/o EUROIMMUN Medizinische Labordiagnostika AG Im Kreppel 1 02747 Herrnhut Germany	Manufacture of immunobiochemical test systems and immunofluorescence test systems for in vitro diagnostics for humans
/07	c/o EUROIMMUN Medizinische Labordiagnostika AG Am Pließnitztal 1 02748 Bernstadt Germany	Manufacture of immunobiochemical test Systems, test systems for the determination of infectious agents and instruments for in vitro diagnostics for humans
/08	c/o EUROIMMUN Medizinische Labordiagnostika AG Schloßstr. 11 91257 Pegnitz Germany	Manufacture of immunofluorescence test systems, installation and service of instruments / software for in vitro diagnostics in humans, trainings
/09	c/o EUROIMMUN Medizinische Labordiagnostika AG Am Flugplatz 4 23560 Lübeck Germany	Design, development, installation, service and sales of immunobiochemical test systems, immunofluorescence test systems, molecular diagnostic / genetic test systems, test systems for the determination of infectious agents and instruments / software for in vitro diagnostics for humans and animals; trainings
/10	c/o EUROIMMUN Medizinische Labordiagnostika AG Gewerbestr. 19 23942 Dassow Germany	Manufacture of sheet metal and other components for instruments for in vitro diagnostics in humans and animals

2021-10-12

TÜV Rheinland Cert GmbH
Am Grauen Stein · 51105 Köln

CE

Declaration of Conformity

EUROIMMUN Medizinische Labordiagnostika AG
Seekamp 31, D-23560 Lübeck, Germany

declare under our sole responsibility that the WESTERBLOT products

Anti-Borrelia burgdorferi-WESTERNBLOT (IgG)	*DY 2132-#### G*
Anti-Borrelia burgdorferi-WESTERNBLOT (IgM)	*DY 2132-#### M*

(product name, order no)

meet the demands of

Directive 98/79/EC on in vitro diagnostic medical devices of 27 October 1998 and its transpositions in national laws which apply to it.

Conformity assessment procedure: Annex III

Lübeck, 04.10.2016
(Place and date of issue)

Dr. Wolfgang Schlumberger
- Member of the Board -

Susanne Aleksandrowicz
- Member of the Board -

Anti-Borrelia burgdorferi-WESTERNBLOT (IgG)
Test instruction

ORDER NO.	ANTIBODIES AGAINST	IG CLASS	SUBSTRATE	FORMAT
DY 2132-3001 G DY 2132-24001 G	Borrelia burgdorferi (complete antigens)	IgG	Antigen coated membrane strips	30 x 01 (30) 240 x 01 (240)

Indication: The Westernblot test kit provides a qualitative in vitro assay for human autoantibodies of the immunoglobulin class IgG against Borrelia in serum or plasma for the diagnosis of Lyme borreliosis and associated diseases (Erythema chronicum migrans, lymphadenosis cutis benigna, acrodermatitis chronica atrophicans, arthritis, carditis, lymphocytic meningoradiculoneuritis and neuroborreliosis).

Principles of the test: The test kit contains test strips with electrophoretically separated antigen extracts of Borrelia burgdorferi. The blot strips will be blocked and incubated in the first reaction step with diluted patient samples. In the case of positive samples, specific antibodies of the class IgG (and IgA, IgM) will bind to the antigens. To detect the bound antibodies, a second incubation is carried out using an enzyme-labelled anti-human IgG (enzyme conjugate) catalysing a colour reaction.

Contents of the test kit:

Component	Format	Format	Symbol
1. Test strips Single strips with electrophoretically separated Borrelia burgdorferi antigens	30 x 1	240 x 1	STRIPS
2. Evaluation matrix with control strip Test strip incubated with a positive control serum	1 pattern	1 pattern per test strip lot	---
3. Enzyme conjugate Alkaline phosphatase-labelled anti-human IgG (goat), 10x concentrate	2 x 3 ml	16 x 3 ml	CONJUGATE 10x
4. Universal buffer 10x concentrate	1 x 100 ml	8 x 100 ml	BUFFER 10x
5. Substrate solution Nitroblue tetrazolium chloride/5-Bromo-4-chloro-3-indolylphosphate (NBT/BCIP), ready for use	1 x 50 ml	8 x 50 ml	SUBSTRATE
6. Adhesive foil	---	8 sheets	---
7. Test instruction	1 booklet	1 booklet	---

LOT Lot description — CE — Storage temperature

IVD In vitro diagnostic medical device — Unopened usable until

Storage and stability: The test kit has to be stored at a temperature between +2°C to +8°C, do not freeze. Unopened, all test kit components are stable until the indicated expiry date.

Waste disposal: Undiluted patient samples and incubated blot strips should be handled as infectious waste. Other reagents do not need to be collected separately, unless stated otherwise in official regulations.

The following components are not provided in the test kits but can be ordered at EUROIMMUN under the respective order numbers.Performance of the test requires an **incubation tray**:
ZD 9895-0130 Incubation tray with 30 channels (black)
ZD 9898-0144 Incubation tray with 44 channels (black, for the EUROBlotOne and EUROBlotCamera system)
For the creation of work protocols and the evaluation of incubated test strips using **EUROLineScan** green paper and adhesive foil are required:
ZD 9880-0101 Green paper (1 sheet)
ZD 9885-0116 Adhesive foil for approx. 16 test strips
ZD 9885-0130 Adhesive foil for approx. 30 test strips
If a **visual evaluation** is to be performed in individual cases, the required evaluation protocol can be ordered under: ZD 2132-0101 Evaluation protocol visual Anti-Borrelia burgdorferi-WESTERNBLOT.

Preparation and stability of the reagents

Note: The bag containing the blot strips is printed with a number in addition to the test kit lot number. This number refers to the strip batch and is also printed on the corresponding evaluation template. These two numbers must match to ensure correct evaluation of test results.
All reagents must be brought to room temperature (+18°C to +25°C) approx. 30 minutes before use. Unopened, reagents are stable until the indicated expiry date when stored at +2°C to +8°C. After initial opening, reagents are stable for 12 months or until the expiry date, if earlier, unless stated otherwise in the instructions. Opened reagents must also be stored at +2°C to +8°C and protected from contamination.

- **Coated test strips:** Ready for use. Open the packing with the test strips only when the strips have reached room temperature to prevent condensation on the strips. After removal of the strips the packing should be sealed tightly and stored at +2°C to +8°C. To ensure correct evaluation of results, the lot number on the bag must match the lot number on the strips as well as on the evaluation matrix.

- **Enzyme conjugate:** The enzyme conjugate is supplied as a 10x concentrate. For the preparation of the ready for use enzyme conjugate the amount required should be removed from the bottle using a clean pipette and diluted 1:10 with ready for use diluted universal buffer. For 1 test strip dilute 0.15 ml anti-human IgG concentrate with 1.35 ml ready for use diluted universal buffer. The ready for use diluted enzyme conjugate should be used at the same working day.

- **Universal buffer:** The universal buffer is supplied as a 10x concentrate. For the preparation of the ready for use universal buffer the amount required should be removed from the bottle using a clean pipette and diluted 1:10 with deionised or distilled water. For the incubation of 1 test strip 1.5 ml buffer concentrate should be diluted with 13.5 ml deionised or distilled water. The ready for use diluted universal buffer should be used at the same working day.

- **Substrate solution:** Ready for use. Close bottle immediately after use, as the contents are sensitive to light.

Warning: The control of human origin has tested negative for HBsAg, anti-HCV, anti-HIV-1 and anti-HIV-2. Nonetheless, all materials should be treated as being a potential infection hazard and should be handled with care. Some of the reagents contain the agent sodium azide in a non-declarable concentration. Avoid skin contact.

Preparation and stability of the serum or plasma samples

Sample material: Human serum or EDTA, heparin or citrate plasma.

Stability: Patient samples to be investigated can generally be stored at +2°C to +8°C for up to 14 days. Diluted samples should be incubated within one working day.

Sample dilution: The **patient samples** for analysis are diluted **1:51** in ready for use diluted universal buffer. For example, add 30 µl of sample to 1.5 ml ready for use diluted universal buffer and mix well by vortexing. Sample pipettes are not suitable for mixing.

Incubation

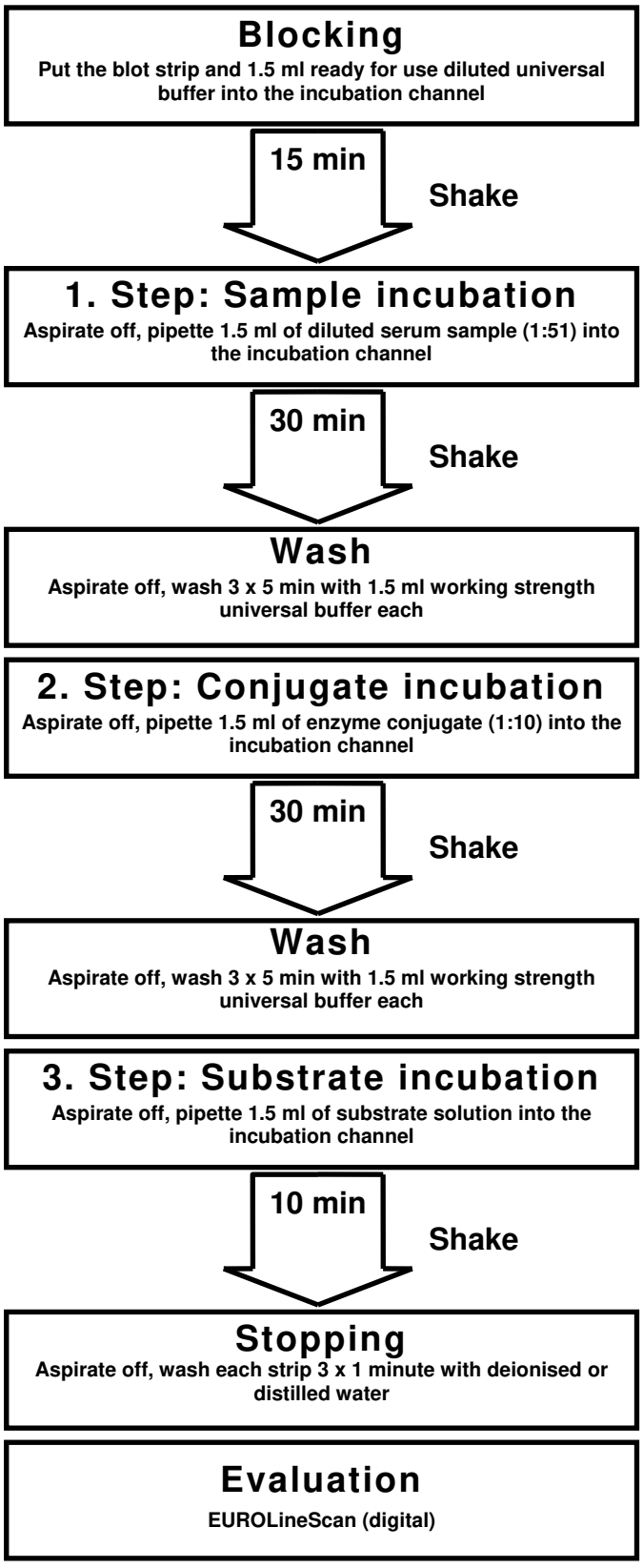
Blocking:	According to the number of serum samples to be tested fill each channel of the incubation tray with 1.5 ml ready for use diluted universal buffer and a blot strip. Remove the required amount of blot strips from the packing using a pair of tweezers. The number on the test strip should be visible. Incubate for **15 minutes** at room temperature (+18°C to +25°C) on a rocking shaker. Afterwards aspirate off all the liquid.
Sample incubation: (1st step)	Fill each channel with 1.5 ml of the diluted serum samples. Incubate at room temperature (+18°C to +25°C) for **30 minutes** on a rocking shaker.
Wash:	Aspirate off the liquid from each channel and wash **3 x 5 minutes** each with 1.5 ml working strength universal buffer on a rocking shaker.
Conjugate incubation: (2nd step)	Pipette 1.5 ml diluted enzyme conjugate (alkaline phosphatase-conjugated anti-human IgG) into each channel. Incubate for **30 minutes** at room temperature (+18°C to +25°C) on a rocking shaker.
Wash:	Aspirate off the liquid from each channel. Wash as described above.
Substrate incubation: (3rd step)	Pipette 1.5 ml substrate solution into the channels of the incubation tray. Incubate for **10 minutes** at room temperature (+18°C to +25°C) on a rocking shaker.
Stopping:	Aspirate off the liquid from each channel and wash each strip **3 x 1 minute** with deionised or distilled water.

For automated incubation with the EUROBlotMaster select the program **Euro02 Inf WB30**.

For automated incubation with the EUROBlotOne select the program **Euro 01/02**.

Anti-Borrelia burgdorferi-WESTERNBLOT (IgG)

Incubation protocol



Anti-Borrelia burgdorferi-WESTERNBLOT (IgG)
Evaluation and interpretation

Handling: For evaluation of incubated test strips we generally recommend using the **EUROLineScan** software. After stopping the reaction using deionised or distilled water, place the incubated test strips onto the adhesive foil of the green work protocol using a pair of tweezers. The position of the test strips can be corrected while they are wet. As soon as all test strips have been placed onto the protocol, they should be pressed hard using filter paper and left to air-dry. After they have dried, the test strips will be stuck to the adhesive foil. The dry test strips are then scanned using a flatbed scanner (EUROIMMUN AG) and evaluated with **EUROLineScan**. Alternatively, imaging and evaluation is possible directly from the incubation trays (EUROBlotCamera and EUROBlotOne). For general information about the EUROLineScan program please refer to the EUROLineScan user manual (EUROIMMUN AG). The code for entering the **Test** in EUROLineScan is **B.b._WB_IgG**.

If a visual evaluation must be performed in exceptional cases, hold the evaluation matrix next to the stuck-on blot strips and position it so that the black band above the number on the blot strips lines up with the alignment bar of the evaluation matrix. **The lot number on the evaluation matrix must match the lot number on the blot strips.** Clearly recognisable bands on the blot strips which concur with the labelled bands on the evaluation matrix are noted in the evaluation protocol.

Antigens: The antigen source for the EUROIMMUN Anti-Borrelia burgdorferi-WESTERNBLOT is provided by a particularly suitable Borrelia burgdorferi strain. The cultured Borrelia have been solubilised using sodium dodecyl sulphate followed by a separation of the solubilised protein using discontinuous polyacrylamide gel electrophoresis according to molecular mass and transfer of the separated proteins to nitrocellulose. From each test kit, 2 control test strips have been removed and incubated with a reference serum. One of these stained strips is included in the kit, the other remains with EUROIMMUN for documentation purposes. Diagnostically relevant antigens have been characterised with monoconal reference antibodies from the German National Reference Laboratory for Borreliae.

Specificity of the antigens on the test strips:

Band	Antigen	Specificity
83 kDa	Membrane-vesical protein, p83	Degradation product of p100, **high specificity**
75 kDa	Heat shock protein, p75	Unspecific
62 kDa	Heat shock protein, p62	Unspecific
57/59 kDa	p57 and p59	Unspecific
50 kDa	p50	Unspecific
47 kDa	p47	Probably genus specific
43 kDa	p43	Unspecific
41 kDa	Flagellin, p41	Genus specific, cross reactivity to other spirochaetaceae and bacteria having flagella
39 kDa	Bmp A, p39	**High specificity**
34 kDa	Osp B, p34	Outer surface protein B, **high specificity**
32 kDa	p32	Unspecific
31 kDa	Osp A, p31	Outer surface protein A, **high specificity**
29 kDa	p29	Probably specific, poorly investigated
28 kDa	p28	Unspecific
25 kDa	Osp C, p25	Outer surface protein C, **high specificity**
21/22 kDa	p21/22	**High specificity**
18 kDa	p18	**Probably specific**
17 kDa	p17	Poorly investigated

In the lower part of the test strip there is a conjugate control membrane chip (IgA, IgG and IgM). Below the conjugate control, there is a membrane chip with a control band (Control).

Attention: A correctly performed determination of antibodies of class IgG against the antigens described above is indicated by a positive reaction of the control band and a positive reaction of the IgG band.

If one of these bands only shows a very weak reaction or none at all, the result is not valid.

Specificity of the antigens: Borrelia burgdorferi antigens can generally be divided into three categories.

Category	Antigens
1	Cross-reacting and undefined antigens with the molecular mass 17 kDa, 28 kDa, 32 kDa, 43 kDa, 47 kDa, 50 kDa, 57 kDa, 59 kDa, 62 kDa and 75 kDa.
2	A genus-specific antigen with a molecular mass of 41 kDa (flagellin).
3	Species-specific and highly specific antigens with the molecular mass 18 kDa, 21/22 kDa, 25 kDa, 31 kDa, 34 kDa, 39 kDa and 83 kDa.

IgG class antibodies against Borrelia burgdorferi

Interpretation of results: In order to evaluate the signals, the band positions and intensity of staining must be taken into consideration, as negative sera sometimes produce weak signals in individual bands. Based on experience, the results of the Borrelia burgdorferi WESTERNBLOT test can be divided into negative, borderline and positive results.

Result	Characteristics
Negative	No bands, or weak intensities of some antigens from categories 1 and 2.
Borderline	A distinctive band from category 3 (antigens in category 3 are shaded grey in the evaluation protocol!) **and** several distinctive signals from categories 1 and 2. It is recommended that a new sample be taken and the test repeated after a few weeks.
Positive	More than one distinctive band from category 3 (antigens in category 3 are shaded grey in the evaluation protocol!). In addition, and particularly in the case of patients in the late stage of the disease, numerous bands from categories 1 and 2 can be observed.

IgM class antibodies against Borrelia burgdorferi

Interpretation of results: In order to evaluate the signals, the band positions and intensity of staining must be taken into consideration. The results of the Borrelia burgdorferi WESTERNBLOT test can be divided into negative, borderline and positive results.

In the early phase of a Borrelia infection IgM antibodies are typically directed against Osp C (p25). IgM antibodies against other specific Borrelia antigens are not considered definitive indicators of a fresh Borrelia infection.

IgM antibodies against flagellin (p41) can represent the initial response of the body to Borrelia burgdorferi. However, an unspecific reaction cannot be excluded, since it is known that antibodies directed against other microorganisms cross react with Borrelia burgdorferi flagellin (p41). For this reason, a single band at the position of flagellin (p41) in IgM detection should not be considered proof of a fresh infection with Borrelia burgdorferi. If only the flagellin band (p41) reacts positively, the test should be repeated several weeks later with a fresh blood sample.

In the serological investigation of a Borrelia infection the determination of antibodies of class IgM often yields unclear results. IgM antibodies can sometimes be found in serum years after an infection or following antibiotic treatment. Therefore, the detection of IgM antibodies does not necessarily indicate a fresh infection. A negative IgM results does not exclude a fresh infection. With a second infection, only antibodies of class IgG and not IgM can be formed.

In the late stage of borreliosis a positive IgM result does not provide any additional information, due to the antibody persistence mentioned above. The cause of such false-positive IgM results often remains unclear. They are observed, for example, in infectious mononucleosis, herpes virus infections and various autoimmune diseases.

For the diagnosis of a fresh Borrelia infection, a positive IgM result should be confirmed with a positive IgG result using a fresh blood sample 3 to 6 weeks later.

Result	Characteristics
Negative	No antigen bands recognisable or weak intensities of some bands of category 1
Borderline	One antigen band of category 2 (flagellin, p41) or a weak band of category 3. It is recommended that a fresh sample be taken and the test repeated after a few weeks. Antibodies against Osp C are characteristic for a fresh infection.
Positiv	At least one distinctive band from category 3 (antigens in category 3 are shaded grey in the evaluation protocol!). Antibodies against Osp C are characteristic for a fresh infection.

For diagnosis, the clinical picture of the patient always needs to be taken into account along with the serological findings.

Test characteristics

Measurement range: The Westernblot is a qualitative method. No measurement range is provided.

Inter- and intra-assay variation: The inter-assay variation was determined by multiple analyses of characterised samples over several days. The intra-assay variation was determined by multiple analyses of characterised samples on one day. In every case, the intensity of the bands was within the specified range. This Westernblot displays excellent inter- and intra-assay reproducibility.

Interference: Haemolytic, lipaemic and icteric sera showed no effect on the analytical results.

Prevalence: Sera from 156 clinically characterised patients and 517 healthy blood donors were investigated with the EUROIMMUN Anti-Borrelia burgdorferi-Westernblot.

		Prevalence		
Clinically characterised sera	**n**	**IgG**	**IgM**	**IgM/IgG**
Erythema migrans	108	64%	67%	84%
Neuroborreliosis	32	85%	44%	88%
Arthritis	10	100%	30%	100%
Acrodermatitis	6	83%	50%	100%

		Prevalence	
Serum samples	**n**	**IgG**	**IgM**
Healthy blood donors *	517	5%	4%

*Medical University of Luebeck

The prevalence of anti-Borrelia antibodies from the healthy blood donor samples agrees with the values from literature [14].

Cross reactivity: The quality of the antigen used (whole antigen, SDS extract) and the antigen source (Borrelia burgdorferi sensu stricto) ensure high specificity of the Westernblot. The determination of cross reactivity is not necessary with the Westernblot, since specific reactions and unspecific or cross reactions can be differentiated directly with this test system.

Clinical significance

The history of Lyme disease, a contagious condition caused by Borrelia burgdorferi and transmitted to humans by ticks, offers infectiologists a formidable lesson on how medicine progresses. Clinical description started in Europe at the turn of the $19^{th}/20^{th}$ century with Pick's description of what was then labelled chronic atrophic acrodermatitis. Fifty years later, Hauser noted the affection was transmitted by ticks. Independently, Afzelius, then Lipschutz, described erythema migrans and its relationship with tick bites. Neurological involvement was also described with the skin signs. These early dermatological descriptions suddenly came into the limelight in 1975 when an epidemia of arthritis occurred in children in Lyme, Connecticut, USA. Many of the affected children had erythema migrans. Based on these observations and an epidemiological analysis of the epidemia, Steele and co-workers defined "Lyme disease" as a rheumatological disorder commonly associated with erythema migrans and sometimes with multiple organ involvement. In 1982 Burgdorfer suggested that ticks transmitted "treponema-like spirochaetes", which were later authentified as the causal agent: Borrelia burgdorferi.

Borrelia burgdorferi as a species of bacteria is belonging to the spirochaetaceae family. This family includes five genera: Borrelia, spirochetes, cristispira, treponema and leptospira. Among about 30 tick species feeding on humans, Ixodes ricinus is the most frequent tick species biting humans in Europe. It is the vector of Borrelia burgdorferi, which causes Lyme disease, of anaplasma phagocytophilum and the tick-borne encephalitis virus. I. ricinus ticks pass through three developmental stages: larvae, nymphs and adults (females and males). The density of this tick species can be very high, reaching in some places more than 300 ticks/100 m^2. Lyme borreliosis is the most frequent tick-transmitted disease in the northern hemisphere. In Europe, especially in Central Europe and Scandinavia there are up to 155 cases per 100,000 individuals caused by the species B. burgdorferi sensu stricto, B. afzelii, and B. garinii. The human seroprevalence rate of antibodies against Borrelia burgdorferi in the normal population of Germany and other Central European countries is about 8% (for IgG); in highly endemic areas even considerably higher with ranges from 18% to 52%. In East Asia, e.g. in an endemic area in China, the seroprevalence amounts to 26%. Persons working in the area of forestry display anti-Borrelia antibodies in about 40%, hunters in more than 50% of cases.

A Borrelia burgdorferi infection can manifest itself in the areas of dermatology, neurology, ophthalmology, rheumatology and internal medicine. The clinical expression of borreliosis can be divided into three stages:

Stage I: The typical primary manifestation of a Borrelia burgdorferi infection is erythema migrans (80%), a reddening of the skin which appears around the area of the tick bite and spreads in a circular manner. The erythema is accompanied by influenza-like general symptoms with fever, shaking chill, headaches and vomiting. Lymphadenopathies are observed in a few cases (lymphadenosis cutis benigna). The leading clinical types of the erythema-free borreliosis (20%) are neurological, arthromyalgic, influenza-like, cardiovascular borreliosis and borreliosis with hepatitis, with regional lymphadenitis and mixtures of these. Stage I can result in spontaneous healing or can develop into a generalised borreliosis. The transition phase is generally symptom-free. IgM antibodies against Borrelia burgdorferi can be detected serologically in 50% to 90% of patients during stage I. Humans produce highly specific antibodies against the outer surface protein C (OspC) shortly after infection with Borrelia burgdorferi. The main antigen VlsE, which is exclusively expressed in vivo, is the most sensitive antigen for IgG antibody detection, whereas OspC is the most sensitive antigen for IgM antibody detection.

Stage II: A variety of symptoms can develop several weeks to some months after receiving the tick bite. In the foreground of these are neurological manifestations: meningitis, encephalitis, asymmetric polyneuritis, cranial nerve pareses, Bannwarth's lymphocytic meningoradiculoneuritis. One of the most frequent neurological symptoms of borreliosis in children is the peripheral facial palsy. Usually the paresis of the facial nerve appears at one side and after several weeks on the opposite side. Also arthritis, particularly of the knee joints, and non-localised pains in the bones, joints and muscles are frequently found. Less frequently are cardiological manifestations such as myocarditis and pericarditis. Antibodies against Borrelia burgdorferi can be detected in 50% to 90% of patients in stage II. In the early phase of this stage mainly IgM antibodies are found but in the late phase often only IgG antibodies occur. However, IgM antibodies can persist for a long time. By additionally determining antibodies against VlsE the serological hit rate can be increased by 20% to 30%. VlsE displays the highest sensitivity of all antigens tested for IgG detection.

Stage III: The typical manifestations of a Borrelia burgdorferi infection in stage III are chronic-relapsing erosive arthritis, acrodermatitis chronica atrophicans and progressive encephalomyelitis, which can proceed in a similar way to multiple sclerosis. Without treatment, the tertiary stage can develop over a period of years to decades after the original infection. In this stage, IgG antibody titers are significantly increased in 90% to 100% of patients, while IgM antibodies are only rarely detectable.

For the diagnosis of neuroborreliosis, the determination of antibodies in cerebrospinal fluid is of decisive importance and far superior to serological testing. An antibody index (AI) discriminates between a blood-derived and a pathological, brain-derived specific antibody fraction in cerebrospinal fluid (CSF) and takes into account individual changes in the blood/CSF barrier function.

Various techniques come into question for the detection of antibodies against Borrelia burgdorferi: ELISA, indirect immunofluorescence (IIFT), passive haemagglutination and immunoblot. Generally, ELISA or IIFT are used as screening test for preliminary characterisation of the serum sample. According to guidelines in the USA and in Germany, serological diagnosis should follow the principle of a two-step procedure: ELISA or indirect immunofluorescence (IIFT) as a first step, which, if reactive, is followed by an immunoblot. In fresh infections it is recommended performing ELISA/IIFT and immunoblot in parallel, since some weak reactions become detectable earlier in the blot than in the screening test. VlsE, the most sensitive antigen for IgG antibody detection, and OspC for IgM antibody detection should be included in the test. By additionally determining antibodies against VlsE the serological hit rate can be increased by 20% compared to whole extract Westernblots. Of all recombinant antigens tested, VlsE possesses the highest sensitivity for the detection of a Borrelia infection.

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Anti-Borrelia burgdorferi-WESTERNBLOT (IgM)
Test instruction

ORDER NO.	ANTIBODIES AGAINST	IG CLASS	SUBSTRATE	FORMAT
DY 2132-3001 M DY 2132-24001 M	Borrelia burgdorferi (complete antigens)	IgM	Antigen coated membrane strips	30 x 01 (30) 240 x 01 (240)

Indication: The Westernblot test kit provides a qualitative in vitro assay for human autoantibodies of the immunoglobulin class IgM against Borrelia in serum or plasma for the diagnosis of Lyme borreliosis. Associated diseases: Erythema chronicum migrans, lymphadenosis cutis benigna, acrodermatitis chronica atrophicans, arthritis, carditis, lymphocytic meningoradiculoneuritis and neuroborreliosis.

Principles of the test: The test kit contains test strips with electrophoretically separated antigen extracts of Borrelia burgdorferi. The blot strips will be blocked and incubated in the first reaction step with diluted patient samples. In the case of positive samples, specific antibodies of the class IgM (and IgA, IgG) will bind to the antigens. To detect the bound antibodies, a second incubation is carried out using an enzyme-labelled anti-human IgM (enzyme conjugate) catalysing a colour reaction.

Contents of the test kit:

Component	Format	Format	Symbol
1. Test strips Single strips with electrophoretically separated Borrelia burgdorferi antigens	30 x 1	240 x 1	STRIPS
2. Evaluation matrix with control strip Test strip incubated with a positive control serum	1 pattern	1 pattern per test strip lot	---
3. Enzyme conjugate Alkaline phosphatase-labelled anti-human IgM (goat), 10x concentrate	2 x 3 ml	16 x 3 ml	CONJUGATE 10x
4. Universal buffer 10x concentrate	1 x 100 ml	8 x 100 ml	BUFFER 10x
5. Substrate solution Nitroblue tetrazolium chloride/5-Bromo-4-chloro-3-indolylphosphate (NBT/BCIP), ready for use	1 x 50 ml	8 x 50 ml	SUBSTRATE
6. Adhesive foil	---	8 sheets	---
7. Test instruction	1 booklet	1 booklet	---

LOT Lot description — CE — Storage temperature

IVD In vitro diagnostic medical device — Unopened usable until

Storage and stability: The test kit has to be stored at a temperature between +2°C to +8°C, do not freeze. Unopened, all test kit components are stable until the indicated expiry date.

Waste disposal: Undiluted patient samples and incubated blot strips should be handled as infectious waste. Other reagents do not need to be collected separately, unless stated otherwise in official regulations.

The following components are not provided in the test kits but can be ordered at EUROIMMUN under the respective order numbers.
Performance of the test requires an **incubation tray**:
ZD 9895-0130 Incubation tray with 30 channels (black)
ZD 9898-0144 Incubation tray with 44 channels (black, for the EUROBlotOne and EUROBlotCamera system)
For the creation of work protocols and the evaluation of incubated test strips using **EUROLineScan** green paper and adhesive foil are required:
ZD 9880-0101 Green paper (1 sheet)
ZD 9885-0116 Adhesive foil for approx. 16 test strips
ZD 9885-0130 Adhesive foil for approx. 30 test strips
If a **visual evaluation** is to be performed in individual cases, the required evaluation protocol can be ordered under: ZD 2132-0101 Evaluation protocol visual Anti-Borrelia-burgdorferi-WESTERNBLOT

Preparation and stability of the reagents

Note: The bag containing the blot strips is printed with a number in addition to the test kit lot number. This number refers to the strip batch and is also printed on the corresponding evaluation template. These two numbers must match to ensure correct evaluation of test results.
All reagents must be brought to room temperature (+18°C to +25°C) approx. 30 minutes before use. Unopened, reagents are stable until the indicated expiry date when stored at +2°C to +8°C. After initial opening, reagents are stable for 12 months or until the expiry date, if earlier, unless stated otherwise in the instructions. Opened reagents must also be stored at +2°C to +8°C and protected from contamination.

- **Coated test strips:** Ready for use. Open the packing with the test strips only when the strips have reached room temperature to prevent condensation on the strips. After removal of the strips the packing should be sealed tightly and stored at +2°C to +8°C. To ensure correct evaluation of results, the lot number on the bag must match the lot number on the strips as well as on the evaluation matrix.

- **Enzyme conjugate:** The enzyme conjugate is supplied as a 10x concentrate. For the preparation of the ready for use enzyme conjugate the amount required should be removed from the bottle using a clean pipette and diluted 1:10 with ready for use diluted universal buffer. For 1 test strip dilute 0.15 ml anti-human IgG concentrate with 1.35 ml ready for use diluted universal buffer. The ready for use diluted enzyme conjugate should be used at the same working day.

- **Universal buffer:** The universal buffer is supplied as a 10x concentrate. For the preparation of the ready for use universal buffer the amount required should be removed from the bottle using a clean pipette and diluted 1:10 with deionised or distilled water. For the incubation of 1 test strip 1.5 ml buffer concentrate should be diluted with 13.5 ml deionised or distilled water. The ready for use diluted universal buffer should be used at the same working day.

- **Substrate solution:** Ready for use. Close bottle immediately after use, as the contents are sensitive to light.

Warning: The control of human origin has tested negative for HBsAg, anti-HCV, anti-HIV-1 and anti-HIV-2. Nonetheless, all materials should be treated as being a potential infection hazard and should be handled with care. Some of the reagents contain the agent sodium azide in a non-declarable concentration. Avoid skin contact.

Preparation and stability of the serum or plasma samples

Sample material: Human serum or EDTA, heparin or citrate plasma.

Stability: Patient samples to be investigated can generally be stored at +2°C to +8°C for up to 14 days. Diluted samples should be incubated within one working day.

Sample dilution: The **patient samples** for analysis are diluted **1:51** in ready for use diluted universal buffer. For example, add 30 µl of sample to 1.5 ml ready for use diluted universal buffer and mix well by vortexing. Sample pipettes are not suitable for mixing.

Incubation

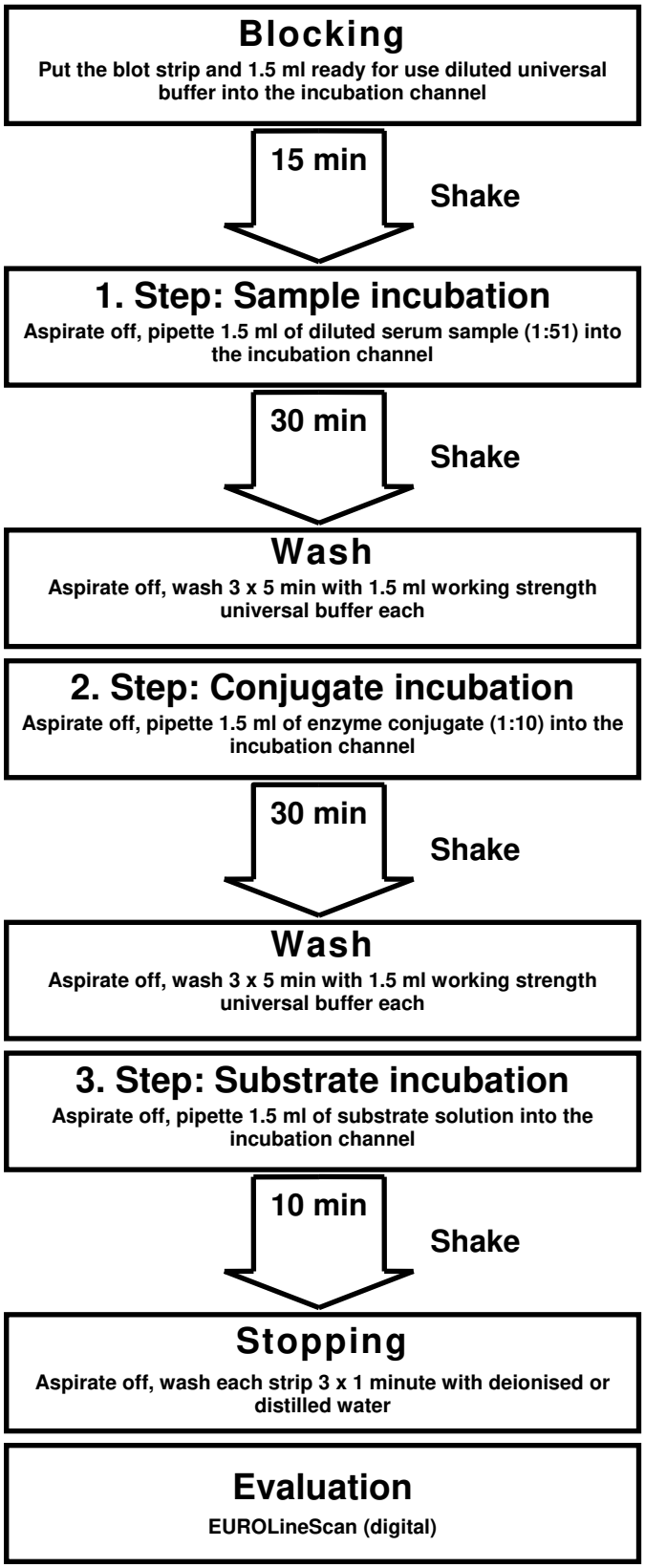
Blocking:	According to the number of serum samples to be tested fill each channel of the incubation tray with 1.5 ml ready for use diluted universal buffer and a blot strip. Remove the required amount of blot strips from the packing using a pair of tweezers. The number on the test strip should be visible. Incubate for **15 minutes** at room temperature (+18°C to +25°C) on a rocking shaker. Afterwards aspirate off all the liquid.
Sample incubation: (1st step)	Fill each channel with 1.5 ml of the diluted serum samples and incubate at room temperature (+18°C to +25°C) for **30 minutes** on a rocking shaker.
Wash:	Aspirate off the liquid from each channel and wash **3 x 5 minutes** each with 1.5 ml working strength universal buffer on a rocking shaker.
Conjugate incubation: (2nd step)	Pipette 1.5 ml diluted enzyme conjugate (alkaline phosphatase-conjugated anti-human IgM) into each channel and incubate for **30 minutes** at room temperature (+18°C to +25°C) on a rocking shaker.
Wash:	Aspirate off the liquid from each channel. Wash as described above.
Substrate incubation: (3rd step)	Pipette 1.5 ml substrate solution into the channels of the incubation tray. Incubate for **10 minutes** at room temperature (+18°C to +25°C) on a rocking shaker.
Stopping:	Aspirate off the liquid from each channel and wash each strip **3 x 1 minute** with deionised or distilled water.

For automated incubation with the EUROBlotMaster select the program **Euro02 Inf WB30**.

For automated incubation with the EUROBlotOne select the program **Euro 01/02**.

Anti-Borrelia-burgdorferi WESTERNBLOT (IgM)

Incubation protocol



Evaluation and Interpretation of the results of the Anti-Borrelia-burgdorferi WESTERNBLOT (IgM)

Handling: For evaluation of incubated test strips we generally recommend using the **EUROLineScan** software. After stopping the reaction using deionised or distilled water, place the incubated test strips onto the adhesive foil of the green work protocol using a pair of tweezers. The position of the test strips can be corrected while they are wet. As soon as all test strips have been placed onto the protocol, they should be pressed hard using filter paper and left to air-dry. After they have dried, the test strips will be stuck to the adhesive foil. The dry test strips are then scanned using a flatbed scanner (EUROIMMUN AG) and evaluated with **EUROLineScan**. Alternatively, imaging and evaluation is possible directly from the incubation trays (EUROBlotCamera and EUROBlotOne). For general information about the EUROLineScan program please refer to the EUROLineScan user manual (EUROIMMUN AG). The code for entering the **Test** in EUROLineScan is **B.b._WB_IgM**.

If a visual evaluation must be performed in exceptional cases, hold the evaluation matrix next to the stuck-on blot strips and position it so that the black band above the number on the blot strips lines up with the alignment bar of the evaluation matrix. **The lot number on the evaluation matrix must match the lot number on the blot strips.** Clearly recognisable bands on the blot strips which concur with the labelled bands on the evaluation matrix are noted in the evaluation protocol.

Antigens: The antigen source for the EUROIMMUN Anti-Borrelia-burgdorferi WESTERNBLOT is provided by a particularly suitable Borrelia burgdorferi strain [2, 3]. The cultured borrelia have been solubilised using sodium dodecyl sulphate followed by a separation of the solubilised protein using discontinuous polyacrylamide gel electrophoresis according to molecular mass and transfer of the separated proteins to nitrocellulose. From each test kit, 2 control test strips have been removed and incubated with a reference serum. One of these stained strips is included in the kit, the other remains with EUROIMMUN for documentation purposes. Diagnostically relevant antigens have been characterised with monoconal reference antibodies from the German National Reference Laboratory for Borreliae [39].

Specificity of the antigens on the test strips: [41, 42, 43]

Band	Antigen	Specificity
83 kDa	Membrane-vesical protein, p 83	Degradation product of p 100, **high specificity**.
75 kDa	Heat shock protein, p 75	Unspecific.
62 kDa	Heat shock protein, p 62	Unspecific.
57/59 kDa	p 57 and p 59	Unspecific.
50 kDa	p 50	Unspecific.
47 kDa	p 47	Probably genus specific.
43 kDa	p 43	Unspecific.
41 kDa	Flagellin, p 41	Genus specific, cross reactivity to other spirochaetaceae and bacteria having flagella.
39 kDa	Bmp A, p 39	**High specificity**.
34 kDa	Osp B, p 34	Outer surface protein B, **high specificity**.
32 kDa	p 32	Unspecific.
31 kDa	Osp A, p 31	Outer surface protein A, **high specificity**.
29 kDa	p 29	Probably specific, poorly investigated.
28 kDa	p 28	Unspecific.
25 kDa	Osp C, p 25	Outer surface protein C, **high specificity**.
21/22 kDa	p 21/22	**High specificity**.
18 kDa	p 18	**Probably specific**.
17 kDa	p 17	Poorly investigated.

In the lower part of the test strip there is a conjugate control membrane chip (IgA, IgG and IgM). Below the conjugate control, there is a membrane chip with a control band (Control).

Attention: A correctly performed determination of antibodies of class IgM against the antigens described above is indicated by a positive reaction of the control band and a positive reaction of the IgM band.

If one of these bands only shows a very weak reaction or none at all, the result is not valid.

Specificity of the antigens: Borrelia burgdorferi antigens can generally be divided into three categories [40].

Category	Antigens
1	Cross-reacting and undefined antigens with the molecular mass 17 kDa, 28 kDa, 32 kDa, 43 kDa, 47 kDa, 50 kDa, 57 kDa, 59 kDa, 62 kDa and 75 kDa.
2	A genus-specific antigen with a molecular mass of 41 kDa (flagellin).
3	Species-specific and highly specific antigens with the molecular mass 18 kDa, 21/22 kDa, 25 kDa, 31 kDa, 34 kDa, 39 kDa and 83 kDa.

IgG class antibodies against Borrelia burgdorferi

Interpretation of results: In order to evaluate the signals, the band positions and intensity of staining must be taken into consideration, as negative sera sometimes produce weak signals in individual bands. Based on experience, the results of the Borrelia burgdorferi WESTERNBLOT test can be divided into negative, borderline and positive results.

Result	Characteristics
Negative	No bands, or weak intensities of some antigens from categories 1 and 2.
Borderline	A distinctive band from category 3 (antigens in category 3 are shaded grey in the evaluation protocol!) and several distinctive signals from categories 1 and 2. It is recommended that a new sample be taken and the test repeated after a few weeks.
Positive	More than one distinctive band from category 3 (antigens in category 3 are shaded grey in the evaluation protocol!). In addition, and particularly in the case of patients in the late stage of the disease, numerous bands from categories 1 and 2 can be observed.

IgM class antibodies against Borrelia burgdorferi

Interpretation of results: In order to evaluate the signals, the band positions and intensity of staining must be taken into consideration. The results of the Borrelia burgdorferi WESTERNBLOT test can be divided into negative, borderline and positive results.

In the early phase of a Borrelia infection IgM antibodies are typically directed against Osp C (p 25). IgM antibodies against other specific Borrelia antigens are not considered definitive indicators of a fresh Borrelia infection.

IgM antibodies against flagellin (p 41) can represent the initial response of the body to Borrelia burgdorferi. However, an unspecific reaction cannot be excluded, since it is known that antibodies directed against other microorganisms cross react with Borrelia burgdorferi flagellin (p 41). For this reason, a single band at the position of flagellin (p 41) in IgM detection should not be considered proof of a fresh infection with Borrelia burgdorferi. If only the flagellin band (p 41) reacts positively, the test should be repeated several weeks later with a fresh blood sample.

In the serological investigation of a Borrelia infection the determination of antibodies of class IgM often yields unclear results. IgM antibodies can sometimes be found in serum years after an infection or following antibiotic treatment. Therefore, the detection of IgM antibodies does not necessarily indicate a fresh infection. A negative IgM result does not exclude a fresh infection. With a second infection, only antibodies of class IgG and not IgM can be formed.

In the late stage of borreliosis a positive IgM result does not provide any additional information, due to the antibody persistence mentioned above. The cause of such false-positive IgM results often remains unclear. They are observed, for example, in infectious mononucleosis, herpes virus infections and various autoimmune diseases [39].

For the diagnosis of a fresh Borrelia infection, a positive IgM result should be confirmed with a positive IgG result using a fresh blood sample 3-6 weeks later.

Result	Characteristics
Negative	No antigen bands recognisable or weak intensities of some bands of category 1
Borderline	One antigen band of category 2 (flagellin, p 41) or a weak band of category 3. It is recommended that a fresh sample be taken and the test repeated after a few weeks. Antibodies against Osp C are characteristic for a fresh infection.
Positive	At least one distinctive band from category 3 (antigens in category 3 are shaded grey in the evaluation protocol!). Antibodies against Osp C are characteristic for a fresh infection.

For diagnosis, the clinical picture of the patient always needs to be taken into account along with the serological findings.

Test characteristics

Measurement range: The Westernblot is a qualitative method. No measurement range is provided.

Inter- and intra-assay variation: The inter-assay variation was determined by multiple analyses of characterised samples over several days. The intra-assay variation was determined by multiple analyses of characterised samples on one day. In every case, the intensity of the bands was within the specified range. This Westernblot displays excellent inter- and intra-assay reproducibility.

Interference: Haemolytic, lipaemic and icteric sera showed no effect on the analytical results.

Prevalence: Sera from 156 clinically characterised patients and 517 healthy blood donors were investigated with the EUROIMMUN Anti-Borrelia burgdorferi-WESTERNBLOT.

		Prevalence		
Clinically characterised sera	**n**	**IgG**	**IgM**	**IgM/IgG**
Erythema migrans	108	64%	67%	84%
Neuroborreliosis	32	85%	44%	88%
Arthritis	10	100%	30%	100%
Acrodermatitis	6	83%	50%	100%

		Prevalence	
Serum samples	**n**	**IgG**	**IgM**
Healthy blood donors *	517	5%	4%

*Medical University of Luebeck

The prevalence of anti-Borrelia antibodies from the healthy blood donor samples agrees with the values from literature [7].

Cross reactivity: The quality of the antigen used (whole antigen, SDS extract) and the antigen source (Borrelia burgdorferi sensu stricto) ensure high specificity of the Westernblot. The determination of cross reactivity is not necessary with the Westernblot, since specific reactions and unspecific or cross reactions can be differentiated directly with this test system.

Clinical significance

The history of Lyme disease, a contagious condition, caused by Borrelia burgdorferi, transmitted to humans by ticks, offers infectiologists a formidable lesson on how medicine progresses. Clinical description started in Europe at the turn of the $19^{th}/20^{th}$ century with Pick's description of what was then labelled chronic atrophic acrodermatitis [1]. Fifty years later Hauser noted the affection was transmitted by ticks [1]. Independently, Afzelius, then Lipschutz, described erythema migrans and its relationship with tick bites. Neurological involvement was also described with the skin signs. These early dermatological descriptions suddenly came into the limelight in 1975 when an epidemic of arthritis occurred in children in Lyme, Connecticut, USA [1]. Many of the affected children had erythema migrans. Based on these observations and an epidemiological analysis of the epidemic, Steele and co-workers defined "Lyme disease" as a rheumatological disorder commonly associated with erythema migrans and sometimes with multiple organ involvement. In 1982 Burgdorfer suggested that tick bites transmitted a Spirochaeta which was later authentified as the causal agent: Borrelia burgdorferi [1, 2, 3].

Borrelia burgdorferi as a species of bacteria is belonging to the spirochaetaceae family. This family includes five genera: borrelia, spirochaetes, cristispira, treponema and leptospira. Among about 30 tick species feeding on humans, Ixodes ricinus is the most frequent tick species biting humans in Europe. It is the vector of Borrelia burgdorferi which causes Lyme disease, and of the tick-borne encephalitis virus [4, 5, 6, 7]. I. ricinus ticks pass through three developmental stages: larvae, nymphs and adults (females and males) [8]. The density of this tick species may be very high, reaching in some places more than 300 ticks/100 m^2 [8]. Lyme borreliosis is the most frequent tick-transmitted disease in the northern hemisphere [9, 10, 11]. In Europe, especially in Central Europe and Scandinavia there are up to 155 cases per 100,000 individuals caused by the species B. burgdorferi sensu stricto, B. afzelii, and B. garinii [4, 6, 10, 11, 12]. The human seroprevalence rates of antibodies against Borrelia burgdorferi in the normal population of Germany and other Central European countries ranges from 18% to 52% [13]. In East Asia, e.g. in China, there are 26% on the average [14]. Persons working in the area of forestry display anti-borrelia antibodies in about 40%, hunters in more than 50% of cases [7, 15].

A Borrelia burgdorferi infection can manifest itself in the areas of dermatology, neurology, ophthalmology, rheumatology and internal medicine [7, 16]. The clinical expression of borreliosis can be divided into three stages:

Stage I: The typical primary manifestation of a Borrelia burgdorferi infection is erythema migrans (80%), a reddening of the skin which appears around the area of the tick bite and spreads in a circular manner [17]. The erythema is accompanied by influenza-like general symptoms with fever, shaking chill, headaches and vomiting [18]. Lymphadenopathies are observed in a few cases (lymphadenosis cutis benigna). The leading clinical types of the erythema-free borreliosis (20%) are neurological, arthromyalgic, influenza-like, cardiovascular, hepatitis, regional lymphadenitis, and mixed [19, 20, 21]. Stage I can result in spontaneous healing or can develop into a generalised borreliosis. The transition phase is generally symptom-free. IgM antibodies against Borrelia burgdorferi can be detected serologically in 50% to 90% of patients in Stage I. The prevalence of specific IgG antibodies is considerably lower [22, 23]. However, serological tests often provide negative results in this stage of the illness [3, 19]. In order to overcome this problem a new test was created including the newly identified VlsE (**v**ariable major protein-**l**ike **s**equence, **e**xpressed), which can be considered as the major antigen for Borrelia serology. Over 85% of IgG-positive sera can be identified at a glance by assessing the VlsE band in incubated Westernblot strips. VlsE allows detection of antibodies against all Borrelia species. The risk of a false negative reaction due to species differences is ten times lower [24]. Humans also produce highly specific borreliacidal antibodies against outer surface protein C (OspC) shortly after infection with Borrelia burgdorferi sensu stricto: immunoglobulin M (IgM) OspC and immunoglobulin G (IgG) OspC borreliacidal antibodies [25]. VlsE is the most sensitive antigen for IgG antibody detection, OspC for IgM antibody detection [3, 26, 27].

Stage II: A variety of symptoms can develop several weeks to some months after receiving the tick bite. In the foreground of these are neurological manifestations: meningitis, encephalitis, asymmetric polyneuritis, cranial nerve pareses, Bannwarth´s lymphocytic meningoradiculoneuritis [20, 28, 29]. One of the most frequent neurological symptoms of borreliosis in children is the peripheral facial palsy. Usually the paresis of the facial nerve appears at one side and after several weeks on the opposite side [20]. Also arthritis, particularly of the knee joints, and non-localised pains in the bones, joints and muscles are frequently found [30]. Rarer are cardiological manifestations, such as myocarditis and pericarditis. Antibodies against Borrelia burgdorferi can be detected in 50% to 90% of patients in stage II [24]. In the early phase of this stage, mainly IgM antibodies are found, but in the late phase, often only IgG antibodies occur. However, IgM antibodies can persist for a long time [3, 30]. By additionally determining antibodies against VlsE the serological hit rate can be increased by 20%-30% [31]. VlsE displays the highest sensitivity of all antigens tested for IgG detection [26].

Stage III: The typical manifestations of a Borrelia burgdorferi infection in stage III are chronic-relapsing erosive arthritis, acrodermatitis chronica atrophicans and progressive encephalomyelitis, which can proceed in a similar way to multiple sclerosis. Without treatment, the tertiary stage can develop over a period of years to decades after the original infection. In this stage, IgG antibodies are significantly increased in 90% to 100% of patients, while IgM antibodies are only rarely detectable [3, 24].

For the diagnosis of neuroborreliosis, the determination of antibodies in cerebrospinal fluid is of decisive importance and far superior to serological testing [21, 29, 32]. An Antibody Index (AI) discriminates between a blood-derived and a pathological, brain-derived specific antibody fraction in cerebrospinal fluid (CSF) and takes into account individual changes in blood/CSF barrier function [33].

Various techniques come into question for the detection of antibodies against Borrelia burgdorferi [34, 35, 36, 37, 38, 39]: ELISA, indirect immunofluorescence, passive haemagglutination and immunoblot [26]. Many investigators employ an ELISA test in parallel with an indirect immunofluorescence test for preliminary characterisation of the serum sample [26, 34]. According to guidelines of the USA and Germany, serological diagnosis should follow the principle of a two-step procedure: ELISA or indirect immunofluorescence (IIFT) as first step: if reactive, followed by Westernblot [3, 24, 40]. In fresh infections it is recommended performing ELISA/IIFT and Westernblot in parallel, since some weak reactions become detectable earlier in Westernblot than in the screening tests [12, 24]. VlsE, the most sensitive antigen for IgG antibody detection, and OspC for IgM antibody detection, should be included in the test [3]. By additionally determining antibodies against VlsE the serological hit rate can be increased by 20% compared to whole extract Westernblots and by 30% compared to recombinant antigen Westernblots [24, 27, 31]. Of all recombinant antigens tested, VlsE possesses the highest sensitivity for the detection of a Borrelia infection [31, 32, 33].

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Declaration of Conformity

EUROIMMUN Medizinische Labordiagnostika AG
Seekamp 31, D-23560 Lübeck, Germany

declare under our sole responsibility that the ELISA-products

Anti-Borrelia plus VlsE ELISA (IgG)	*EI 2132-9601-2 G*
CSF: Anti-Borrelia plus VlsE ELISA (IgG)	*EI 2132-9601-L G*
CSQ pair of controls Anti-Borrelia (IgG)	*EI 2132-0208-8 L G*

(product name, order no)

in combination with automated analyzer for ELISA

EUROIMMUN Analyzer I

meet the demands of

Directive 98/79/EC on in vitro diagnostic medical devices of 27 October 1998

Lübeck, 26.05.2010

(Place and date of issue)

Wolfgang Schlumberger, PhD
- Member of the Board -

Susanne Aleksandrowicz
- Member of the Board -

Declaration of Conformity

EUROIMMUN Medizinische Labordiagnostika AG
Seekamp 31, D-23560 Lübeck, Germany

declare under our sole responsibility that the ELISA products

Anti-Borrelia ELISA (IgG)	*EI 2132-9601 G*
Anti-Borrelia ELISA (IgM)	*EI 2132-9601 M*
CSF: Anti-Borrelia ELISA (IgM)	*EI 2132-9601-L M*

(product name, order no)

meet the demands of

Directive 98/79/EC on in vitro diagnostic medical devices of 27 October 1998

Lübeck, 25.04.2008
(Place and date of issue)

Wolfgang Schlumberger, PhD
- Member of the Board -

Susanne Aleksandrowicz
- Member of the Board -

Anti-Borrelia plus VlsE ELISA (IgG)
Test instruction

ORDER NO.	ANTIBODIES AGAINST	IG CLASS	SUBSTRATE	FORMAT
EI 2132-9601-2 G	Borrelia burgdorferi, Borrelia afzelii, Borrelia garinii, Borrelia VlsE Antigen	IgG	Ag-coated microplate wells	96 x 01 (96)

Indications: The ELISA test kit provides a semiquantitative or quantitative in vitro assay for human antibodies of the IgG class against Borrelia antigens in serum or plasma for the diagnosis of infection with Borrelia and associated diseases: Erythema chronicum migrans, lymphadenosis cutis benigna, acrodermatitis chronica atrophicans, arthritis, carditis, lymphocytic meningoradiculitis and neuro-borreliosis.

Application: Clinical diagnosis of borreliosis can be achieved by determination of Borrelia-specific antibodies of classes IgG and IgM. The Anti-Borrelia plus VlsE ELISA (IgG) is based on an optimised lysate mixture of the most releveant human pathogenic Borrelia strains and also contains recombinant VlsE. Due to its complete antigen spectrum, the ELISA offers a very high sensitivity and is therefore ideally suited for antibody screening. Samples with positive or borderline ELISA results should always be further investigated using immunoblot.

Principle of the test: The test kit contains microtiter strips each with 8 break-off reagent wells coated with a mix of whole antigen extracts of Borrelia burgdorferi sensu stricto, Borrelia afzelii, Borrelia garinii and recombinant VlsE of Borrelia burgdorferi. In the first reaction step, diluted patient samples are incubated in the wells. In the case of positive samples, specific IgG antibodies will bind to the antigens. To detect the bound antibodies, a second incubation is carried out using an enzyme-labelled anti-human IgG (enzyme conjugate) catalysing a colour reaction.

Contents of the test kit:

Component	Colour	Format	Symbol
1. Microplate wells coated with antigens 12 microplate strips each containing 8 individual break-off wells in a frame, ready for use	---	12 x 8	STRIPS
2. Calibrator 1 200 RU/ml (IgG, human), ready for use	dark red	1 x 2.0 ml	CAL 1
3. Calibrator 2 20 RU/ml (IgG, human), ready for use	red	1 x 2.0 ml	CAL 2
4. Calibrator 3 2 RU/ml (IgG, human), ready for use	light red	1 x 2.0 ml	CAL 3
5. Positive control (IgG, human), ready for use	blue	1 x 2.0 ml	POS CONTROL
6. Negative control (IgG, human), ready for use	green	1 x 2.0 ml	NEG CONTROL
7. Enzyme conjugate peroxidase-labelled anti-human IgG (rabbit), ready for use	green	1 x 12 ml	CONJUGATE
8. Sample buffer ready for use	light blue	1 x 100 ml	SAMPLE BUFFER
9. Wash buffer 10x concentrate	colourless	1 x 100 ml	WASH BUFFER 10x
10. Chromogen/substrate solution TMB/H_2O_2, ready for use	colourless	1 x 12 ml	SUBSTRATE
11. Stop solution 0.5 M sulphuric acid, ready for use	colourless	1 x 12 ml	STOP SOLUTION
12. Test instruction	---	1 booklet	
13. Quality control certificate	---	1 protocol	

LOT Lot description
IVD In vitro diagnostic medical device
CE
Storage temperature
Unopened usable until

Preparation and stability of the reagents

Note: All reagents must be brought to room temperature (+18°C to +25°C) approx. 30 minutes before use. After first use, the reagents are stable until the indicated expiry date if stored at +2°C to +8°C and protected from contamination, unless stated otherwise below.

- **Coated wells:** Ready for use. Tear open the resealable protective wrapping of the microplate at the recesses above the grip seam. Do not open until the microplate has reached room temperature to prevent the individual strips from moistening. Immediately replace the remaining wells of a partly used microplate in the protective wrapping and tightly seal with the integrated grip seam (Do not remove the desiccant bags).
  Once the protective wrapping has been opened for the first time, the wells coated with antigens can be stored in a dry place and at a temperature between +2°C and +8°C for 4 months.

- **Calibrators and controls:** Ready for use. The reagents must be mixed thoroughly before use.

- **Enzyme conjugate:** Ready for use. The enzyme conjugate must be mixed thoroughly before use.

- **Sample buffer:** Ready for use.

- **Wash buffer:** The wash buffer is a 10x concentrate. If crystallisation occurs in the concentrated buffer, warm it to 37°C and mix well before diluting. The quantity required should be removed from the bottle using a clean pipette and diluted with deionised or distilled water (1 part reagent plus 9 parts distilled water).
  For example: For 1 microplate strip, 5 ml concentrate plus 45 ml water.
  The working strength wash buffer is stable for 4 weeks when stored at +2°C to +8°C and handled properly.

- **Chromogen/substrate solution:** Ready for use. Close the bottle immediately after use, as the contents are sensitive to light. The chromogen/substrate solution must be clear on use. Do not use the solution if it is blue coloured.

- **Stop solution:** Ready for use.

Storage and stability: The test kit has to be stored at a temperature between +2°C to +8°C. Do not freeze. Unopened, all test kit components are stable until the indicated expiry date.

Waste disposal: Patient samples, calibrators, controls and incubated microplate strips should be handled as infectious waste. All reagents must be disposed of in accordance with local disposal regulations.

Warning: The calibrators and controls of human origin have tested negative for HBsAg, anti-HCV, anti-HIV-1 and anti-HIV-2. Nonetheless, all materials should be treated as being a potential infection hazard and should be handled with care. Some of the reagents contain the agent sodium azide in a non-declarable concentration. Avoid skin contact.

Preparation and stability of the patient samples

Samples: Human serum or EDTA, heparin or citrate plasma.

Stability: Patient samples to be investigated can generally be stored at +2°C to +8°C for up to 14 days. Diluted samples must be incubated within one working day.

Sample dilution: Patient samples are diluted **1:101** with sample buffer.
Example: Add 10 µl of sample to 1.0 ml sample buffer and mix well by vortexing (sample pipettes are not suitable for mixing).

NOTE: The calibrators and controls are prediluted and ready for use, do not dilute them.

Incubation

For **semiquantative analysis** incubate **calibrator 2** along with the positive and negative controls and patient samples. For **quantitative analysis** incubate **calibrators 1, 2 and 3** along with the positive and negative controls and patient samples.

(Partly) manual test performance

Sample incubation: (1st step)
Transfer 100 µl of the calibrators, positive and negative controls or diluted patient samples into the individual microplate wells according to the pipetting protocol.
Incubate for **30 minutes** at room temperature (+18°C to +25°C).

Washing:
Manual: Empty the wells and subsequently wash 3 times using 300 µl of working strength wash buffer for each wash.
Automatic: Wash the reagent wells 3 times with 450 µl of working strength wash buffer (program setting: e.g. TECAN Columbus Washer "Overflow Modus").

Leave the wash buffer in each well for 30 to 60 seconds per washing cycle, then empty the wells. After washing (manual and automated tests), thoroughly dispose of all liquid from the microplate by tapping it on absorbent paper with the openings facing downwards to remove all residual wash buffer.

Note: Residual liquid (> 10 µl) remaining in the reagent wells after washing can interfere with the substrate and lead to false low extinction values. Insufficient washing (e.g., less than 3 wash cycles, too small wash buffer volumes, or too short residence times) can lead to false high extinction values.
Free positions on the microplate strip should be filled with blank wells of the same plate format as that of the parameter to be investigated.

Conjugate incubation: (2nd step)
Pipette 100 µl of enzyme conjugate (peroxidase-labelled anti-human IgG) into each of the microplate wells.
Incubate for **30 minutes** at room temperature (+18°C to +25°C).

Washing:
Empty the wells. Wash as described above.

Substrate incubation: (3rd step)
Pipette 100 µl of chromogen/substrate solution into each of the microplate wells.
Incubate for **15 minutes** at room temperature (+18°C to +25°C), protect from direct sunlight.

Stopping:
Pipette 100 µl of stop solution into each of the microplate wells in the same order and at the same speed as the chromogen/substrate solution was introduced.

Measurement:
Photometric measurement of the colour intensity should be made at a **wavelength of 450 nm** and a reference wavelength between 620 nm and 650 nm **within 30 minutes of adding the stop solution**. Prior to measuring, slightly shake the microplate to ensure a homogeneous distribution of the solution.

Test performance using fully automated analysis devices

Sample dilution and test performance are carried out fully automatically using an analysis device. The incubation conditions programmed in the respective software authorised by EUROIMMUN may deviate slightly from the specifications given in the ELISA test instruction. However, these conditions were validated in respect of the combination of the EUROIMMUN Analyzer I, Analyzer I-2P or the DSX from Dynex and this EUROIMMUN ELISA. Validation documents are available on enquiry.
Automated test performance using other fully automated, open system analysis devices is possible. However, the combination should be validated by the user.

Pipetting protocol

	1	2	3	4	5	6	7	8	9	10	11	12
A	**C 2**	**P 6**	**P 14**	**P 22**			**C 1**	**P 4**	**P 12**	**P 20**		
B	**pos.**	**P 7**	**P 15**	**P 23**			**C 2**	**P 5**	**P 13**	**P 21**		
C	**neg.**	**P 8**	**P 16**	**P 24**			**C 3**	**P 6**	**P 14**	**P 22**		
D	**P 1**	**P 9**	**P 17**				**pos.**	**P 7**	**P 15**	**P 23**		
E	**P 2**	**P 10**	**P 18**				**neg.**	**P 8**	**P 16**	**P 24**		
F	**P 3**	**P 11**	**P 19**				**P 1**	**P 9**	**P 17**			
G	**P 4**	**P 12**	**P 20**				**P 2**	**P 10**	**P 18**			
H	**P 5**	**P 13**	**P 21**				**P 3**	**P 11**	**P 19**			

The pipetting protocol for microtiter strips 1-4 is an example for the **semiquantitative analysis** of 24 patient sample (P 1 to P 24).
The pipetting protocol for microtiter strips 7-10 is an example for the **quantitative analysis** of 24 patient sample (P 1 to P 24).

The calibrators (C 1 to C 3), the positive (pos.) and negative (neg.) controls, and the patient samples have each been incubated in one well. The reliability of the ELISA test can be improved by duplicate determinations for each sample.
The wells can be broken off individually from the strips. Therefore, the number of tests performed can be matched to the number of samples, minimising reagent wastage.
Both positive and negative controls serve as internal controls for the reliability of the test procedure. They should be assayed with each test run.

Calculation of results

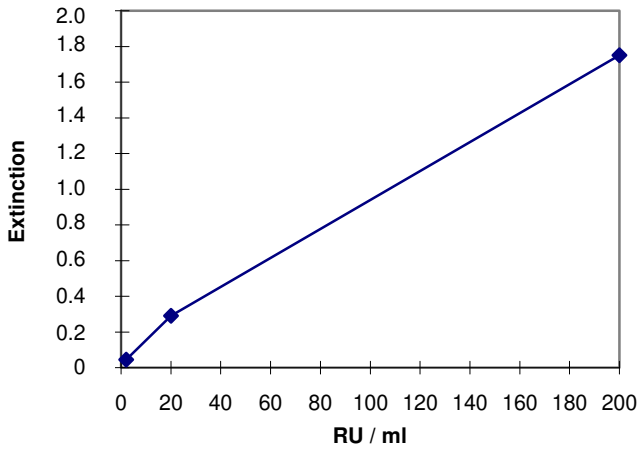
Semiquantitative: Results can be evaluated semiquantitatively by calculating a ratio of the extinction value of the control or patient sample over the extinction value of the calibrator 2. Calculate the ratio according to the following formula:

$$\frac{\textbf{Extinction of the control or patient sample}}{\textbf{Extinction of calibrator 2}} = \textbf{Ratio}$$

EUROIMMUN recommends interpreting results as follows:

Ratio <0.8:	**negative**
Ratio ≥0.8 to <1.1:	**borderline**
Ratio ≥1.1:	**positive**

Quantitative: The standard curve from which the concentration of antibodies in the patient samples can be taken is obtained by point-to-point plotting of the extinction values measured for the 3 calibration sera against the corresponding units (linear/linear). Use “point-to-point” plotting for calculation of the standard curve by computer. The following plot is an example of a typical calibration curve. Please do not use this curve for the determination of antibody concentrations in patient samples.



If the extinction for a patient sample lies above the value of calibrator 1 (200 RU/ml), the result should be reported as “>200 RU/ml“. It is recommended that the sample be retested at a dilution of e.g. 1:400. The result in RU/ml read from the calibration curve for this sample must then be multiplied by a factor of 4. The upper limit of the normal range of non-infected persons (cut-off value) recommended by EUROIMMUN is 20 relative units (RU)/ml. EUROIMMUN recommends interpreting results as follows:

<16 RU/ml:	**negative**
≥16 to <22 RU/ml:	**borderline**
≥22 RU/ml:	**positive**

For duplicate determinations the mean of the two values should be taken. If the two values deviate substantially from one another, EUROIMMUN recommends to retest the samples.

A negative serological result does not exclude an infection. Particularly in the early phase of an infection, antibodies may not yet be present or are only present in such small quantities that they are not detectable. In case of a borderline result, a secure evaluation is not possible. If there is a clinical suspicion and a negative test result, we recommend clarification by means of other diagnostic methods and/or the serological investigation of a follow-up sample. A positive result indicates that there has been contact with the pathogen. In the determination of pathogen-specific IgM antibodies, polyclonal stimulation of the immune system or antibody persistence may affect the diagnostic relevance of positive findings. Significant titer increases (exceeding factor 2) and/or seroconversion in a follow-up sample taken after 7 to 10 days can indicate an acute infection. To investigate titer changes, sample and follow-up sample should be incubated in adjacent wells of the ELISA microplate within the same test run. For diagnosis, the clinical picture of the patient always needs to be taken into account along with the serological findings.

Test characteristics

Calibration: As no international reference serum exists for antibodies against Borrelia, the calibration is performed in relative units (RU)/ml.

For every group of tests performed, the extinction values of the calibrators and the relative units and/or ratio determined for the positive and negative controls must lie within the limits stated for the relevant test kit lot. A quality control certificate containing these reference values is included. If the values specified for the controls are not achieved, the test results may be inaccurate and the test should be repeated.

The binding activity of the antibodies and the activity of the enzyme used are temperature-dependent. It is therefore recommended using a thermostat in all three incubation steps. The higher the room temperature (+18°C to +25°C) during the incubation steps, the greater will be the extinction values. Corresponding variations apply also to the incubation times. However, the calibrators are subject to the same influences, with the result that such variations will be largely compensated in the calculation of the result.

Antigen: The antigen source is provided by particularly suitable Borrelia strains (Borrelia burgdorferi sensu stricto, Borrelia afzelii, Borrelia garinii) as well as recombinant VlsE (**v**arible major protein-**l**ike **s**equence, **e**xpressed) of Borrelia burgdorferi. VlsE is a newly characterised surface protein of Borrelia which is expressed exclusively in vivo and which contains conserved and highly immunogenic epitopes.The cultured borrelia have been solubilised by using sodium dodecyl sulphate. The used antigen mixture contains all relevant proteins.

Linearity: The linearity of the Anti-Borrelia plus VlsE ELISA (IgG) was determined by assaying 4 serial dilutions of different patient samples. The coefficient of determination R^2 for all sera was > 0.95. The Anti-Borrelia plus VlsE ELISA (IgG) is linear at least in the tested concentration range (8 RU/ml to 124 RU/ml).

Detection limit: The lower detection limit is defined as the mean value of an analyte-free sample plus three times the standard deviation and is the smallest detectable antibody titer. The lower detection limit of the Anti-Borrelia plus VlsE ELISA (IgG) is 0.7 RU/ml.

Cross reactivity: Cross reactivity of the Anti-Borrelia plus VlsE ELISA (IgG) was evaluated in a study performed on 256 patient sera with antibodies against Treponema pallidum and 16 patient sera with antibodies against Leptospira. 95 sera of the Anti-Treponema pallidum-positive sera and 1 serum of the Anti-Leptospira-positive sera presented reactivity. Therefore, the cross reactivity amounts to 37.1% and. 6.3%, respectively. Cross reactions with antibodies against other spirochaetes cannot be excluded. The Anti-Borrelia plus VlsE ELISA is designed as a screening test which should be followed by a confirmatory test (Western- or line blot). Further sera from patients with different possible influencing factors were investigated. An overview of these results can be found in the following table.

Possible influencing factors	n	Anti-Borrelia plus VlsE ELISA (IgG) positive
Acute EBV infection	33	0%
Anti-HSV 1	12	0%
Anti-CMV	12	0%
Anti-VZV	12	0%
Anti-Adenovirus	12	0%
Anti-RSV	12	0%
Anti-Parainfluenza	12	0%
Anti-Influenza-A virus	12	0%
Anti-Influenza-B virus	12	0%
Anti-Mycoplasma pneumoniae	12	0%
Anti-Measles virus	12	0%
Anti-Mumps virus	12	0%
Anti-Rubella virus	12	0%
Anti-Toxoplasma gondii	11	0%
Anti-Chlamydia pneumoniae	11	0%
Anti-Helicobacter pylori	12	0%
ANA + DNS (AAb)	36	2.8%
Rheuma factor	37	0%
Neurological diseases	54	3.7%

Interference: Haemolytic, lipaemic and icteric samples showed no influence on the result up to a concentration of 10 mg/ml for haemoglobin, 20 mg/ml for triglycerides and 0.4 mg/ml for bilirubin in this ELISA.

Reproducibility: The reproducibility of the test was investigated by determining the intra- and inter-assay coefficients of variation (CV) using 3 sera. The intra-assay CVs are based on 20 determinations and the inter-assay CVs on 4 determinations performed in 6 different test runs.

Intra-assay variation, n = 20		
Serum	**Mean value (RU/ml)**	**CV (%)**
1	52	3.5
2	97	3.5
3	128	3.6

Inter-assay variation, n = 4 x 6		
Serum	**Mean value (RU/ml)**	**CV (%)**
1	55	3.6
2	103	3.7
3	138	3.8

Specificity and sensitivity: Sera of 165 patients with suspected borreliosis were analysed using the EUROIMMUN Anti-Borrelia plus VlsE ELISA (IgG) and the EUROIMMUN Anti-Borrelia EUROLINE-Westernblot (IgG) as a reference method. The test showed a specificity of 90.2% and a sensitivity of 100%.

n = 165		**EUROIMMUN EUROLINE-Westernblot**		
		positive	borderline	negative
ELISA	positive	60	11	9
	borderline	0	1	1
	negative	0	0	83

Borderline results are not included by the calculation of specificity and sensitivity.

Clinical study: 138 patients with clinically characterised borreliosis in different disease stages were screened with the Anti-Borrelia plus VlsE-ELISA (IgG) and Anti-Borrelia-ELISA (IgM). Sensitivities between 89 to 96% were found.

n = 138		**EUROIMMUN Anti-Borrelia plus VlsE-ELISA (IgG), EUROIMMUN Anti-Borrelia-ELISA (IgM)**		
		IgG positive	IgM positive	IgG and/or IgM positive
Clinic	erythema migrans, n = 97	60	68	86 (88.7%)
	mono- or poly- arthritis, n = 26	22	5	25 (96.2%)
	neuroborreliosis, n = 15	13	4	14 (93.3%)

All ELISA negative results were confirmed as negative by an Anti-Borrelia Westernblot.

Reference range: The levels of the anti-Borrelia antibodies (IgG) were analysed with this EUROIMMUN ELISA in a panel of 500 healthy blood donors. With a cut-off of 20 RU/ml, 5% of the blood donors were anti-Borrelia positive (IgG), wich reflects the known percentage of infections in adults.

Clinical significance

The history of Lyme disease, a contagious condition caused by Borrelia burgdorferi and transmitted to man by ticks, offers infectiologists a formidable lesson on how medicine progresses. Clinical description started in Europe at the turn of the 19th/20th century with Pick's description of what was then labelled chronic atrophic acrodermatitis. Fifty years later, Hauser noted the affection was transmitted by ticks. Independently, Afzelius, then Lipschutz, described erythema migrans and its relationship with tick bites. Neurological involvement was also described with the skin signs. These early dermatological descriptions suddenly came into the limelight in 1975 when an epidemia of arthritis occurred in children in Lyme, Connecticut, USA. Many of the affected children had erythema migrans. Based on these observations and an epidemiological analysis of the epidemia, Steele and co-workers defined "Lyme disease" as a rheumatological disorder commonly associated with erythema migrans and sometimes with multiple organ involvement. In 1982 Burgdorfer suggested that ticks transmitted "treponema-like spirochaetes", which were later authentified as the causal agent: Borrelia burgdorferi.

Borrelia burgdorferi as a species of bacteria is belonging to the spirochaetaceae family. This family includes five genera: borrelia, spirochaetes, cristispira, treponema and leptospira. Among about 30 tick species feeding on humans, Ixodes ricinus is the most frequent tick species biting humans in Europe. It is the vector of Borrelia burgdorferi, which causes Lyme disease, of anaplasma phagocytophilum and the tick-borne encephalitis virus. I. ricinus ticks pass through three developmental stages: larvae, nymphs and adults (females and males). The density of this tick species can be very high, reaching in some places more than 300 ticks/100 m^2. Lyme borreliosis is the most frequent tick-transmitted disease in the northern hemisphere. In Europe, especially in Central Europe and Scandinavia there are up to 155 cases per 100,000 individuals caused by the species B. burgdorferi sensu stricto, B. afzelii, and B. garinii (in rare cases also by B. spielmanii and B. bavariensis). The human seroprevalence rate of antibodies against Borrelia burgdorferi in the normal popu-lation of Germany and other Central European countries is about 8% (for IgG); in highly endemic areas even considerably higher with ranges from 18% to 52%. In East Asia, e.g. in an endemic area in China, the seroprevalence amounts to 26%. Persons working in the area of forestry display anti-Borrelia antibodies in about 40%, hunters in more than 50% of cases.

A Borrelia burgdorferi infection can manifest itself in the areas of dermatology, neurology, ophthalmology, rheumatology and internal medicine. The clinical expression of borreliosis can be divided into three stages:

Stage I: The typical primary manifestation of a Borrelia burgdorferi infection is erythema migrans (80%), a reddening of the skin which appears around the area of the tick bite and spreads in a circular manner. The erythema is accompanied by influenza-like general symptoms with fever, shaking chill, headaches and vomiting. Lymphadenopathies are observed in a few cases (lymphadenosis cutis benigna). The leading clinical types of the erythema-free borreliosis (20%) are neurological, arthromyalgic, influenza-like, cardiovascular borreliosis and borreliosis with hepatitis, with regional lymphadenitis and mixtures of these. Stage I can result in spontaneous healing or can develop into a generalised borreliosis. The transition phase is generally symptom-free. IgM antibodies against Borrelia burgdorferi can be detected serologically in 50% to 90% of patients during stage I. Humans produce highly specific antibodies against the outer surface protein C (OspC) shortly after infection with Borrelia burgdorferi. The main antigen VlsE, which is exclusively expressed in vivo, is the most sensitive antigen for IgG antibody detection, whereas OspC is the most sensitive antigen for IgM antibody detection.

Stage II: A variety of symptoms can develop several weeks to some months after receiving the tick bite. In the foreground of these are neurological manifestations: meningitis, encephalitis, asymmetric polyneuritis, cranial nerve pareses, Bannwarth's lymphocytic meningoradiculoneuritis. One of the most frequent neurological symptoms of borreliosis in children is the peripheral facial palsy. Usually the paresis of the facial nerve appears on one side and after several weeks on the opposite side. Also arthritis, particularly of the knee joints, and non-localised pains in the bones, joints and muscles are frequently found. Less frequently are cardiological manifestations such as myocarditis and pericarditis. Antibodies against Borrelia burgdorferi can be detected in 50% to 90% of patients in stage II. In the early phase of this stage mainly IgM antibodies are found but in the late phase often only IgG antibodies occur. However, IgM antibodies can persist for a long time. By additionally determining antibodies against VlsE the serological hit rate can be increased by 20% to 30%. VlsE displays the highest sensitivity of all antigens tested for IgG detection.

Stage III: The typical manifestations of a Borrelia burgdorferi infection in stage III are chronic-relapsing erosive arthritis, acrodermatitis chronica atrophicans and progressive encephalomyelitis, which can proceed in a similar way to multiple sclerosis. Without treatment, the tertiary stage can develop over a period of years to decades after the original infection. In this stage, IgG antibody titers are significantly increased in 90% to 100% of patients, while IgM antibodies are only rarely detectable.

For the diagnosis of neuroborreliosis, the determination of antibodies in cerebrospinal fluid is of decisive importance and far superior to serological testing. An antibody index (AI) discriminates between a blood-derived and a pathological, brain-derived specific antibody fraction in cerebrospinal fluid (CSF) and takes into account individual changes in the blood/CSF barrier function.

Various techniques come into question for the detection of antibodies against Borrelia burgdorferi: ELISA, indirect immunofluorescence (IIFT), passive haemagglutination and immunoblot. Generally, ELISA or IIFT are used as screening test for preliminary characterisation of the serum sample. According to guidelines in the USA and in Germany, serological diagnosis should follow the principle of a two-step procedure: ELISA or indirect immunofluorescence (IIFT) as a first step, which, if reactive, is followed by an immunoblot. In fresh infections it is recommended performing ELISA/IIFT and immuno-blot in parallel, since some weak reactions become detectable earlier in the blot than in the screening test. VlsE, the most sensitive antigen for IgG antibody detection, and OspC for IgM antibody detection should be included in the test. By additionally determining antibodies against VlsE the serological hit rate can be increased by 20% compared to whole extract Westernblots. Of all recombinant antigens tested, VlsE possesses the highest sensitivity for the detection of a Borrelia infection.

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Anti-Borrelia ELISA (IgM)
Test instruction

ORDER NO.	ANTIBODIES AGAINST	IG CLASS	SUBSTRATE	FORMAT
EI 2132-9601 M	Borrelia burgdorferi, Borrelia afzelii, Borrelia garinii	IgM	Ag-coated microplate wells	96 x 01 (96)

Indications: The ELISA test kit provides a semiquantitative or quantitative in vitro assay for human antibodies of the IgM class against Borrelia antigens in serum or plasma for the diagnosis of Infection with Borrelia. Associated diseases: Erythema chronicum migrans, lymphadenosis cutis benigna, acrodermatitis chronica atrophicans, arthritis, carditis, lymphocytic meningoradiculitis, neuroborreliosis.

Application: Clinical diagnosis of borreliosis can be achieved by determination of Borrelia-specific antibodies of classes IgG and IgM. The Anti-Borrelia ELISA (IgM) is based on an optimised lysate mixture of the most releveant human pathogenic Borrelia strains. Specific cultivation methods help to ensure a large proportion of OspC in the test. Due to its complete antigen spectrum, the ELISA offers a very high sensitivity and is therefore ideally suited for antibody screening. Samples with positive or borderline ELISA results should always be further investigated using immunoblot.

Principle of the test: The test kit contains microtiter strips each with 8 break-off reagent wells coated with antigen extracts of Borrelia burgdorferi sensu stricto, Borrelia afzelii and Borrelia garinii. In the first reaction step, diluted patient samples are incubated with the wells. In the case of positive samples, specific IgM antibodies (also IgA and IgG) will bind to the antigens. To detect the bound antibodies, a second incubation is carried out using an enzyme-labelled anti-human IgM (enzyme conjugate) catalysing a colour reaction.

Contents of the test kit:

Component	Colour	Format	Symbol
1. Microplate wells coated with antigens 12 microplate strips each containing 8 individual break-off wells in a frame, ready for use	---	12 x 8	STRIPS
2. Calibrator 1, 200 RU/ml (IgM, human), ready for use	dark red	1 x 2.0 ml	CAL 1
3. Calibrator 2, 20 RU/ml (IgM, human), ready for use	red	1 x 2.0 ml	CAL 2
4. Calibrator 3, 2 RU/ml (IgM, human), ready for use	light red	1 x 2.0 ml	CAL 3
5. Positive control, (IgM, human), ready for use	blue	1 x 2.0 ml	POS CONTROL
6. Negative control, (IgM, human), ready for use	green	1 x 2.0 ml	NEG CONTROL
7. Enzyme conjugate peroxidase-labelled anti-human IgM (goat), ready for use	red	1 x 12 ml	CONJUGATE
8. Sample buffer containing IgG/RF absorbent (anti-human IgG antibody preparation obtained from goat), ready for use	green	1 x 100 ml	SAMPLE BUFFER
9. Wash buffer 10x concentrate	colourless	1 x 100 ml	WASH BUFFER 10x
10. Chromogen/substrate solution TMB/H_2O_2, ready for use	colourless	1 x 12 ml	SUBSTRATE
11. Stop solution 0.5 M sulphuric acid, ready for use	colourless	1 x 12 ml	STOP SOLUTION
12. Test instruction	---	1 booklet	
13. Quality control certificate	---	1 protocol	

LOT	Lot description		Storage temperature
IVD	In vitro diagnostic medical device	CE	Unopened usable until

Preparation and stability of the reagents

Note: All reagents must be brought to room temperature (+18°C to +25°C) approx. 30 minutes before use. After first use, the reagents are stable until the indicated expiry date if stored at +2°C to +8°C and protected from contamination, unless stated otherwise below.

- **Coated wells:** Ready for use. Tear open the resealable protective wrapping of the microplate at the recesses above the grip seam. Do not open until the microplate has reached room temperature (+18°C to +25°C) to prevent the individual strips from moistening. Immediately replace the remaining wells of a partly used microplate in the protective wrapping and tightly seal with the integrated grip seam (Do not remove the desiccant bags).
  Once the protective wrapping has been opened for the first time, the wells coated with antigens can be stored in a dry place and at a temperature between +2°C and +8°C for 4 months.

- **Calibrators and controls:** Ready for use. The reagents must be mixed thoroughly before use.

- **Enzyme conjugate:** Ready for use. The enzyme conjugate must be mixed thoroughly before use.

- **Sample buffer:** Ready for use. The green coloured sample buffer contains IgG/RF absorbent. Serum or plasma samples diluted with this sample buffer are only to be used for the determination of IgM antibodies.

- **Wash buffer:** The wash buffer is a 10x concentrate. If crystallisation occurs in the concentrated buffer, warm it to 37°C and mix well before diluting. The quantity required should be removed from the bottle using a clean pipette and diluted with deionised or distilled water (1 part reagent plus 9 parts distilled water).
  For example: For 1 microplate strip, 5 ml concentrate plus 45 ml water.
  The working strength wash buffer is stable for 4 weeks when stored at +2°C to +8°C and handled properly.

- **Chromogen/substrate solution:** Ready for use. Close the bottle immediately after use, as the contents are sensitive to light. The chromogen/substrate solution must be clear on use. Do not use the solution if it is blue coloured.

- **Stop solution:** Ready for use.

Storage and stability: The test kit has to be stored at a temperature between +2°C to +8°C. Do not freeze. Unopened, all test kit components are stable until the indicated expiry date.

Waste disposal: Patient samples, calibrators, controls and incubated microplate strips should be handled as infectious waste. All reagents must be disposed of in accordance with local disposal regulations.

Warning: The calibrators and controls of human origin have tested negative for HBsAg, anti-HCV, anti-HIV-1 and anti-HIV-2. Nonetheless, all materials should be treated as being a potential infection hazard and should be handled with care. Some of the reagents contain the agent sodium azide in a non-declarable concentration. Avoid skin contact.

Preparation and stability of the patient samples

Samples: Human serum or EDTA, heparin or citrate plasma.

Stability: Patient samples to be investigated can generally be stored at +2°C to +8°C for up to 14 days. Diluted samples should be incubated within one working day.

Introduction: Before the determination of specific antibodies of class IgM, antibodies of class IgG should be removed from the patient sample. This procedure must be carried out in order to prevent any rheumatoid factors from reacting with specifically bound IgG, which would lead to false positive IgM test results, and to prevent that specific IgG displace IgM from the antigen, which would lead to false IgM negative test results.

Functional principle: The sample buffer (green coloured!) contains an anti-human antibody preparation from goat. IgG of a serum or plasma sample is bound with high specificity by these antibodies and precipitated. If the sample also contains rheumatoid factors, these will be absorbed by the IgG/anti-human IgG complex.

Separation properties:
- All IgG subclasses are bound and precipitated by the anti-human IgG antibodies.
- Human serum IgG in concentrations of up to 15 mg per ml are removed (average serum IgG concentration in adults: 12 mg per ml).
- Rheumatoid factors are also removed.
- The recovery rate of the IgM fraction is almost 100%.

Performance: The **patient samples** for analysis are diluted **1:101** with green coloured sample buffer. For example, add 10 µl sample to 1.0 ml sample buffer and mix well by vortexing. Sample pipettes are not suitable for mixing Incubate the mixture for at least **10 minutes** at room temperature (+18°C to +25°C). Subsequently, it can be pipetted into the microplate wells according to the pipetting protocol.

Notes:
- Antibodies of the class IgG should not be analysed with this mixture.
- It is possible to check the efficacy of the IgG/RF absorbent for an individual patient sample by performing an IgG test in parallel to the IgM test using the mixture. If the IgG test is negative, the IgM result can be considered as reliable.
- The calibrators and controls are ready for use, do not dilute them.

Incubation

For **semiquantative analysis** incubate **calibrator 2** along with the positive and negative controls and patient samples. For **quantitative analysis** incubate **calibrators 1, 2 and 3** along with the positive and negative controls and patient samples.

(Partly) manual test performance

Sample incubation: (1st step)
Transfer 100 µl of the calibrators, positive and negative controls or diluted patient samples into the individual microplate wells according to the pipetting protocol. Incubate for **30 minutes** at room temperature (+18°C to +25°C).

Washing:
Manual: Empty the wells and subsequently wash 3 times using 300 µl of working strength wash buffer for each wash.
Automatic: Wash the reagent wells 3 times with 450 µl of working strength wash buffer (program setting: e.g. TECAN Columbus Washer "Overflow Modus").

Leave the wash buffer in each well for 30 to 60 seconds per washing cycle, then empty the wells. After washing (manual and automated tests), thoroughly dispose of all liquid from the microplate by tapping it on absorbent paper with the openings facing downwards to remove all residual wash buffer.

Note: Residual liquid (> 10 µl) remaining in the reagent wells after washing can interfere with the substrate and lead to false low extinction values.
Insufficient washing (e.g. less than 3 wash cycles, too small wash buffer volumes, or too short residence times) can lead to false high extinction values.
Free positions on the microplate strip should be filled with blank wells of the same plate format as that of the parameter to be investigated.

Conjugate incubation: (2nd step)
Pipette 100 µl of enzyme conjugate (peroxidase-labelled anti-human IgM) into each of the microplate wells. Incubate for **30 minutes** at room temperature (+18°C to +25°C).

Washing:
Empty the wells. Wash as described above.

Substrate incubation: (3rd step)
Pipette 100 µl of chromogen/substrate solution into each of the microplate wells. Incubate for **15 minutes** at room temperature (+18°C to +25°C), protect from direct sunlight.

Stopping:
Pipette 100 µl of stop solution into each of the microplate wells in the same order and at the same speed as the chromogen/substrate solution was introduced.

Measurement:
Photometric measurement of the colour intensity should be made at a **wavelength of 450 nm** and a reference wavelength between 620 nm and 650 nm **within 30 minutes of adding the stop solution.** Prior to measuring, carefully shake the microplate to ensure a homogeneous distribution of the solution.

Test performance using fully automated analysis devices

Sample dilution and test performance are carried out fully automatically using an analysis device. The incubation conditions programmed in the respective software authorised by EUROIMMUN may deviate slightly from the specifications given in the ELISA test instruction. However, these conditions were validated in respect of the combination of the EUROIMMUN Analyzer I, Analyzer I-2P or the DSX from Dynex and this EUROIMMUN ELISA. Validation documents are available on enquiry.
Automated test performance using other fully automated, open system analysis devices is possible. However, the combination should be validated by the user.

Pipetting protocol

	1	2	3	4	5	6	7	8	9	10	11	12
A	**C 2**	**P 6**	**P 14**	**P 22**			**C 1**	**P 4**	**P 12**	**P 20**		
B	**pos.**	**P 7**	**P 15**	**P 23**			**C 2**	**P 5**	**P 13**	**P 21**		
C	**neg.**	**P 8**	**P 16**	**P 24**			**C 3**	**P 6**	**P 14**	**P 22**		
D	**P 1**	**P 9**	**P 17**				**pos.**	**P 7**	**P 15**	**P 23**		
E	**P 2**	**P 10**	**P 18**				**neg.**	**P 8**	**P 16**	**P 24**		
F	**P 3**	**P 11**	**P 19**				**P 1**	**P 9**	**P 17**			
G	**P 4**	**P 12**	**P 20**				**P 2**	**P 10**	**P 18**			
H	**P 5**	**P 13**	**P 21**				**P 3**	**P 11**	**P 19**			

The pipetting protocol for microtiter strips 1-4 is an example of the **semiquantitative determination** of antibodies in 24 patient samples (P 1 to P 24).
The pipetting protocol for microtiter strips 7-10 is an example of the **quantitative determination** of antibodies in 24 patient samples (P 1 to P 24).

Calibrators (C 1 to C 3), positive (pos.) and negative (neg.) control as well as the patient samples have been incubated in one well each. The reliability of the ELISA test can be improved by duplicate determinations of each sample.
The wells can be broken off individually from the strips. This makes it possible to adjust the number of test substrates used to the number of samples to be examined and minimises reagent wastage.
Both positive and negative controls serve as internal controls for the reliability of the test procedure. They should be assayed with each test run.

Calculation of results

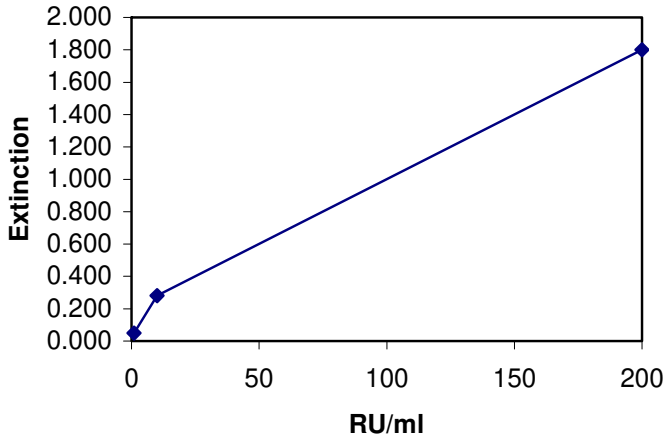
Semiquantitative: Results can be evaluated semiquantitatively by calculating a ratio of the extinction value of the control or patient sample over the extinction value of calibrator 2. Calculate the ratio according to the following formula:

$$\frac{\textbf{Extinction of the control or patient sample}}{\textbf{Extinction of calibrator 2}} = \textbf{Ratio}$$

EUROIMMUN recommends interpreting results as follows:

Ratio <0.8:	**negative**
Ratio ≥0.8 to <1.1:	**borderline**
Ratio ≥1.1:	**positive**

Quantitative: The standard curve from which the concentration of antibodies in the patient samples can be taken is obtained by point-to-point plotting of the extinction values measured for the 3 calibrators against the corresponding units (linear/linear). Use “point-to-point” plotting for calculation of the standard curve by computer. The following plot is an example of a typical calibration curve. Please do not use this curve for the determination of antibody concentrations in patient samples.



If the extinction for a patient sample lies above the value of calibrator 1 (200 RU/ml), the result should be reported as “>200 RU/ml“. It is recommended that the sample be retested at a dilution of e.g. 1:400. The result in RU/ml read from the calibration curve for this sample must then be multiplied by a factor of 4. The upper limit of the reference range of non-infected persons (cut-off value) recommended by EUROIMMUN is 20 relative units RU/ml. EUROIMMUN recommends interpreting results as follows:

<16 RU/ml:	**negative**
≥16 to <22 RU/ml:	**borderline**
≥22 RU/ml:	**positive**

For duplicate determinations the mean of the two values should be taken. If the two values deviate substantially from one another, EUROIMMUN recommends to retest the samples.

A negative serological result does not exclude an infection. Particularly in the early phase of an infection, antibodies may not yet be present or are only present in such small quantities that they are not detectable. In case of a borderline result, a secure evaluation is not possible. If there is a clinical suspicion and a negative test result, we recommend clarification by means of other diagnostic methods and/or the serological investigation of a follow-up sample. A positive result indicates that there has been contact with the pathogen. In the determination of pathogen-specific IgM antibodies, polyclonal stimulation of the immune system or antibody persistence may affect the diagnostic relevance of positive findings. Significant titer increases (exceeding factor 2) and/or seroconversion in a follow-up sample taken after 7-10 days can indicate an acute infection. To investigate titer changes, sample and follow-up sample should be incubated in adjacent wells of the ELISA microplate within the same test run. For diagnosis, the clinical picture of the patient always needs to be taken into account along with the serological findings.

Test characteristics

Calibration: As no international reference serum exists for antibodies against Borrelia, the calibration is performed in relative units (RU)/ml.

For every group of tests performed, the extinction values of the calibrators and the relative units and/or ratio values determined for the positive and negative controls must lie within the limits stated for the relevant test kit lot. A quality control certificate containing these reference values is included. If the values specified for the controls are not achieved, the test results may be inaccurate and the test should be repeated.

The binding activity of the antibodies and the activity of the enzyme used are temperature-dependent. It is therefore recommended using a thermostat in all three incubation steps. The higher the room temperature (+18°C to +25°C) during the incubation steps, the greater will be the extinction values. Corresponding variations apply also to the incubation times.
However, the calibrators are subject to the same influences, with the result that such variations will be largely compensated in the calculation of the result.

Antigen: The antigen source is provided by particularly suitable Borrelia strains (Borrelia burgdorferi sensu stricto, Borrelia afzelii and Borrelia garinii). The cultured bacteria were solubilised using sodium dodecyl sulphate. The used antigen mixture contains all relevant proteins.

Linearity: The linearity of the Anti-Borrelia ELISA (IgM) was determined by assaying 4 serial dilutions of different patient samples. The coefficient of determination R^2 for all sera was > 0.95. The Anti-Borrelia ELISA (IgM) is linear at least in the tested concentration range (3 RU/ml to 177 RU/ml).

Detection limit: The lower detection limit is defined as the mean value of an analyte-free sample plus three times the standard deviation and is the smallest detectable antibody titer. The lower detection limit of the Anti-Borrelia ELISA (IgM) is 1.3 RU/ml.

Cross reactivity: Cross reactivity of the Anti-Borrelia ELISA (IgM) was evaluated in a study performed on 263 patient sera with antibodies against Treponema pallidum and 18 patient sera with antibodies against Leptospira. 26 sera of the Anti-Treponema pallidum-positive sera and 3 sera of the Anti-Leptospira-positive sera presented reactivity. Therefore, the cross reactivity amounts to 9.9% and 16.7%, respectively. Cross reactions with antibodies against other spirochaetes cannot be excluded. The Anti-Borrelia ELISA is designed as a screening test which should be followed by a confirmatory test (Western- or line blot). Further sera from patients with different possible influencing factors were investigated. An overview of these results can be found in the following table.

Possible influencing factors	n	Anti-Borrelia ELISA (IgM) positive
Acute EBV infection	27	0%
Anti-CMV	19	0%
Anti-Measles virus	13	0%
Anti-Mumps virus	12	0%
Anti-Toxoplasma gondii	14	0%
Anti-VZV	15	0%
Anti-Rubella virus	10	0%
Anti-HSV	5	0%
Anti-HAV	7	0%
Anti-HBV	8	0%
Anti-HAMA	4	0%
ANA + DNS (AAb)	45	0%
Rheuma factor	39	0%
Neurological diseases	54	1.9%

Interference: Haemolytic, lipaemic and icteric samples showed no influence on the result up to a concentration of 10 mg/ml for haemoglobin, 20 mg/ml for triglycerides and 0.4 mg/ml for bilirubin in this ELISA.

Reproducibility: The reproducibility of the test was investigated by determining the intra- and inter-assay coefficients of variation (CV) using 3 sera. The intra-assay CVs are based on 20 determinations and the inter-assay CVs on 4 determinations performed in 6 different test runs.

Intra-assay variation, n = 20		
Serum	**Mean value (RU/ml)**	**CV (%)**
1	75	4.3
2	103	2.9
3	115	3.4

Inter-assay variation, n = 4 x 6		
Serum	**Mean value (RU/ml)**	**CV (%)**
1	78	6.9
2	109	6.2
3	122	4.7

Specificity and sensitivity: Sera from 150 patients with suspected borreliosis were analysed using the EUROIMMUN Anti-Borrelia ELISA (IgM) and the EUROIMMUN Anti-Borrelia burgdorferi Westernblot (IgM) as a reference method. The test showed a specificity of 96.4% and a sensitivity of 100%.

n = 150		**EUROIMMUN Westernblot**		
		positive	borderline	negative
ELISA	positive	34	5	4
	negative	0	1	106

Clinical study: 138 patients with clinically characterised borreliosis in different disease stages were screened with the Anti-Borrelia plus VlsE-ELISA (IgG) and Anti-Borrelia-ELISA (IgM). Sensitivities between 89-96% were found.

n = 138		**EUROIMMUN Anti-Borrelia plus VlsE-ELISA (IgG), EUROIMMUN Anti-Borrelia-ELISA (IgM)**		
		IgG positive	IgM positive	IgG und/oder IgM positive
Clinic	erythema migrans, n = 97	60	68	86 (88.7%)
	mono- or poly- arthritis, n = 26	22	5	25 (96.2%)
	neuroborreliosis, n = 15	13	4	14 (93.3%)

All ELISA negative results were confirmed as negative by an Anti-Borrelia Westernblot.

Reference range: The levels of the anti-Borrelia antibodies (IgM) were analysed with this EUROIMMUN ELISA in 500 healthy blood donors. With a cut-off of 20 RU/ml, 1.6% of the blood donors were anti-Borrelia positive (IgM).

Clinical significance

The history of Lyme disease, a contagious condition caused by Borrelia burgdorferi and transmitted to man by ticks, offers infectiologists a formidable lesson on how medicine progresses. Clinical description started in Europe at the turn of the 19^{th}/20^{th} century with Pick's description of what was then labelled chronic atrophic acrodermatitis. Fifty years later, Hauser noted the affection was transmitted by ticks. Independently, Afzelius, then Lipschutz, described erythema migrans and its relationship with tick bites. Neurological involvement was also described with the skin signs. These early dermatological descriptions suddenly came into the limelight in 1975 when an epidemia of arthritis occurred in children in Lyme, Connecticut, USA. Many of the affected children had erythema migrans. Based on these observations and an epidemiological analysis of the epidemia, Steele and co-workers defined "Lyme disease" as a rheumatological disorder commonly associated with erythema migrans and sometimes with multiple organ involvement. In 1982 Burgdorfer suggested that ticks transmitted "Treponema-like spirochaetes", which were later authentified as the causal agent: Borrelia burgdorferi.

Borrelia burgdorferi as a species of bacteria is belonging to the spirochaetaceae family. This family includes five genera: borrelia, spirochaetes, cristispira, treponema and leptospira. Among about 30 tick species feeding on humans, Ixodes ricinus is the most frequent tick species biting humans in Europe. It is the vector of Borrelia burgdorferi, which causes Lyme disease, of anaplasma phagocytophilum and the tick-borne encephalitis virus. I. ricinus ticks pass through three developmental stages: larvae, nymphs and adults (females and males). The density of this tick species can be very high, reaching in some places more than 300 ticks/100 m^2. Lyme borreliosis is the most frequent tick-transmitted disease in the northern hemisphere. In Europe, especially in Central Europe and Scandinavia there are up to 155 cases per 100,000 individuals caused by the species B. burgdorferi sensu stricto, B. afzelii, and B. garinii (in rare cases also by B. spielmanii and B. bavariensis). The human seroprevalence rate of antibodies against Borrelia burgdorferi in the normal popu-lation of Germany and other Central European countries is about 8% (for IgG); in highly endemic areas even considerably higher with ranges from 18% to 52%. In East Asia, e.g. in an endemic area in China, the seroprevalence amounts to 26%. Persons working in the area of forestry display anti-Borrelia antibodies in about 40%, hunters in more than 50% of cases.

A Borrelia burgdorferi infection can manifest itself in the areas of dermatology, neurology, ophthal-mology, rheumatology and internal medicine. The clinical expression of borreliosis can be divided into three stages:

Stage I: The typical primary manifestation of a Borrelia burgdorferi infection is erythema migrans (80%), a reddening of the skin which appears around the area of the tick bite and spreads in a circular manner. The erythema is accompanied by influenza-like general symptoms with fever, shaking chill, headaches and vomiting. Lymphadenopathies are observed in a few cases (lymphadenosis cutis benigna). The leading clinical types of the erythema-free borreliosis (20%) are neurological, arthromyalgic, influenza-like, cardiovascular borreliosis and borreliosis with hepatitis, with regional lymphadenitis and mixtures of these. Stage I can result in spontaneous healing or can develop into a generalised borreliosis. The transition phase is generally symptom-free. IgM antibodies against Borrelia burgdorferi can be detected serologically in 50% to 90% of patients during stage I. Humans produce highly specific antibodies against the outer surface protein C (OspC) shortly after infection with Borrelia burgdorferi. The main antigen VlsE, which is exclusively expressed in vivo, is the most sensitive antigen for IgG antibody detection, whereas OspC is the most sensitive antigen for IgM antibody detection.

Stage II: A variety of symptoms can develop several weeks to some months after receiving the tick bite. In the foreground of these are neurological manifestations: meningitis, encephalitis, asymmetric poly-neuritis, cranial nerve pareses, Bannwarth's lymphocytic meningoradiculoneuritis. One of the most frequent neurological symptoms of borreliosis in children is the peripheral facial palsy. Usually the paresis of the facial nerve appears on one side and after several weeks on the opposite side. Also arthritis, particularly of the knee joints, and non-localised pains in the bones, joints and muscles are frequently found. Less frequently are cardiological manifestations such as myocarditis and pericarditis. Antibodies against Borrelia burgdorferi can be detected in 50% to 90% of patients in stage II. In the early phase of this stage mainly IgM antibodies are found but in the late phase often only IgG antibodies occur. However, IgM antibodies can persist for a long time. By additionally determining antibodies against VlsE the serological hit rate can be increased by 20% to 30%. VlsE displays the highest sensi-tivity of all antigens tested for IgG detection.

Stage III: The typical manifestations of a Borrelia burgdorferi infection in stage III are chronic-relapsing erosive arthritis, acrodermatitis chronica atrophicans and progressive encephalomyelitis, which can proceed in a similar way to multiple sclerosis. Without treatment, the tertiary stage can develop over a period of years to decades after the original infection. In this stage, IgG antibody titers are significantly increased in 90% to 100% of patients, while IgM antibodies are only rarely detectable.

For the diagnosis of neuroborreliosis, the determination of antibodies in cerebrospinal fluid is of decisive importance and far superior to serological testing. An antibody index (AI) discriminates between a blood-derived and a pathological, brain-derived specific antibody fraction in cerebrospinal fluid (CSF) and takes into account individual changes in the blood/CSF barrier function.

Various techniques come into question for the detection of antibodies against Borrelia burgdorferi: ELISA, indirect immunofluorescence (IIFT), passive haemagglutination and immunoblot. Generally, ELISA or IIFT are used as screening test for preliminary characterisation of the serum sample. According to guidelines in the USA and in Germany, serological diagnosis should follow the principle of a two-step procedure: ELISA or indirect immunofluorescence (IIFT) as a first step, which, if reactive, is followed by an immunoblot. In fresh infections it is recommended performing ELISA/IIFT and immuno-blot in parallel, since some weak reactions become detectable earlier in the blot than in the screening test. VlsE, the most sensitive antigen for IgG antibody detection, and OspC for IgM antibody detection should be included in the test. By additionally determining antibodies against VlsE the serological hit rate can be increased by 20% compared to whole extract Westernblots. Of all recombinant antigens tested, VlsE possesses the highest sensitivity for the detection of a Borrelia infection.

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CE

Declaration of Conformity

EUROIMMUN Medizinische Labordiagnostika AG
Seekamp 31, 23560 Lübeck, Germany

declares under its sole responsibility as manufacturer that the Blot product:

Anti-Echinococcus EUROLINE WB (IgG) *DY 2321-####-1 G*

(product name, order number)

meet the following demands of:

Directive 98/79/EC on in vitro diagnostic medical devices of 27 October 1998 and its transpositions in national laws which apply to it.

Conformity assessment procedure: Annex III

This Declaration of Conformity is valid based on the respective currently valid version of technical documentation.

Lübeck, 25.04.2022
(Place and date of issue)

Dr. Ewald Müller-Kunert
- Head of Quality Management and Regulatory Affairs -

Susanne Aleksandrowicz
- Member of the Executive Board -

Anti-Echinococcus EUROLINE WB (IgG)
Test instruction

ORDER NO.	ANTIBODIES AGAINST	IG CLASS	SUBSTRATE	FORMAT
DY 2321-1601-1 G	Echinococcus antigen extract (whole antigen) plus Em18, Em95 and EgAgB	IgG	Antigen coated membrane strips	16 x 01 (16)

Indication: The EUROLINE-WB test kit provides a qualitative in vitro assay for human antibodies of the immunoglobulin class IgG against Echinococcus granulosus and Echinococcus multilocularis antigens in serum or plasma for the diagnosis of echinococcosis.

Application: The Anti-Echinococcus EUROLINE-WB (IgG) is excellently suited for result confirmation of serological screening tests for the detection of Echinococcus-specific antibodies (IHA, IIFT, ELISA). The use of an Echinococcus whole antigen extract and biochemically produced single antigens of Echinococcus granulosus and Echinococcus multilocularis in the Anti-Echinococcus EUROLINE-WB (IgG) also enables differentiation between cystic and alveolar echinococcosis, caused by Echinococcus granulosus and multilocularis, respectively.

Test principle: The test kit contains blot strips with electrophoretically separated antigen extract of Echinococcus. Additionally, each blot has a membrane chip coated with the biochemically produced antigens Em18, Em95 and EgAgB. In the first reaction step the blot strips are incubated with diluted patient samples. In the case of positive samples, specific antibodies of class IgG (and IgA, IgM) will bind to the antigens. To detect the bound antibodies, a second incubation is carried out using an enzyme-labelled anti-human IgG (enzyme conjugate) catalysing a colour reaction.

Contents of the test kit:

Component	Format	Symbol
1. Test strips Single strips with electrophoretically separated Echinococcus antigen extract plus Em18, Em95 and EgAgB	16 x 1	STRIPS
2. Evaluation matrix with control strip Test strip incubated with a positive control serum	1 pattern	---
3. Enzyme conjugate Alkaline phosphatase-labelled anti-human IgG (goat), 10x concentrate	1 x 3 ml	CONJUGATE 10x
4. Universal buffer 10x concentrate	1 x 50 ml	BUFFER 10x
5. Substrate solution Nitroblue tetrazolium chloride/5-Bromo-4-chloro-3-indolylphosphate (NBT/BCIP), ready for use	1 x 30 ml	SUBSTRATE
6. Incubation tray	2 x 8 channels	TRAY
7. Test instruction	1 booklet	---

LOT	Lot description		Storage temperature
IVD	In vitro diagnostic medical device	CE	Unopened usable until

Storage and stability: The test kit has to be stored at a temperature between +2°C to +8°C, do not freeze. Unopened, all test kit components are stable until the indicated expiry date.

Waste disposal: Undiluted patient sera and incubated blot strips should be handled as infectious waste. Other reagents do not need to be collected separately, unless stated otherwise in official regulations.

The following components are not provided in the test kits but can be ordered at EUROIMMUN under the respective order numbers. Performance of the test requires an **incubation tray**:
ZD 9895-0130 Incubation tray with 30 channels (black)
ZD 9898-0144 Incubation tray with 44 channels (black, for the EUROBlotOne and EUROBlotCamera system)
For the creation of work protocols and the evaluation of incubated test strips using **EUROLineScan** green paper and adhesive foil are required:
ZD 9880-0101 Green paper (1 sheet)
ZD 9885-0116 Adhesive foil for approx. 16 test strips
ZD 9885-0130 Adhesive foil for approx. 30 test strips
If a **visual evaluation** is to be performed in individual cases, the required evaluation protocol can be ordered under: ZD 2321-0101-1 Evaluation protocol visual Anti-Echinococcus EUROLINE-WB.

Preparation and stability of the reagents

Note: The bag containing the blot strips is printed with a number in addition to the test kit lot number. This number refers to the strip batch and is also printed on the corresponding evaluation template. These two numbers must match to ensure correct evaluation of test results.
All reagents must be brought to room temperature (+18°C to +25°C) approx. 30 minutes before use. Unopened, reagents are stable until the indicated expiry date when stored at +2°C to +8°C. After initial opening, reagents are stable for 12 months or until the expiry date, if earlier, unless stated otherwise in the instructions. Opened reagents must also be stored at +2°C to +8°C and protected from contamination.

- **Coated test strips:** Ready for use. Open the packing with the test strips only when the strips have reached room temperature to prevent condensation on the strips. After removal of the strips the packing should be sealed tightly and stored at +2°C to +8°C. To ensure correct evaluation of results, the lot number on the bag must match the lot number on the strips as well as on the evaluation matrix.

- **Enzyme conjugate:** The enzyme conjugate is supplied as a 10x concentrate. For the preparation of the ready for use enzyme conjugate the amount required should be removed from the bottle using a clean pipette and diluted 1:10 with ready for use diluted universal buffer. For 1 test strip dilute 0.15 ml anti-human IgG concentrate with 1.35 ml ready for use diluted universal buffer. The ready for use diluted enzyme conjugate should be used at the same working day.

- **Universal buffer:** The universal buffer is supplied as a 10x concentrate. For the preparation of the ready for use universal buffer the amount required should be removed from the bottle using a clean pipette and diluted 1:10 with deionised or distilled water. For the incubation of 1 test strip 1.5 ml buffer concentrate should be diluted with 13.5 ml deionised or distilled water. The ready for use diluted universal buffer should be used at the same working day.

- **Substrate solution:** Ready for use. Close bottle immediately after use, as the contents are sensitive to light .

Warning: The control of human origin has tested negative for HBsAg, anti-HCV, anti-HIV-1 and anti-HIV-2. Nonetheless, all materials should be treated as being a potential infection hazard and should be handled with care. Some of the reagents contain the agent sodium azide in a non-declarable concentration. Avoid skin contact.

Preparation and stability of the serum or plasma samples

Sample material: Human serum or EDTA, heparin or citrate plasma.

Stability: Patient samples to be investigated can generally be stored at +2°C to +8°C for up to 14 days. Diluted samples should be incubated within one working day.

Sample dilution: The **patient samples** for analysis are diluted **1:51** in the ready for use diluted universal buffer. For example, add 30 µl of sample to 1.5 ml ready for use diluted universal buffer and mix well by vortexing. Sample pipettes are not suitable for mixing.

Incubation

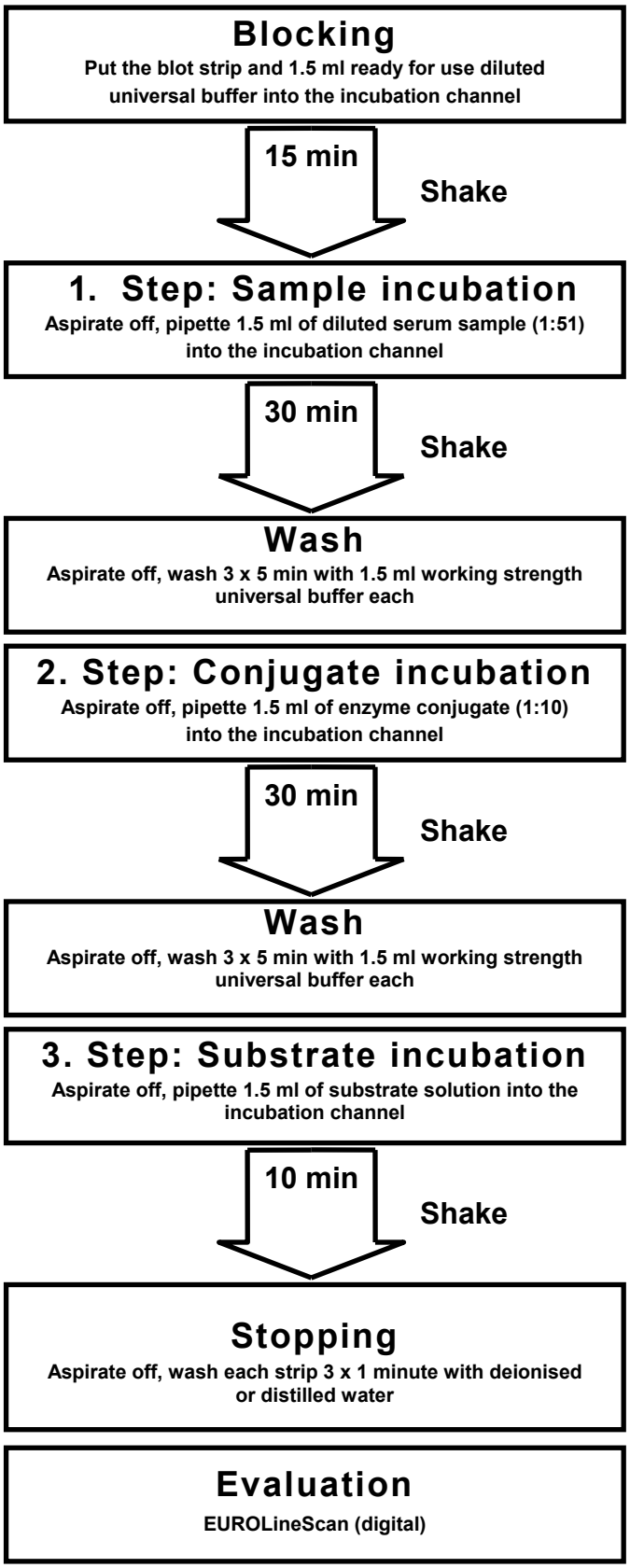
Blocking:	According to the number of serum samples to be tested fill each channel of the incubation tray with 1.5 ml ready for use diluted universal buffer and a blot strip. Remove the required amount of blot strips from the packing using a pair of tweezers. The number on the test strip should be visible. Incubate for **15 minutes** at room temperature (+18°C to +25°C) on a rocking shaker. Afterwards aspirate off all the liquid.
Sample incubation: (1st step)	Fill each channel with 1.5 ml of the diluted serum samples and incubate at room temperature (+18°C to +25°C) for **30 minutes** on a rocking shaker.
Wash:	Aspirate off the liquid from each channel and wash **3 x 5 minutes** each with 1.5 ml working strength universal buffer on a rocking shaker.
Conjugate incubation: (2nd step)	Pipette 1.5 ml ready for use diluted enzyme conjugate (alkaline phosphatase-conjugated anti-human IgG) into each channel and incubate for **30 minutes** at room temperature (+18°C to +25°C) on a rocking shaker.
Wash:	Aspirate off the liquid from each channel. Wash as described above.
Substrate incubation: (3rd step)	Pipette 1.5 ml substrate solution into the channels of the incubation tray. Incubate for **10 minutes** at room temperature (+18°C to +25°C) on a rocking shaker.
Stopping:	Aspirate off the liquid from each channel and wash each strip **3 x 1 minute** with deionised or distilled water.

For automated incubation with the EUROBlotMaster select the program **Euro02 Inf WB 30**.

For automated incubation with the EUROBlotOne select the program **Euro 01/02**.

Anti-Echinococcus EUROLINE-WB (IgG)

Incubation protocol



Evaluation and Interpretation of the results of the Anti-Echinococcus EUROLINE-WB (IgG)

Handling: For evaluation of incubated test strips we generally recommend using the **EUROLineScan** software. After stopping the reaction using deionised or distilled water, place the incubated test strips onto the adhesive foil of the green work protocol using a pair of tweezers. The position of the test strips can be corrected while they are wet. As soon as all test strips have been placed onto the protocol, they should be pressed hard using filter paper and left to air-dry. After they have dried, the test strips will be stuck to the adhesive foil. The dry test strips are then scanned with a flatbed scanner (EUROIMMUN AG) and evaluated with **EUROLineScan**. Alternatively, imaging and evaluation is possible directly from the incubation trays (EUROBlotCamera and EUROBlotOne). For general information about the EUROLineScan program please refer to the EUROLineScan user manual (EUROIMMUN AG). The code for entering the **Test** in EUROLineScan is **Echino_EL-WB_IgG**.

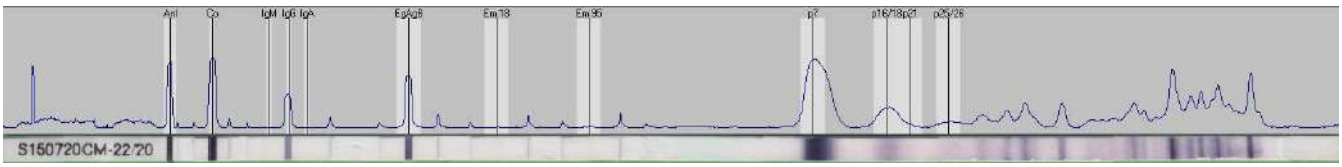
If a visual evaluation must be performed in exceptional cases, hold the evaluation matrix next to the stuck-on blot strips and position it so that the black band above the number on the blot strips lines up with the alignment bar of the evaluation matrix. **The lot number on the evaluation matrix must match the lot number on the blot strips.** Clearly recognisable bands on the blot strips which concur with the labelled bands on the evaluation matrix are noted in the evaluation protocol.

Antigens: The antigen source for the **EUROIMMUN Anti-Echinococcus EUROLINE-WB** is provided by a particularly suitable Echinococcus antigen preparation. This antigen has been solubilised using sodium dodecyl sulphate followed by a separation of the solubilised protein using discontinuous polyacrylamide gel electrophoresis according to molecular mass and transfer of the separated proteins to nitrocellulose. Additional membrane strips containing the antigens Em 95, Em 18 and EgAgB are applied to each nitrocellulose membrane.

Control blot strips were taken from each nitrocellulose membrane and incubated with a reference serum. One of these stained strips is included in the kit.

Specificity of the antigens on the test strips:

Band	Antigen	Specificity
24-26 kDa	p25/26	Unspecific
21 kDa	p21	Specific for Echinococcus and other parasites
16-18 kDa	p16/18	Specific for Echinococcus
7 kDa	p7	Specific for Echinococcus
Em 95	Em 95	Specific for Echinococcus multilocularis
Em 18	Em 18	Specific for Echinococcus multilocularis
EgAgB	EgAgB	Specific for Echinococcus



In the lower part of the test strip there is a conjugate control membrane chip (IgA, IgG and IgM). Below the conjugate control, there is a membrane chip with a control band (Control).

Attention: A correctly performed determination of antibodies of class IgG against the antigens described above is indicated by a positive reaction of the control band and a positive reaction of the IgG band.

If one of these bands only shows a very weak reaction or none at all, the result is not valid.

IgG class antibodies against Echinococcus

In order to evaluate the signals, the band position and the intensity of the staining must be taken into account, as negative sera can also in some cases produce weak signals at individual band positions.

The defined antigens on the Anti-Echinococcus EUROLINE-WB can be divided into 5 categories:

Category	Antigens
1	antigen: p25/26
2	genus-specific Echinococcus antigen: EgAgB
3	Echinococcus antigen: p21
4	Echinococcus antigens: p7 and p16/18
5	Echinococcus multilocularis antigens: Em18 and Em95

Result interpretation: The results obtained with the Anti-Echinococcus EUROLINE-WB can be classified into negative, borderline and positive.

Result	Characteristics
Negative	No bands, or one positive antigen band of category 1, or one borderline antigen band of category 2.
Borderline	Positive antigen band of category 2, or at least one borderline antigen band of category 3, 4 or 5. It is recommended that a fresh sample be taken and the test be repeated after a few weeks.
Positive	At least one positive antigen band of category 3, 4 or 5. If one antigen band of category 3 is positive, either alone or in combination with an antigen band of category 1, cross reactivity may have occurred due to an Ascaris or Anisakis infection.

The Anti-Echinococcus EUROLINE-WB allows a serological differentiation between an infection with Echinococcus granulosus and Echinococcus multilocularis in many cases. The differentiation is automatically made during evaluation with EUROLineScan.

For differentiation the following applies:

Result	Characteristics
Echinococcus granulosus	Positive antigen band of category 2 and additionally at least one positive antigen band of category 3 or 4.
Echinococcus multilocularis	At least one positive antigen band of category 5. Further positive antigen bands from the other categories may also occur.

For diagnosis, the clinical picture of the patient always needs to be taken into account along with the serological findings.

Test characteristics

Measurement range: The EUROLINE-WB is a qualitative method. No measurement range is provided.

Inter- and intra-assay variation: The inter-assay variation was determined by multiple analyses of characterised samples over several days. The intra-assay variation was determined by multiple analyses of characterised samples on one day. In every case, the intensity of the bands was within the specified range. This EUROLINE-WB displays excellent inter- and intra-assay reproducibility.

Interference: Haemolytic, lipaemic and icteric sera showed no effect on the analytical results.

Cross reactions: The cross reactivity of the EUROLINE-WB was investigated using patient samples with the following parasite infections:

Species	Number of samples	Number of positive results
Ascaris lumbricoides	10	3
Anisakis simplex	16	1
Filarioidea	5	0
Strongyloides stercoralis	10	0
Schistosoma ssp.	9	0
Multi-Helminth infection	2	0
Plasmodium ssp.	7	0
Toxocara canis	10	0
Taenia solium	7	0
Trichinella spiralis	13	0
Fasciola hepatica	17	0
Entamoeba histolytica	11	0
Leishmania ssp.	5	0

Specificity and sensitivity: A panel of 107 defined patient samples with a positive Echinococcus result and a control panel with serum samples from 50 healthy blood donors and 50 tumour patients were analysed using the Anti-Echinococcus EUROLINE-WB (IgG). The samples had been provided by the Institute of Parasitology of the University of Bern, Switzerland.

n = 207		Characterisation: Institute of Parasitology, University of Bern	
		positive	negative
Anti-Echinococcus EUROLINE-WB (IgG)	positive	99	0
	negative	8	100

The Anti-Echinococcus EUROLINE-WB (IgG) has a specificity of 100% and a sensitivity of 93%.

Of the 99 Echinococcus samples that were positive in the Anti-Echinococcus EUROLINE-WB (IgG) 80 samples could be differentiated further:

Echinococcus IgG positive: n = 99	Number of samples
Echinococcus granulosus	47
Echinococcus multilocularis	33
No differentiation possible	19

The rate of differentiation between Echinococcus granulosus and Echinococcus multilocularis is 81%.

Clinical significance

Echinococcosis is an infectious disease caused by parasites of the genus *Echinococcus*. In Europe, the dog tapeworm (*E. granulosus*), causing cystic echinococcosis (CE), and the fox tapeworm (*E. multilocularis*), causing alveolar echinococcosis (AE), are most important from the medical point of view.
The development of all *Echinococcus* species includes an obligatory host change: the definitive hosts are carnivores, the intermediate hosts mostly herbivores. The mature worms of the fox tapeworm, *E. multilocularis*, live in the intestine of their definitive hosts (in Europe, mainly red foxes, seldom dogs and cats) who release the mature cestode eggs with their droppings. These eggs are very robust and may be infectious for several months if the conditions are good. The intermediate hosts (small mammals like field mice or European water voles) take the eggs in with their food. The larvae of the worms then develop in their inner organs (mostly in the liver).
Humans can be infected with *E. granulosus* by smear infection, through dealing with contaminated soil or consuming contaminated foods. Also here, the eggs are dispersed with the definitive hosts' droppings (mostly dogs, at times cats) and stay infectious for months. The larvae develop in liquid-filled blisters (hyatides) which are found in liver, lung, other organs and also in the skeletal system of the intermediate host (e.g. hoofed animals such as cows or sheep).
Humans are incidental hosts for fox and dog tapeworms. Human infection usually takes place by intake of cestodes after contact with infected hosts (e.g. smear infections or via the animal's fur).
The clinical image of both echinococcoses (CE and AE) differs in the different developmental behaviours of both parasites in the human body. The clinical appearance of AE corresponds to that of a malignoma. After haematogenous transport of the cestodes into the liver, infection of the liver takes place (usually unnoticed for a long time) and an alveolar tumour develops.
In infections with *E. granulosus*, larvae are released in the human intestine. They are transported haematogenously via the portal vein first into the liver and then into other organs, e.g. the lungs. The clinical course may vary significantly and is characterised by the slowly growing cysts and their different localisation.
In humans, both diseases remain asymptomatic for many years, until after 10 to 15 years they show symptoms like cholestatic icterus, epigastric pains, fatigue, weight loss and hepatomegaly. By invasion and destruction of the healthy liver tissue, an untreated echinococcosis may lead to the patient's death. Differential diagnosis from cysts, malignant and benign tumours, abscesses and the distinction between AE and CE are important for a diagnosis.

Imaging techniques, such as sonography, CT and MRT are used for diagnosis. The use of serological test systems for the detection of parasite-specific antibodies in serum or plasma helps to confirm results from imaging techniques. If whole antigens of *Echinococcus* are used, an echinococcosis can be detected with good sensitivity by ELISA or IFT tests.
The use of species-specific antigens in Westernblot and ELISA tests enables in many cases the serological differentiation of *E. granulosus* and *E. multilocularis.*

A negative result in this serological investigation does not exclude an infection. In liver CE, serum antibodies can be detected in 80 to 94% of cases. In lung echinococcosis, the prevalence amounts to only 65 to 70%.
In practice, molecular detection methods (PCR for the detection of *Echinococcus* DNA and RNA) have not proven to deliver.

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CE

Declaration of Conformity

EUROIMMUN Medizinische Labordiagnostika AG
Seekamp 31, D-23560 Lübeck, Germany

declare under our sole responsibility that the ELISA products

Anti-West Nile Virus ELISA (IgG)	*EI 2662-9601 G*
Anti-West Nile Virus ELISA (IgM)	*EI 2662-9601 M*
Avidity: Anti-West Nile Virus ELISA (IgG)	*EI 2662-9601-1 G*

(product name, order no)

meet the demands of

Directive 98/79/EC on in vitro diagnostic medical devices of 27 October 1998 and its transpositions in national laws which apply to it.

Conformity assessment procedure: Annex III

Lübeck, 13.10.2016
(Place and date of issue)

Susanne Aleksandrowicz
- Member of the Board -

Dr. Wolfgang Schlumberger
- Member of the Board -

Anti-West Nile Virus ELISA (IgG)
Test instruction

ORDER NO.	ANTIBODIES AGAINST	IG CLASS	SUBSTRATE	FORMAT
EI 2662-9601 G	West Nile virus	IgG	Ag-coated microplate wells	96 x 01 (96)

Principle of the test: The ELISA test kit provides a semiquantitative or quantitative in vitro assay for human antibodies of the IgG class against West Nile virus in serum or plasma. The test kit contains microtiter strips each with 8 break-off reagent wells coated with West Nile virus antigens. In the first reaction step, diluted patient samples are incubated in the wells. In the case of positive samples, specific IgG (also IgA and IgM) antibodies will bind to the antigens. To detect the bound antibodies, a second incubation is carried out using an enzyme-labelled anti-human IgG (enzyme conjugate) catalysing a colour reaction.

Contents of the test kit:

Component	Colour	Format	Symbol
1. Microplate wells coated with antigens: 12 microplate strips each containing 8 individual break-off wells in a frame, ready for use	---	12 x 8	STRIPS
2. Calibrator 1 200 RU/ml (IgG, human), ready for use	dark red	1 x 2.0 ml	CAL1
3. Calibrator 2 20 RU/ml (IgG, human), ready for use	red	1 x 2.0 ml	CAL2
4. Calibrator 3 2 RU/ml, (IgG, human), ready for use	light red	1 x 2.0 ml	CAL3
5. Positive control (IgG, human), ready for use	blue	1 x 2.0 ml	POS CONTROL
6. Negative control (IgG, human), ready for use	green	1 x 2.0 ml	NEG CONTROL
7. Enzyme conjugate peroxidase-labelled anti-human IgG (rabbit), ready for use	green	1 x 12 ml	CONJUGATE
8. Sample buffer ready for use	light blue	1 x 100 ml	SAMPLE BUFFER
9. Wash buffer 10x concentrate	colourless	1 x 100 ml	WASH BUFFER 10x
10. Chromogen/substrate solution TMB/H_2O_2, ready for use	colourless	1 x 12 ml	SUBSTRATE
11. Stop solution 0.5 M sulphuric acid, ready for use	colourless	1 x 12 ml	STOP SOLUTION
12. Test instruction	---	1 booklet	
13. Quality control certificate	---	1 protocol	
14. Protective foil	---	2 pieces	

LOT Lot description
IVD In vitro diagnostics
CE
Storage temperature
Unopened usable until

Storage and stability: The test kit has to be stored at a temperature between +2°C to +8°C. Do not freeze. Unopened, all test kit components are stable until the indicated expiry date.

Waste disposal: Patient samples, calibrators, controls and incubated microplate strips should be handled as infectious waste. All reagents must be disposed of in accordance with local disposal regulations.

Preparation and stability of the reagents

Note: All reagents must be brought to room temperature (+18°C to +25°C) approx. 30 minutes before use. After first use, the reagents are stable until the indicated expiry date if stored at +2°C to +8°C and protected from contamination, unless stated otherwise below.
The thermostat adjusted ELISA incubator must be set at 37°C +/- 1°C.

- **Coated wells:** Ready for use. Tear open the resealable protective wrapping of the microplate at the recesses above the grip seam. Do not open until the microplate has reached room temperature to prevent the individual strips from moistening. Immediately replace the remaining wells of a partly used microplate in the protective wrapping and tightly seal with the integrated grip seam (Do not remove the desiccant bag).
  Once the protective wrapping has been opened for the first time, the wells coated with antigens can be stored in a dry place and at a temperature between +2°C and +8°C for 4 months.

- **Calibrators and controls:** Ready for use. The reagents must be mixed thoroughly before use.

- **Enzyme conjugate:** Ready for use. The enzyme conjugate must be mixed thoroughly before use.

- **Sample buffer:** Ready for use.

- **Wash buffer:** The wash buffer is a 10x concentrate. If crystallisation occurs in the concentrated buffer, warm it to 37°C and mix well before diluting. The quantity required should be removed from the bottle using a clean pipette and diluted with deionized or distilled water (1 part reagent plus 9 parts distilled water).
  For example: For 1 microplate strip, 5 ml concentrate plus 45 ml water.
  The working strength diluted wash buffer is stable for 4 weeks when stored at +2°C to +8°C and handled properly.

- **Chromogen/substrate solution:** Ready for use. Close the bottle immediately after use, as the contents are sensitive to light. The chromogen/substrate solution must be clear on use. Do not use the solution if it is blue coloured.

- **Stop solution:** Ready for use.

Warning: The controls used have been tested negative for HBsAg, anti-HCV, anti-HIV-1 and anti-HIV-2 using enzyme immunoassays and indirect immunofluorescence methods. Nonetheless, all materials should be treated as being a potential infection hazard and should be handled with care. Some of the reagents contain the toxic agent sodium azide. Avoid skin contact.

Preparation and stability of the patient samples

Samples: Human serum or EDTA, heparin or citrate plasma.

Stability: Patient samples to be investigated can generally be stored at +2°C to +8°C for up to 14 days. Diluted samples should be incubated within one working day.

Sample dilution: Patient samples are diluted **1:101** in sample buffer. For example: dilute 10 µl serum in 1.0 ml sample buffer and mix well by vortexing (sample pipettes are not suitable for mixing).

NOTE: Calibrators and controls are prediluted and ready for use, do not dilute them.

Incubation

For **semiquantative analysis** incubate **calibrator 2** along with the positive and negative controls and patient samples. For **quantitative analysis** incubate **calibrators 1, 2 and 3** along with the positive and negative controls and patient samples.

(Partly) manual test performance

Sample incubation: (1st step)
Transfer 100 µl of the calibrator, positive and negative controls or diluted patient samples into the individual microplate wells according to the pipetting protocol.
For manual processing of microplate wells, cover the finished test plate with the protective foil. When using an automated microplate processor for incubation, follow the instrument manufacturer's recommendations with regard to microwell plate sealing.
Incubate **60 minutes at 37°C ± 1°C**.

Washing:
Manual: Remove the protective foil and empty the wells and subsequently wash 3 times using 300 µl of working strength wash buffer for each wash.
Automatic: Remove the protective foil and empty the wells and subsequently wash 3 times with 450 µl of working strength wash buffer (program setting: e.g. TECAN Columbus Washer "Overflow Modus").

Leave the wash buffer in each well for 30 to 60 seconds per washing cycle, then empty the wells. After washing (manual and automated tests), thoroughly dispose of all liquid from the microplate by tapping it on absorbent paper with the openings facing downwards to remove all residual wash buffer.

Note: Residual liquid (> 10 µl) in the reagent wells after washing can interfere with the substrate and lead to false low extinction values. Insufficient washing (e.g., less than 3 wash cycles, too small wash buffer volumes, or too short reaction times) can lead to false high extinction values.
Free positions on the microplate strip should be filled with blank wells of the same plate format as that of the parameter to be investigated.

Conjugate incubation: (2nd step)
Pipette 100 µl of enzyme conjugate (peroxidase-labelled anti-human IgG) into each of the microplate wells.
When using an automated microplate processor for incubation, follow the instrument manufacturer´s recommendations with regard to microwell plate sealing.
Incubate **30 minutes at room temperature (+18°C to +25°C)**

Washing:
Empty the wells. Wash as described above.

Substrate incubation: (3rd step)
Pipette 100 µl of chromogen/substrate solution into each of the microplate wells. Incubate for **15 minutes at room temperature** (+18°C to +25°C) (protect from direct sunlight).

Stopping the reaction:
Pipette 100 µl of stop solution into each of the microplate wells in the same order and at the same speed as the chromogen/substrate solution was introduced.

Measurement:
Photometric measurement of the colour intensity should be made at a wavelength of 450 nm and a reference wavelength between 620 nm and 650 nm **within 30 minutes of adding the stop solution.** Prior to measuring, slightly shake the microplate to ensure a homogeneous distribution of the solution.

Test performance using fully automated analysis devices

Sample dilution and test performance are carried out fully automatically using the analysis device. The incubation conditions programmed in the respective software authorised by EUROIMMUN may deviate slightly from the specifications given in the ELISA test instruction. However, these conditions were validated in respect of the combination of the EUROIMMUN Analyzer I, Analyzer I-2P or the DSX from Dynex and this EUROIMMUN ELISA. Validation documents are available on inquiry. Automated test performance using other fully automated, open system analysis devices is possible, however, the combination should be validated by the user.

Pipetting protocol

	1	2	3	4	5	6	7	8	9	10	11	12
A	**C 2**	**P 6**	**P 14**	**P 22**			**C 1**	**P 4**	**P 12**	**P 20**		
B	**pos.**	**P 7**	**P 15**	**P 23**			**C 2**	**P 5**	**P 13**	**P 21**		
C	**neg.**	**P 8**	**P 16**	**P 24**			**C 3**	**P 6**	**P 14**	**P 22**		
D	**P 1**	**P 9**	**P 17**				**pos.**	**P 7**	**P 15**	**P 23**		
E	**P 2**	**P 10**	**P 18**				**neg.**	**P 8**	**P 16**	**P 24**		
F	**P 3**	**P 11**	**P 19**				**P 1**	**P 9**	**P 17**			
G	**P 4**	**P 12**	**P 20**				**P 2**	**P 10**	**P 18**			
H	**P 5**	**P 13**	**P 21**				**P 3**	**P 11**	**P 19**			

The pipetting protocol for microtiter strips 1-4 is an example for the **semiquantitative analysis** of 24 patient samples (P 1 to P 24).
The pipetting protocol for microtiter strips 7-10 is an example for the **quantitative analysis** of 24 patient samples (P 1 to P 24).

The calibrators (C 1 to C 3), the positive (pos.) and negative (neg.) controls, and the patient samples have each been incubated in one well. The reliability of the ELISA test can be improved by duplicate determinations for each sample.
The wells can be broken off individually from the strips. This makes it possible to adjust the number of test substrates used to the number of samples to be examined and minimizes reagent wastage.
Both positive and negative controls serve as internal controls for the reliability of the test procedure. They should be assayed with each test run.

Calculation of results

Semiquantitative: Results can be evaluated semiquantitatively by calculating a ratio of the extinction value of the control or patient sample over the extinction value of the calibrator 2. Calculate the ratio according the following formula:

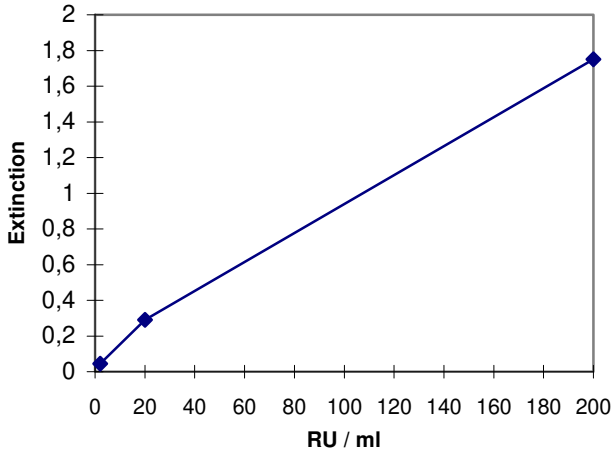
$$\frac{\textbf{Extinction of the control or patient sample}}{\textbf{Extinction of calibrator 2}} = \textbf{Ratio}$$

EUROIMMUN recommends interpreting results as follows:

Ratio <0.8:	**negative**
Ratio ≥0.8 to <1.1:	**borderline**
Ratio ≥1.1:	**positive**

In cases of borderline test results, an additional patient sample should be taken 7 days later and re-tested in parallel with the first patient sample. The results of both samples allow proper evaluation of titer changes.

Quantitative: The standard curve from which the concentration of antibodies in the patient samples can be taken is obtained by point-to-point plotting of the extinction values measured for the 3 calibration sera against the corresponding units (linear/linear). Use “point-to-point” plotting for calculation of the standard curve by computer. The following plot is an example of a typical calibration curve. Please do not use this curve for the determination of antibody concentrations in patient samples.



If the extinction of a serum sample lies above the value of calibrator 1 (200 RU/ml). The result should be given as “>200 RU/ml“. It is recommended that the sample be re-tested at a dilution of 1:400. The result in RU/ml read from the calibration curve for this sample must then be multiplied by a factor of 4.

The upper limit of the normal range of non-infected persons (**cut-off value**) recommended by EUROIMMUN is **20 relative units (RU)/ml**. EUROIMMUN recommends interpreting results as follows:

<16 RU/ml:	**negative**
≥16 to <22 RU/ml:	**borderline**
≥22 RU/ml:	**positive**

Evaluation information: For duplicate determinations the mean of the two values should be taken. If the two values deviate substantially from one another the sample should be retested.

For the interpretation of borderline results an investigation using further tests (e.g. avidity determination of antibody class IgG) can be helpful. Diagnosis can be secured by the determination of the titer change in two serum samples taken at an interval of at least 7 days and analysed in parallel.

For diagnosis, the clinical symptoms of the patient should always be taken into account along with the serological results.

Test characteristics

Calibration: As no quantificated international reference serum exists for antibodies against West Nile virus, the calibration is performed in relative units (RU).

For every group of tests performed, the extinction values of the calibrators and the relative units and/or ratios determined for the positive and negative controls must lie within the limits stated for the relevant test kit lot. A quality control certificate containing these reference values is included. If the values specified for the controls are not achieved, the test results may be inaccurate and the test should be repeated.
The activity of the enzyme used is temperature-dependent and the extinction values may vary if a thermostat is not used. The higher the room temperature during substrate incubation, the greater will be the extinction values. Corresponding variations apply also to the incubation times. However, the calibration sera are subject to the same influences, with the result that such variations will be largely compensated in the calculation of the result.

Antigen: The antigen source is a recombinant, detergent-extracted glycoprotein E of WNV from the membrane fraction of human cells.

Linearity: The linearity of the Anti-West Nile Virus ELISA (IgG) was determined by assaying 4 serial dilutions of different patient samples. The coefficient of determination R^2 for all sera was > 0.95. The Anti-West Nile Virus ELISA (IgG) is linear at least in the tested concentration range (10 RU/ml to 160 RU/ml).

Detection limit: The lower detection limit is defined as the mean value of an analyte-free samples plus three times the standard deviation and is the smallest detectable antibody titer. The lower detection limit of the Anti-West Nile Virus ELISA (IgG) is 0.4 RU/ml.

Cross reactivity: Cross reactivities to other flaviviruses cannot be excluded. They were recognized with anti-TBE positive and anti-Dengue virus positive samples.

Antibodies against	n	Anti-West Nile Virus ELISA (IgG)
Adenovirus	12	0%
Chlamydia pneumoniae	12	0%
CMV	12	0%
EBV-CA	12	0%
Helicobacter pylori	12	0%
HSV-1	12	0%
Influenza virus A	12	0%
Influenza virus B	12	0%
Measles virus	12	0%
Mumps virus	12	0%
Mycoplasma pneumoniae	12	0%
Parainfluenza virus Pool	12	0%
RSV	12	0%
Rubella virus	12	0%
Toxoplasma gondii	9	0%
VZV	12	0%

Interference: Haemolytic, lipaemic and icteric samples showed no influence on the result up to a concentration of 10 mg/ml for haemoglobin, 20 mg/ml for triglycerides and 0.4 mg/ml for bilirubin in this ELISA.

Reproducibility: The reproducibility of the test was investigated by determining the intra- and inter-assay coefficients of variation using 3 sera. The intra-assay CVs are based on 20 determinations and the inter-assay CVs on 4 determinations performed in 6 different test runs.

Intra-assay variation, n = 20		
Serum	**Mean value (RU/ml)**	**CV (%)**
1	101	6.1
2	104	3.3
3	149	2.7

Inter-assay variation, n = 4 x 6		
Serum	**Mean value (RU/ml)**	**CV (%)**
1	103	5.1
2	120	4.9
3	171	4.2

Sensitivity and specificity: Samples from 295 patients (origin: Europe) were investgated using the EUROIMMUN Anti-West Nile Virus ELISA and a neutralization test (NT) (performed by RKI, Berlin) as a reference method. The specificity was 96.9%, with a sensitivity of 99.5%. Values for 4 of the samples were borderline and were not included in the calculation.

Reference range: The levels of anti-West Nile virus antibodies (IgG) were analyzed with this EUROIMMUN ELISA in a panel of 500 healthy blood donors. With a cut-off of 20 RU/ml, 1.0% of the blood donors were anti-West Nile virus positive (IgG).

Clinical significance

West Nile virus (WNV) is an enveloped single-stranded RNA virus of the Flaviviridae family [1]. This family comprises around 100 virus types that are presently categorized into the three known species Flavivirus, Pestivirus and Hepacivirus [1, 2, 3, 4, 5]. West Nile virus received its name in 1937 when it was first isolated from a blood sample of an elderly woman living in the West Nile district in Uganda, who had fever of unknown cause accompanied by neurological disorders [6]. Further isolates were achieved only in 1951 from the sera of children with weak, unspecific symptoms, namely in Egypt where the virus is endemically distributed. At that time mice and embryonated hen's eggs were used for virus detection [1].

WNV is present not only in tropical areas, but also in moderate climate regions [2, 3, 4, 5]. Significant epidemics were observed in 1951/52 and 1957 in Israel and 1974 and 1983/84 in South Africa [1]. In the mid 90's the virus changed its virulence causing an epidemic accumulation of WNV encephalitis in Algeria (1994), Rumania (1996/97), the Czech Republic (1997), the Democratic Republic of Congo (1998), Russia, North America (1999) and Israel (2000) [2, 3, 7, 8, 9, 10, 11, 12].

In the USA 149 infections with 18 cases of death were recorded from 1999 to 2001. In 2002 this number rose to 4156 infections and 284 deaths, in 2003 to 9858 infections and 262 deaths [7, 8, 10]. Currently the virus has been detected in seven Canadian provinces, in 48 USA states and in Mexico, as well as in Puerto Rico, the Dominican Republic, Jamaica, Guadeloupe and El Salvador [3, 9, 11].

Since 1958, when antibodies against the WNV were first detected in the sera of two Albanians, repeated outbreaks of West Nile fever have occurred in Southern and Eastern Europe and meanwhile also in Central and West Europe [8, 9, 10, 12, 14, 15]. Its emergence and rapid spread is credited to world climate change, long-distance travel and globalization of economic trade [9, 12, 13, 15, 16, 17]. Consequent monitoring of West Nile activity by controlling sera of exposed persons is essential [15, 17, 18, 19]. Seroprevalence studies in endemic regions have shown an infection spread of up to 40% [14]. West Nile virus is therefore the Flavivirus with the largest distribution area [2, 3, 4, 5, 9].

WNV is transmitted by a number of mosquitoes. In the Mediterranean region and in Africa mosquitoes of the Culex univittatus complex species are the main arthropod hosts, while in North America WNV could be detected in 37 mosquito species, with Culex pipiens being the main vector [1, 2, 3, 4, 7, 9]. In India Culex vishnui and in France Culex modestus were identified as the main vectors [1]. In total WNV could be found in more than 40 mosquito and in several tick species [3, 4, 5].

Birds represent the vertebrate reservoir [1, 20, 21]. Alone in the USA WNV has been found in more than 162 species of birds. Many of them showed clinical symptoms and thousands of birds died after contracting a natural infection [7, 8, 10, 20, 21]. Birds that survive develop lifelong immunity. Acting as coincidental hosts mammals can also become infected when bitten by an infected mosquito [10, 13, 14]. Transmission has additionally been documented via breast milk, bone marrow transplantations, liver and heart transplants, blood transfusions, lab accidents such as open wounds during handling of infected brain tissue as well as transplacental transmission [2, 3, 4, 5, 8, 22, 23, 24, 25, 26, 27, 28]. Other than humans, mostly only horses became ill after an infection [2, 13, 21]. As well as WNV infection via a mosquito bite, a second natural infection source is possible in animals, namely via feeding on infected prey [3, 5]. An experimental infection of cats was successfully achieved by feeding them infected mice [20, 21].

70% to 80% of the humans infected with WNV showed no symptoms [8]. In the remaining 20%-30%, signs of sudden flu-like symptoms appear after an incubation period of 2-6 days with fever ranging from 38.5 to 40°C lasting for 3-5 days, nausea, shivering, head and back aches, joint and muscle pain and other unspecific symptoms such as loss of appetite, dizziness, vomiting, diarrhoea, coughing and a sore throat [1, 2, 3, 4, 5].

Typical for epidemical occurring fever are exanthema on the breast, back and upper extremities and general lymph node swelling [1, 3, 26]. Severe clinical cases of WNV infections are characterized by myocarditis, pancreatitis and hepatitis and since 1996, also neurological disorders, as WNV is now capable of crossing the blood-brain barrier [3]. The neurological symptoms begin after a short febrile prodome phase approximately 1-7 days after infection and become manifest in the form of encephalitis and meningoencephalitis accompanied by stiffness, spasms and shivering as the result of damage done to the basal ganglia [3, 8, 29, 30].

Another widespread symptom is general muscle weakness similar to the Guillain-Barré syndrome and also polio-like paralysis [29, 30]. Approximately 4%-14% of the hospitalized patient cases are fatal [29]. High risk factors are old age and a weak immune system [1, 3, 29].

An infection with WNV during pregnancy can cause miscarriages, congenital meningitis, birth defects in approx. 10% of the cases and in an additional 10% of newborns growth disturbances [3, 8, 27, 28].

The diagnosis of WNV can be performed by virus detection or by detection of specific antibodies [1, 4, 17, 18, 19]. As virus isolation from serum or cerebrospinal fluid or virus detection using Real-Time Reverse Transcription-Polymerase Chain Reaction (RT-PCR) is usually unsuccessful due to short viraemia and low virus titers, the detection of specific WNV antibodies using ELISA and IFA has gained importance [11, 17, 18, 19, 31, 32, 33, 34, 35, 36, 37, 38].

Specific IgM antibodies in serum can be determined using ELISA or IIFT [19]. Antibodies of class IgM are detectable in serum from the second day after initial symptoms of the illness occur. A four-fold increase in titer of the respective class of antibody is considered proof of a WNV infection.

If the IgM test is negative, even though the symptoms indicate a WNV infection, a second serum sample should be taken and tested for IgM antibodies a few days later. A combination of ELISA and IIFT provides close to 100% reliability [17, 18, 31, 32, 33, 34]. Anti-WNV IgM antibodies persist for 2 to 3 months, often for more than a year [4, 17, 18, 19, 22].

Antibodies of class IgG are detectable approx. 2 days after the appearance of IgM antibodies [11, 19, 32, 33, 34, 40]. Two to four weeks after a positive IgM result the infection can be confirmed and its severity and prognosis evaluated using a qualitative and quantitative test for the detection of specific WNV IgG antibodies in the patient serum [34].

For the reliable differentiation between acute and past infections the detection of low-avidity IgG antibodies gives evidence for a primary or an acute WNV infection, while high-avidity antibodies indicate a past or reactivated WNV infection [39, 40, 41]. EUROIMMUN offers additional test systems for determination of IgG avidity in both ELISA and IIFT formats. The detection of low-avidity antibodies using ELISA and IIFT in parallel is possible for WNV as it is for Toxoplasma gondii, rubella virus, EBV-EA, EBV-CA and Corona virus [39, 40, 41].

As the degree of similarity within the Flavivirus family is high antibody cross reactions can occur [19, 31, 42]. Therefore samples that are positive for specific IgM and/or IgG antibodies against WNV should be titrated and investigated on all relevant Flavivirus IIFT substrates for cross reactions. By comparing the titer strengths the initial result can be confirmed or disproved by the second detection and an infection with another Flavivirus identified as the source of illness [17, 32, 34].

To supplement and extend the current Anti-West Nile Virus ELISA and Anti-West Nile Virus IIFT (each IgG or IgM or avidity) BIOCHIP Mosaics and Profiles for the detection (IIFT) of infections with Flaviviruses and the BIOCHIP Mosaic Fever Profile 1: South-East Asia have been developed. With these tests specific antibodies (IgG and IgM) against several infectious agents can be investigated simultaneously [36, 37, 38, 43, 44, 45]. These supplementary tests allow similar or ambiguous disease symptoms and potential cross reactions to be clarified and differential diagnostic issues to be addressed [1].

A specific antiviral therapy for WNV encephalitis is not available at present [1, 2, 3, 4, 5, 14, 35]. Intensive medical care is the only possibility to positively influence the illness. Eradication of WNV is impossible due to the natural bird-mosquito cycle [1]. A vaccine with formalin inactivated WNV is only available for horses [2, 10]. Therefore public education, individual precautionary measures and protection against insect bites are essential contributions to preventing WNV infections [2, 3, 4, 5, 14, 16].

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Anti-West Nile Virus ELISA (IgM)
Test instruction

ORDER NO.	ANTIBODIES AGAINST	IG CLASS	SUBSTRATE	FORMAT
EI 2662-9601 M	West Nile Virus	IgM	Ag-coated microplate wells	96 x 01 (96)

Principle of the test: The ELISA test kit provides a semiquantitative in vitro assay for human antibodies of the IgM class against West Nile virus in serum or plasma. The test kit contains microtiter strips each with 8 break-off reagent wells coated with West Nile virus antigens. In the first reaction step, diluted patient samples are incubated in the wells. In the case of positive samples, specific IgM (also IgA and IgG) antibodies will bind to the antigens. To detect the bound antibodies, a second incubation is carried out using an enzyme-labelled anti-human IgM (enzyme conjugate) catalysing a colour reaction.

Contents of the test kit:

Component	Colour	Format	Symbol
1. Microplate wells coated with antigens: 12 microplate strips each containing 8 individual break-off wells in a frame, ready for use	---	12 x 8	STRIPS
2. Calibrator (IgM, human), ready for use	dark red	1 x 2.0 ml	CAL
3. Positive control (IgM, human), ready for use	blue	1 x 2.0 ml	POS CONTROL
4. Negative control (IgM, human), ready for use	green	1 x 2.0 ml	NEG CONTROL
5. Enzyme conjugate peroxidase-labelled anti-human IgM (goat), ready for use	red	1 x 12 ml	CONJUGATE
6. Sample buffer buffer containing IgG/RF-Absorbent (Anti-human IgG antibody preparation obtained from goat), ready for use	green	1 x 100 ml	SAMPLE BUFFER
7. Wash buffer 10x concentrate	colourless	1 x 100 ml	WASH BUFFER 10x
8. Chromogen/substrate solution TMB/H_2O_2, ready for use	colourless	1 x 12 ml	SUBSTRATE
9. Stop solution 0.5 M sulphuric acid, ready for use	colourless	1 x 12 ml	STOP SOLUTION
10. Test instruction	---	1 booklet	
11. Quality control certificate	---	1 protocol	
12. Protective foil	---	3 pieces	
LOT Lot description IVD In vitro diagnostics	CE		Storage temperature Unopened usable until

Storage and stability: The test kit has to be stored at a temperature between +2°C to +8°C. Do not freeze. Unopened, all test kit components are stable until the indicated expiry date.

Waste disposal: Patient samples, calibrators, controls and incubated microplate strips should be handled as infectious waste. All reagents must be disposed of in accordance with local disposal regulations.

Preparation and stability of the reagents

Note: All reagents must be brought to room temperature (+18°C to +25°C) approx. 30 minutes before use. After first use, the reagents are stable until the indicated expiry date if stored at +2°C to +8°C and protected from contamination, unless stated otherwise below.
The thermostat adjusted ELISA incubator must be set at 37°C +/- 1°C.

- **Coated wells:** Ready for use. Tear open the resealable protective wrapping of the microplate at the recesses above the grip seam. Do not open until the microplate has reached room temperature to prevent the individual strips from moistening. Immediately replace the remaining wells of a partly used microplate in the protective wrapping and tightly seal with the integrated grip seam (Do not remove the desiccant bag).
  Once the protective wrapping has been opened for the first time, the wells coated with antigens can be stored in a dry place and at a temperature between +2°C and +8°C for 4 months.

- **Calibrator and controls:** Ready for use. The reagents must be mixed thoroughly before use.

- **Enzyme conjugate:** Ready for use. The enzyme conjugate must be mixed thoroughly before use.

- **Sample buffer:** Ready for use. The green coloured sample buffer contains IgG/RF absorbent. Serum or plasma samples diluted with this sample buffer are only to be used for the determination of IgM antibodies.

- **Wash buffer:** The wash buffer is a 10x concentrate. If crystallization occurs in the concentrated buffer, warm it to 37°C and mix well before diluting. The quantity required should be removed from the bottle using a clean pipette and diluted with deionized or distilled water (1 part reagent plus 9 parts distilled water).
  For example: For 1 microplate strip, 5 ml concentrate plus 45 ml water.
  The working strength diluted wash buffer is stable for 4 weeks when stored at +2°C to +8°C and handled properly.

- **Chromogen/substrate solution:** Ready for use. Close the bottle immediately after use, as the contents are sensitive to light. The chromogen/substrate solution must be clear on use. Do not use the solution if it is blue coloured.

- **Stop solution:** Ready for use.

Warning: The control sera used have been tested negative for HBsAg, anti-HCV, anti-HIV-1 and anti-HIV-2 using enzyme immunoassays and indirect immunofluorescence methods. Nonetheless, all materials should be treated as being a potential infection hazard and should be handled with care. Some of the reagents contain the toxic agent sodium azide. Avoid skin contact.

Preparation and stability of the patient samples

Samples: Human serum or EDTA, heparin or citrate plasma.

Stability: Patient samples to be investigated can generally be stored at +2°C to +8°C for up to 14 days. Diluted samples should be incubated within one working day.

Introduction: Before the determination of specific antibodies of class IgM, antibodies of class IgG should be removed from the patient sample. This procedure must be carried out in order to prevent any rheumatoid factors from reacting with specifically bound IgG, which would lead to false positive IgM test results, and to prevent specific IgG displacing IgM from the antigen, which would lead to false IgM negative test results.

Functional principle: The sample buffer (green coloured!) contains an anti-human antibody preparation from goat. IgG from a serum sample is bound with high specificity by these antibodies and precipitated. If the sample also contains rheumatoid factors, these will be absorbed by the IgG/anti-human IgG complex.

Separation properties:
- All IgG subclasses are bound and precipitated by the anti-human IgG antibodies.
- Human serum IgG in concentrations of up to 15 mg per ml are removed (average serum IgG concentration in adults: 12 mg per ml).
- Rheumatoid factors are also removed.
- The recovery rate of the IgM fraction is almost 100%.

Performance: The **patient samples** for analysis are diluted **1:101** with sample buffer. For example, add 10 µl serum to 1.0 ml sample buffer and mix well. Incubate the mixture for at least **10 minutes** at room temperature. Subsequently, it can be pipetted into the microplate wells according to the pipetting protocol.

Notes:
- Antibodies of the class IgG should not be analyzed with this mixture.
- It is possible to check the efficacy of the IgG/RF absorbent for an individual patient sample by performing an IgG test in parallel to the IgM test using the mixture. If the IgG test is negative, the IgM result can be considered as reliable.
- The calibrator and controls containing IgM antibodies are pre-diluted and ready for use, do not dilute them.

Incubation

(Partly) manual test performance

Sample incubation: (1st step)
Transfer 100 µl of the calibrator, positive and negative controls or diluted patient samples into the individual microplate wells according to the pipetting protocol.
For manual processing of microplate wells, cover the finished test plate with the protective foil. When using an automated microplate processor for incubation, follow the instrument manufacturer´s recommendations with regard to microwell plate sealing.
Incubate **60 minutes at 37°C ± 1°C**.

Washing:
Manual: Remove the protective foil and empty the wells and subsequently wash 3 times using 300 µl of working strength wash buffer for each wash.
Automatic: Remove the protective foil and empty the wells and subsequently wash 3 times with 450 µl of working strength wash buffer (program setting: e.g. TECAN Columbus Washer "Overflow Modus").

Leave the wash buffer in each well for 30 to 60 seconds per washing cycle, then empty the wells. After washing (manual and automated tests), thoroughly dispose of all liquid from the microplate by tapping it on absorbent paper with the openings facing downwards to remove all residual wash buffer.

Note: Residual liquid (> 10 µl) in the reagent wells after washing can interfere with the substrate and lead to false low extinction values. Insufficient washing (e.g., less than 3 wash cycles, too small wash buffer volumes, or too short reaction times) can lead to false high extinction values.
Free positions on the microplate strip should be filled with blank wells of the same plate format as that of the parameter to be investigated.

Conjugate incubation: (2nd step)
Pipette 100 µl of enzyme conjugate (peroxidase-labelled anti-human IgM) into each of the microplate wells.
When using an automated microplate processor for incubation, follow the instrument manufacturer´s recommendations with regard to microwell plate sealing.
Incubate **30 minutes at room temperature (+18°C to +25°C)**.

Washing:
Empty the wells. Wash as described above.

Substrate incubation: (3. step)
Pipette 100 µl of chromogen/substrate solution into each of the microplate wells. Incubate for **15 minutes at room temperature** (+18°C to +25°C) (protect from direct sunlight).

Stopping the reaction: (3rd step)
Pipette 100 µl of stop solution into each of the microplate wells in the same order and at the same speed as the chromogen/substrate solution was introduced.

Measurement:
Photometric measurement of the colour intensity should be made at a wavelength of 450 nm and a reference wavelength between 620 nm and 650 nm **within 30 minutes of adding the stop solution.** Prior to measuring, slightly shake the microplate to ensure a homogeneous distribution of the solution.

Test performance using fully automated analysis devices

Sample dilution and test performance are carried out fully automatically using the analysis device. The incubation conditions programmed in the respective software authorised by EUROIMMUN may deviate slightly from the specifications given in the ELISA test instruction. However, these conditions were validated in respect of the combination of the Analyzer I, Analyzer I-2P and the DSX from Dynex and this EUROIMMUN ELISA. Validation documents are available on inquiry. Automated test performance using other fully automated, open system analysis devices is possible, however, the combination should be validated by the user.

Pipetting protocol

	1	2	3	4	5	6	7	8	9	10	11	12
A	**C**	**P 6**	**P 14**	**P 22**								
B	**pos.**	**P 7**	**P 15**	**P 23**								
C	**neg.**	**P 8**	**P 16**	**P 24**								
D	**P 1**	**P 9**	**P 17**									
E	**P 2**	**P 10**	**P 18**									
F	**P 3**	**P 11**	**P 19**									
G	**P 4**	**P 12**	**P 20**									
H	**P 5**	**P 13**	**P 21**									

The above pipetting protocol is an example of the **semiquantitative analysis** of antibodies in 24 patient samples (P 1 to P 24).

Calibrator (C), positive (pos.) and negative (neg.) control as well as the patient samples have been incubated in one well each. The reliability of the ELISA test can be improved by duplicate determinations of each sample.
The wells can be broken off individually from the strips. This makes it possible to adjust the number of test substrates used to the number of samples to be examined and minimizes reagent wastage.
Both positive and negative controls serve as internal controls for the reliability of the test procedure. They should be assayed with each test run.

Calculation of results

The extinction value of the calibrator defines the upper limit of the reference range of non-infected persons (**cut-off**) recommended by EUROIMMUN. Values above the indicated cut-off are to be considered as positive, those below as negative.

Semiquantitative: Results can be evaluated semiquantitatively by calculating a ratio of the extinction value of the control or patient sample over the extinction value of calibrator. Use the following formula to calculate the ratio:

$$\frac{\textbf{Extinction of the control/patient sample}}{\textbf{Extinction of calibrators}} = \textbf{Ratio}$$

EUROIMMUN recommends interpreting results as follows:

Ratio <0.8:	**negative**
Ratio ≥0.8 to <1.1:	**borderline**
Ratio ≥1.1:	**positive**

Evaluation information: For duplicate determinations the mean of the two values should be taken. If the two values deviate substantially from one another the sample should be retested.

For the interpretation of borderline results an investigation using further tests (e.g. avidity determination of antibody class IgG) can be helpful. Diagnosis can be secured by the determination of the titer change in two serum samples taken at an interval of at least 7 days and analysed in parallel.

For diagnosis, the clinical symptoms of the patient should always be taken into account along with the serological results.

Test characteristics

Calibration: As no quantificated international reference serum exists for antibodies of the IgM class against West Nile virus, results are provided in the form of ratios which are a relative measure for the concentration of antibodies.

For every group of tests performed, the extinction values of the calibrators and the ratios determined for the positive and negative controls must lie within the limits stated for the relevant test kit lot. A quality control certificate containing these reference values is included. If the values specified for the control sera are not achieved, the test results may be inaccurate and the test should be repeated.

The activity of the enzyme used is temperature-dependent and the extinction values may vary if a thermostat is not used. The higher the room temperature during substrate incubation, the greater will be the extinction values. Corresponding variations apply also to the incubation times. However, the calibrators are subject to the same influences, with the result that such variations will be largely compensated in the calculation of the result.

Antigen: The antigen source is a recombinant, detergent-extracted glycoprotein E of WNV from the membrane fraction of human cells.

Detection limit: The lower detection limit is defined as the mean value of an analyte-free sample plus three times the standard deviation and is the smallest detectable antibody titer. The lower detection limit of the Anti-West Nile ELISA (IgM) is a ratio value of 0.03.

Cross reactivity: Cross reactivities to other flaviviruses cannot be excluded. They were recognized with anti-TBE positive and anti-Dengue virus positive samples.

Antibodies against	n	Anti-West Nile ELISA (IgM)
Borrelia burgdoferi	9	0%
CMV	8	0%
EBV-CA	9	0%
HSV-1/2	2	0%
Measles virus	10	0%
Mumps virus	9	0%
Parvovirus B19	8	0%
Rubella virus	10	0%
Toxoplasma gondii	10	0%
VZV	4	0%

Interference: Haemolytic, lipaemic and icteric samples showed no influence on the result up to a concentration of 10 mg/ml for haemoglobin, 20 mg/ml for triglycerides and 0.4 mg/ml for bilirubin in this ELISA.

Reproducibility: The reproducibility of the test was investigated by determining the intra- and inter-assay coefficients of variation using 3 sera. The intra-assay CVs are based on 20 determinations and the inter-assay CVs on 4 determinations performed in 6 different test runs.

Intra-assay variation, n = 20		
Serum	**Mean value (Ratio)**	**CV (%)**
1	1.9	5.9
2	2.0	2.2
3	2.4	4.0

***Inter-assay variation,* n = 4 x 6**		
Serum	**Mean value (Ratio)**	**CV (%)**
1	1.9	3.9
2	2.1	9.4
3	2.4	6.8

Sensitivity and specificity: Study I: For the determination of the sensitivity 18 clinically and serologically precharacterised sera (Robert Koch Institute, Berlin, Germany) were tested with the EUROIMMUN Anti-West Nile Virus ELISA (IgM). The sensitivity amounted to 94.4%.

Study II: 99 patient sera, characterised as positive at the Saskatchewan Disease Control Laboratory (Canada) using several serological methods, were investigated with the EUROIMMUN Anti-West Nile Virus ELISA (IgM). The sensitivity was 93.7%. For the determination of the specificity 500 blood donor samples were investigated. The specificity was 99.8%.

Reference range: The levels of anti-West Nile virus antibodies (IgM) were analyzed with this EUROIMMUN ELISA in a panel of 500 healthy blood donors. With a cut-off ratio of 1.0, 0.2% of the blood donors were anti-West Nile virus positive (IgM).

Clinical significance

West Nile virus (WNV) is an enveloped single-stranded RNA virus of the Flaviviridae family [1]. This family comprises around 100 virus types that are presently categorized into the three known species Flavivirus, Pestivirus and Hepacivirus [1, 2, 3, 4, 5]. West Nile virus received its name in 1937 when it was first isolated from a blood sample of an elderly woman living in the West Nile district in Uganda, who had fever of unknown cause accompanied by neurological disorders [6]. Further isolates were achieved only in 1951 from the sera of children with weak, unspecific symptoms, namely in Egypt where the virus is endemically distributed. At that time mice and embryonated hen's eggs were used for virus detection [1].

WNV is present not only in tropical areas, but also in moderate climate regions [2, 3, 4, 5]. Significant epidemics were observed in 1951/52 and 1957 in Israel and 1974 and 1983/84 in South Africa [1]. In the mid 90's the virus changed its virulence causing an epidemic accumulation of WNV encephalitis in Algeria (1994), Rumania (1996/97), the Czech Republic (1997), the Democratic Republic of Congo (1998), Russia, North America (1999) and Israel (2000) [2, 3, 7, 8, 9, 10, 11, 12]. In the USA 149 infections with 18 cases of death were recorded from 1999 to 2001. In 2002 this number rose to 4156 infections and 284 deaths, in 2003 to 9858 infections and 262 deaths [7, 8, 10]. Currently the virus has been detected in seven Canadian provinces, in 48 USA states and in Mexico, as well as in Puerto Rico, the Dominican Republic, Jamaica, Guadeloupe and El Salvador [3, 9, 11].

Since 1958, when antibodies against the WNV were first detected in the sera of two Albanians, repeated outbreaks of West Nile fever have occurred in Southern and Eastern Europe and meanwhile also in Central and West Europe [8, 9, 10, 12, 14, 15]. Its emergence and rapid spread is credited to world climate change, long-distance travel and globalization of economic trade [9, 12, 13, 15, 16, 17]. Consequent monitoring of West Nile activity by controlling sera of exposed persons is essential [15, 17, 18, 19]. Seroprevalence studies in endemic regions have shown an infection spread of up to 40% [14]. West Nile virus is therefore the Flavivirus with the largest distribution area [2, 3, 4, 5, 9].

WNV is transmitted by a number of mosquitoes. In the Mediterranean region and in Africa mosquitoes of the Culex univittatus complex species are the main arthropod hosts, while in North America WNV could be detected in 37 mosquito species, with Culex pipiens being the main vector [1, 2, 3, 4, 7, 9]. In India Culex vishnui and in France Culex modestus were identified as the main vectors [1]. In total WNV could be found in more than 40 mosquito and in several tick species [3, 4, 5].

Birds represent the vertebrate reservoir [1, 20, 21]. Alone in the USA WNV has been found in more than 162 species of birds. Many of them showed clinical symptoms and thousands of birds died after contracting a natural infection [7, 8, 10, 20, 21]. Birds that survive develop lifelong immunity. Acting as coincidental hosts mammals can also become infected when bitten by an infected mosquito [10, 13, 14]. Transmission has additionally been documented via breast milk, bone marrow transplantations, liver and heart transplants, blood transfusions, lab accidents such as open wounds during handling of infected brain tissue as well as transplacental transmission [2, 3, 4, 5, 8, 22, 23, 24, 25, 26, 27, 28]. Other than humans, mostly only horses became ill after an infection [2, 13, 21]. As well as WNV infection via a mosquito bite, a second natural infection source is possible in animals, namely via feeding on infected prey [3, 5]. An experimental infection of cats was successfully achieved by feeding them infected mice [20, 21].

70% to 80% of the humans infected with WNV showed no symptoms [8]. In the remaining 20%-30%, signs of sudden flu-like symptoms appear after an incubation period of 2-6 days with fever ranging from 38.5 to 40°C lasting for 3-5 days, nausea, shivering, head and back aches, joint and muscle pain and other unspecific symptoms such as loss of appetite, dizziness, vomiting, diarrhoea, coughing and a sore throat [1, 2, 3, 4, 5].

Typical for epidemical occurring fever are exanthema on the breast, back and upper extremities and general lymph node swelling [1, 3, 26]. Severe clinical cases of WNV infections are characterized by myocarditis, pancreatitis and hepatitis and since 1996, also neurological disorders, as WNV is now capable of crossing the blood-brain barrier [3]. The neurological symptoms begin after a short febrile prodome phase approximately 1-7 days after infection and become manifest in the form of encephalitis and meningoencephalitis accompanied by stiffness, spasms and shivering as the result of damage done to the basal ganglia [3, 8, 29, 30].

Another widespread symptom is general muscle weakness similar to the Guillain-Barré syndrome and also polio-like paralysis [29, 30]. Approximately 4%-14% of the hospitalized patient cases are fatal [29]. High risk factors are old age and a weak immune system [1, 3, 29].

An infection with WNV during pregnancy can cause miscarriages, congenital meningitis, birth defects in approx. 10% of the cases and in an additional 10% of newborns growth disturbances [3, 8, 27, 28].

The diagnosis of WNV can be performed by virus detection or by detection of specific antibodies [1, 4, 17, 18, 19]. As virus isolation from serum or cerebrospinal fluid or virus detection using Real-Time Reverse Transcription-Polymerase Chain Reaction (RT-PCR) is usually unsuccessful due to short viraemia and low virus titers, the detection of specific WNV antibodies using ELISA and IFA has gained importance [11, 17, 18, 19, 31, 32, 33, 34, 35, 36, 37, 38].

Specific IgM antibodies in serum can be determined using ELISA or IIFT [19]. Antibodies of class IgM are detectable in serum from the second day after initial symptoms of the illness occur. A four-fold increase in titer of the respective class of antibody is considered proof of a WNV infection.

If the IgM test is negative, even though the symptoms indicate a WNV infection, a second serum sample should be taken and tested for IgM antibodies a few days later. A combination of ELISA and IIFT provides close to 100% reliability [17, 18, 31, 32, 33, 34]. Anti-WNV IgM antibodies persist for 2 to 3 months, often for more than a year [4, 17, 18, 19, 22].

Antibodies of class IgG are detectable approx. 2 days after the appearance of IgM antibodies [11, 19, 32, 33, 34, 40]. Two to four weeks after a positive IgM result the infection can be confirmed and its severity and prognosis evaluated using a qualitative and quantitative test for the detection of specific WNV IgG antibodies in the patient serum [34].

For the reliable differentiation between acute and past infections the detection of low-avidity IgG antibodies gives evidence for a primary or an acute WNV infection, while high-avidity antibodies indicate a past or reactivated WNV infection [39, 40, 41].

EUROIMMUN offers additional test systems for determination of IgG avidity in both ELISA and IIFT formats. The detection of low-avidity antibodies using ELISA and IIFT in parallel is possible for WNV as it is for Toxoplasma gondii, rubella virus, EBV-EA, EBV-CA and Corona virus [39, 40, 41].

As the degree of similarity within the Flavivirus family is high antibody cross reactions can occur [19, 31, 42]. Therefore samples that are positive for specific IgM and/or IgG antibodies against WNV should be titrated and investigated on all relevant Flavivirus IIFT substrates for cross reactions. By comparing the titer strengths the initial result can be confirmed or disproved by the second detection and an infection with another Flavivirus identified as the source of illness [17, 32, 34].

To supplement and extend the current Anti-West Nile Virus ELISA and Anti-West Nile Virus IIFT (each IgG or IgM or avidity) BIOCHIP Mosaics and Profiles for the detection (IIFT) of infections with Flaviviruses and the BIOCHIP Mosaic Fever Profile 1: South-East Asia have been developed. With these tests specific antibodies (IgG and IgM) against several infectious agents can be investigated simultaneously [36, 37, 38, 43, 44, 45]. These supplementary tests allow similar or ambiguous disease symptoms and potential cross reactions to be clarified and differential diagnostic issues to be addressed [1].

A specific antiviral therapy for WNV encephalitis is not available at present [1, 2, 3, 4, 5, 14, 35]. Intensive medical care is the only possibility to positively influence the illness. Eradication of WNV is impossible due to the natural bird-mosquito cycle [1]. A vaccine with formalin inactivated WNV is only available for horses [2, 10]. Therefore public education, individual precautionary measures and protection against insect bites are essential contributions to preventing WNV infections [2, 3, 4, 5, 14, 16].

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MINISTERIO DE SANIDAD, CONSUMO Y BIENESTAR SOCIAL

agencia española de medicamentos y productos sanitarios

ENAC CERTIFICACIÓN Nº 39/C-SG055

LA AGENCIA ESPAÑOLA DE MEDICAMENTOS Y PRODUCTOS SANITARIOS

THE AGENCIA ESPAÑOLA DE MEDICAMENTOS Y PRODUCTOS SANITARIOS

otorga el certificado número

grants the certificate no.

2013 11 0039 EN

según la norma

in accordance with the standard

UNE-EN ISO 13485:2018
(EN ISO 13485: 2016 & ISO 13485: 2016)

Productos Sanitarios: Sistemas de Gestión de Calidad – Requisitos para fines reglamentarios

Medical devices – Quality management systems - Requirements for regulatory purposes

a la empresa / *to the company*

Dia.Pro Diagnostic Bioprobes S.r.l.

Sede social y de fabricación/ *Headquarters and manufacturing facility*

Via G. Carducci, 27-20099-Sesto San Giovanni-Milano-Italy

Para las siguientes actividades / *For the following activities:*

Diseño, desarrollo y producción de reactivos y productos reactivos, calibradores y materiales de control para inmunoquímica, microbiología, inmunología infecciosa y técnicas de biología molecular.

Diseño, desarrollo, producción y servicio técnico de instrumentos y software para diagnóstico *in vitro*.

Design, development and manufacturing of reagents, reagent products, calibrators and control materials for immunochemistry, microbiology, infectious immunology and molecular biology techniques.

Design and development, management of production and technical servicing of instruments and software for "in vitro" diagnostic.

Modificaciones de alcance: Ver Anexo I / *see Annex I*

Fecha de validez/ *Date of validity:* **Desde/** *From:* **8-03-2019** **Hasta/***To:* **17-12-2021**

Certificación inicial/ *Initial certification date:* **27-11-2013**

Renovación / *Renewal of certification date:* **8-03-2019**

Madrid, 08 de marzo de 2019

DIRECTORA DE LA AGENCIA ESPAÑOLA DE MEDICAMENTOS Y PRODUCTOS SANITARIOS

agencia española de medicamentos y productos sanitarios

Fdo. Mª Jesús Lamas Díaz

13485 CERTIFICADO Revisión 17/12/2018

Agencia Española de Medicamentos y Productos Sanitarios

Fecha de la firma: 08/03/2019

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ENAC
CERTIFICACIÓN
Nº 39/C-SG055

ANEXO I / *ANNEX I*

CERTIFICADO UNE-EN ISO 13485:2018/ *UNE-EN ISO 13485:2018 CERTIFICATE*

Modificaciones del alcance / *Scope modifications:*

Fecha/*Date*	**Descripción de la modificación**/ *Modification description*
18-12-2018	Cambio en la descripción del tipo de técnica en el ámbito tecnológico (inmunología infecciosa y técnicas de biología molecular). Cambio del nivel de detalle en la descripción del ámbito tecnológico *Change in the description of the method of analysis in the technological scope (infectious immunology and molecular biology techniques).* *Change in the level of detail of the technological scope description.*
8-03-2019	Ampliación del ámbito tecnológico para incluir: Inmunoquímica y microbiología Instrumentos y software para diagnóstico "in vitro". Modificación del alcance para incluir la actividad de asistencia técnica para Instrumentos y software para diagnóstico "in vitro". *Extension of technological scope:* *Immunochemistry and Microbiology* *Instruments and software for "in vitro" diagnostic* *Modification of the scope to include the activity of technical servicing of instruments and software for "in vitro" diagnostic*

Madrid, 08 de marzo de 2019
DIRECTORA DE LA AGENCIA ESPAÑOLA DE MEDICAMENTOS Y PRODUCTOS SANITARIOS

agencia española de medicamentos y productos sanitarios

Fdo. Mª Jesús Lamas Díaz

Agencia Española de Medicamentos y Productos Sanitarios
Fecha de la firma: 08/03/2019
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MINISTERIO DE SANIDAD, CONSUMO Y BIENESTAR SOCIAL

agencia española de medicamentos y productos sanitarios

CERTIFICADO CE DE SISTEMA DE GARANTÍA DE CALIDAD TOTAL
de acuerdo con el Anexo IV (excepto punto 4) de la Directiva 98/79/CE

EC FULL QUALITY ASSURANCE SYSTEM CERTIFICATE
in accordance with Annex IV (except Section 4) of Directive 98/79/EC

PRÓRROGA/*EXTENSION* — Fecha inicial/ *Initial date*: **11/12/2003**
Fecha de última prórroga/ *Last extension date*: **27/11/2013**

Certificado nº/*Certificate no*	**Fecha de validez/*Date of validity***	**ON nº/*NB no***
2003 12 0388 CT	**Desde/*From* 27/11/2018 Hasta/*To* 18/11/2023**	**0318**

A favor de /*In favour of:*

Fabricante/*Manufacturer*:
Nombre/*Name:* DIA. Pro Diagnostic Bioprobes S.r.l.
Dirección/*Address:* Via G. Carducci, 27 -20099- Sesto San Giovanni – Milano (Italy).
Representante autorizado ante la UE/*Authorized EU representative*:
Nombre/*Name:* Idem Dirección/*Address:* Idem

Para los productos/*For the products:*

Categoría/*Category:* Productos Sanitarios para Diagnóstico "In Vitro" / *In Vitro Diagnostic Medical Devices*
Grupo genérico/*Generic group*: Diagnóstico de enfermedades infecciosas / *Diagnostic of infectious diseases*
Tipo/*Type*: Especificados en Anexos de este Certificado/*Specified in Annexes to this Certificate.*

Elaborado en/*In the facilities:*

Dia. Pro Diagnostic Bioprobes S.r.l.
Via G. Carducci, 27 -20099- Sesto San Giovanni – Milano (Italy).

Este certificado debe ir acompañado por certificado de examen de diseño: SI
This certificate must be accompanied by design examination certificate: YES

Este certificado es consecuencia de la auditoria del Sistema Completo de Garantía de Calidad y del examen de la documentación técnica contenida en el expediente nº 2003 05 0240, y garantiza que los productos descritos cumplen los requisitos de la Directiva. / *This certificate is issued on the full quality assurance system audit, and the examination of the technical documentation contained in dossier nº 2003 05 02405, and guarantees that the described products fulfil the requirements of the Directive.*

Madrid, 26 de noviembre de 2018
DIRECTORA DE LA AGENCIA ESPAÑOLA DE MEDICAMENTOS Y PRODUCTOS SANITARIOS

agencia española de medicamentos y productos sanitarios

Fdo. Mª Jesús Lamas Díaz

Firmado digitalmente por: Agencia Española de Medicamentos y Productos Sanitarios
Fecha de la firma: 26/11/2018
Localizador: X9GVDEF5C3

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ORGANISMO NOTIFICADO 0318

ANEXO Nº/*ANNEX NO:* I
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EC FULL QUALITY ASSURANCE SYSTEM CERTIFICATE
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Fecha de última prórroga/ *Last extension date*: **27/11/2013**

Certificado nº/*Certificate no*	Fecha de validez/*Date of validity*	ON nº/*NB no*
2003 12 0388 CT	**Desde/*From* 27/11/2018 Hasta/*To* 18/11/2023**	**0318**

A favor de/*In favour of:*

Fabricante/*Manufacturer*:
Nombre/*Name:* Dia. Pro Diagnostic Bioprobes S.r.l.
Dirección/*Address:* Via G. Carducci, 27 -20099- Sesto San Giovanni – Milano (Italy).
Representante autorizado ante la UE/*Authorized EU representative*:
Nombre/*Name:* Idem Dirección/*Address:* Idem

Tipo de producto / *Device type*: Reactivos y productos reactivos, calibradores y materiales de control para el diagnóstico de enfermedades infecciosas / *Reagents, and reagent products, calibrators and control materials for diagnostic of human infectious diseases.*

Clasificación/*Classification:* Lista A, anexo II / *List A, Annex II*

1. **Reactivos y productos reactivos para la determinación, confirmación y cuantificación de marcadores de infección en muestras humanas mediante técnicas de Inmunoabsorción enzimática (ELISA)/** *Reagents and reactive products for the determination, confirmation and quantification of infection markers in human samples by Enzyme-linked immunosorbent assay (ELISA)* **[NANDO: IVD 0201; IVD 0202; IVD 0203]**

1.1. HBs Ag one

- SAG1.CE (192 tests)
- SAG1.CE.96 (96 tests)
- SAG1.CE.480 (480 tests)
- SAG1.CE.960 (960 tests)

Descrito en el certificado / *Described in the certificate* 2003 12 0389 ED

1.2. HBs Ab

- SAB.CE (96 tests)

Descrito en el certificado / *Described in the certifícate* 2003 12 0390 ED

1.3. HBc Ab

- BCAB.CE (96 tests)

Descrito en el certificado / *Described in the certifícate* 2003 12 0391 ED

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1.4. HBc IgM

- BCM.CE (96 tests)

Descrito en el certificado / *Described in the certifícate* 2004 03 0424 ED

1.5. HBe Ag & Ab

- HBE.CE (96 tests)

Descrito en el certificado / *Described in the certifícate* 2004 03 0425 ED

1.6. HBs Ag Confirmation

- SCONF.CE (20 tests)
- SCONF.CE.40 (40 tests)

Descrito en el certificado / *Described in the certifícate* 2006 11 0511 ED

1.7. HBs Ag one Version ULTRA

- SAG1ULTRA.CE (192 tests)
- SAG1ULTRA.CE.96 (96 tests)
- SAG1ULTRA.CE.480 (480 tests)
- SAG1ULTRA.CE.960 (960 tests)
- SAG1ULTRA.CE.DB (192 tests)

Descrito en el certificado / *Described in the certifícate* 2008 12 0588 ED

1.8. HCV Ab

- CVAB.CE (192 tests)
- CVAB.CE.96 (96 tests)
- CVAB.CE.480 (480 tests)
- CVAB.CE.960 (960 tests)
- CVAB.CE.DB (192 tests)

Descrito en el certificado / *Described in the certifícate* 2003 12 0392 ED

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1.9. HCV Ab Confirmation

- CCONF.CE (12 tests)

Descrito en el certificado / *Described in the certifícate* 2005 09 0485 ED

1.10. HCV IgM

- CVM.CE (96 tests)

Descrito en el certificado / *Described in the certifícate* 2007 09 0532 ED

1.11. HCV Ab (Format 20)

- CVAB.CE.EG (192 tests)
- CVAB.CE.EG.96 (96 tests)
- CVAB.CE.EG.480 (480 tests)
- CVAB.CE.EG.960 (960 tests)

Descrito en el certificado / *Described in the certifícate* 2015 10 0842 ED

1.12. HDV Ab

- DAB.CE (96 tests)

Descrito en el certificado / *Described in the certificate* 2003 12 0393 ED

1.13. HDV Ag

- DAG.CE (96 tests)

Descrito en el certificado / *Described in the certifícate* 2003 12 0394 ED

1.14. HDV IgM

- DIM.CE (96 tests)

Descrito en el certificado / *Described in the certifícate* 2003 12 0395 ED

ANEXO Nº/*ANNEX NO:* I
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EC FULL QUALITY ASSURANCE SYSTEM CERTIFICATE
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Fecha de última prórroga/ *Last extension date*: **27/11/2013**

Certificado nº/*Certificate no*	Fecha de validez/*Date of validity*	ON nº/*NB no*
2003 12 0388 CT	**Desde/*From* 27/11/2018 Hasta/*To* 18/11/2023**	**0318**

1.15. HTLV I & II Ab

- HTLVAB.CE (192 tests)
- HTLVAB.CE.96 (96 tests)
- HTLVAB.CE.480 (480 tests)
- HTLVAB.CE.960 (960 tests)

Descrito en el certificado/ *Described in the certifícate* 2005 12 0493 ED

1.16. HTLV I & II Ab Version ULTRA

- HTLVABULTRA.CE (192 tests)
- HTLVABULTRA.CE.96 (96 tests)
- HTLVABULTRA.CE.480 (480 tests)
- HTLVABULTRA.CE.960 (960 tests)
- HTLVABULTRA.CE.DB (192 tests)

Descrito en el certificado/ *Described in the certifícate* 2011 11 0775 ED

1.17. HIV Ab & Ag

- IVCOMB.CE (192 tests)
- IVCOMB.CE.96 (96 tests)
- IVCOMB.CE.480 (480 tests)
- IVCOMB.CE.960 (960 tests)
- IVCOMB.CE.DB (192 tests)

Descrito en el certificado/ *Described in the certifícate* 2008 02 0539 ED

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ANEXO Nº/*ANNEX NO:* I
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PRÓRROGA/*EXTENSION* — Fecha inicial/ *Initial date*: **11/12/2003**
Fecha de última prórroga/ *Last extension date*: **27/11/2013**

Certificado nº/*Certificate no*	Fecha de validez/*Date of validity*	ON nº/*NB no*
2003 12 0388 CT	**Desde/*From* 27/11/2018 Hasta/*To* 18/11/2023**	**0318**

2. Reactivos y productos reactivos para la determinación, confirmación y cuantificación de marcadores de infección en muestras humanas mediante técnicas de PCR en tiempo real/ *Reagents and reactive products for the determination, confirmation and quantification of infection markers in human samples by Real-Time PCR* **[NANDO: IVD 0203]**

2.1. HBV DNA Quantitation (QT)

- HBVDNAQT.CE (50 tests)
- HBVDNAQT.CE.25 (25 tests)
- HBVDNAQT.CE.100 (100 tests)
- HBVDNAQT.CE.150 (150 tests)

Descrito en el certificado / *Described in the certifícate* 2012 09 0790 ED

2.2. HDV RNA Quantitation (QT)

- DRNA.CE (50 tests)
- DRNA.CE.25 (25 tests)
- DRNA.CE.100 (100 tests)
- DRNA.CE.150 (150 tests)

Descrito en el certificado / *Described in the certifícate* 2009 11 0660 ED

3. Reactivos y productos reactivos para la determinación, confirmación y cuantificación de marcadores de infección en muestras humanas mediante ensayos de quimioluminiscencia (CLIA)/ *Reagents and reactive products for the determination, confirmation and quantification of infection markers in human samples by Chemiluminescence Immunoassay (CLIA)* **[NANDO: IVD 0201; IVD 0202; IVD 0203]**

3.1. DIA.CHEMILUX HCV Ab

- RACVAB.CE (100 tests)

Descrito en el certificado / *Described in the certifícate* 2015 01 0834 ED

3.2. DIA.CHEMILUX HBs Ag

- RASAG.CE (100 tests)

Descrito en el certificado / *Described in the certifícate* 2015 10 0841 ED

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Fecha de última prórroga/ *Last extension date*: **27/11/2013**

Certificado nº/*Certificate no*	Fecha de validez/*Date of validity*	ON nº/*NB no*
2003 12 0388 CT	**Desde/*From* 27/11/2018 Hasta/*To* 18/11/2023**	**0318**

3.3. DIA.CHEMILUX HIV Ab & Ag

- RAIVCOMB.CE (100 tests) — Descrito en el certificado / *Described in the certificate* 2016 02 0844 ED

3.4. DIA.CHEMILUX HBc Ab

- RABCAB.CE (100 tests) — Descrito en el certificado / *Described in the certificate* 2017 07 0863 ED

3.5. DIA.CHEMILUX HTLV I & II Ab

- RAHTLVAB.CE (100 tests) — Descrito en el certificado / *Described in the certificate* 2018 11 0878 ED

Este certificado ampara todas las marcas de estos productos incluidas por el fabricante en su declaración de conformidad. / *This certificate covers all trademarks of these products included by the manufacturer in his declaration of conformity*.

Madrid, 26 de noviembre de 2018
DIRECTORA DE LA AGENCIA ESPAÑOLA DE MEDICAMENTOS Y PRODUCTOS SANITARIOS

agencia española de medicamentos y productos sanitarios

Fdo. Mª Jesús Lamas Díaz

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agencia española de medicamentos y productos sanitarios

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PRÓRROGA/*EXTENSION* — Fecha inicial/ *Initial date*: **10/05/2014**
Fecha de última prórroga/ *Last extension date*: **27/11/2013**

Certificado nº/*Certificate no*	**Fecha de validez/*Date of validity***	**ON nº/*NB no***
2004 05 0442 CT	**Desde/*From* 26/11/2018 Hasta/*To* 18/11/2023**	**0318**

A favor de /*In favour of:*

Fabricante/*Manufacturer*:
Nombre/*Name:* Dia. Pro Diagnostic Bioprobes S.r.l.
Dirección/*Address:* Via G. Carducci, 27 -20099- Sesto San Giovanni – Milano (Italy).
Representante autorizado ante la UE/*Authorized EU representative*:
Nombre/*Name:* Idem Dirección/*Address:* Idem

Para los productos/*For the products:*

Categoría/*Category:* Productos Sanitarios para Diagnóstico "In Vitro" / *In Vitro Diagnostic Medical Devices*
Grupo genérico/*Generic group*: Diagnóstico de enfermedades infecciosas / *Diagnostic of infectious diseases*
Tipo/*Type*: Especificados en Anexos de este Certificado/*Specified in Annexes to this Certificate.*

Elaborado en/*In the facilities:*

Dia. Pro Diagnostic Bioprobes S.r.l.
Via G. Carducci, 27 -20099- Sesto San Giovanni – Milano (Italy).

Este certificado debe ir acompañado por certificado de examen de diseño: NO
This certificate must be accompanied by design examination certificate: NO

Este certificado es consecuencia de la auditoria del Sistema Completo de Garantía de Calidad y del examen de la documentación técnica contenida en el expediente nº 2003 05 0240, y garantiza que los productos descritos cumplen los requisitos de la Directiva. / *This certificate is issued on the full quality assurance system audit, and the examination of the technical documentation contained in dossier nº 2003 05 02405, and guarantees that the described products fulfil the requirements of the Directive.*

Madrid, 23 de noviembre de 2018
DIRECTORA DE LA AGENCIA ESPAÑOLA DE MEDICAMENTOS Y PRODUCTOS SANITARIOS

agencia española de medicamentos y productos sanitarios

Fdo. Mª Jesús Lamas Díaz

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Fecha de la firma: 23/11/2018
Localizador: YD6VVGJ021

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ORGANISMO NOTIFICADO 0318

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Certificado nº/*Certificate no*	Fecha de validez/*Date of validity*	ON nº/*NB no*
2004 05 0442 CT	**Desde/*From* 26/11/2018 Hasta/*To* 18/11/2023**	**0318**

A favor de/*In favour of:*

Fabricante/*Manufacturer:*
Nombre/*Name:* Dia. Pro Diagnostic Bioprobes S.r.l.
Dirección/*Address:* Via G. Carducci, 27 -20099- Sesto San Giovanni – Milano (Italy).
Representante autorizado ante la UE/*Authorized EU representative:*
Nombre/*Name:* Idem Dirección/*Address:* Idem

Tipo de producto / *Device type*: Reactivos y productos reactivos, calibradores y materiales de control para el diagnóstico de enfermedades infecciosas / *Reagents, and reagent products, calibrators and control materials for diagnostic of human infectious diseases.*

Clasificación/*Classification:* Lista B, anexo II / *List B, Annex II*

1. **Reactivos y productos reactivos para la determinación, confirmación y cuantificación de marcadores de infección en muestras humanas mediante técnicas de Inmunoabsorción enzimática (ELISA)/** *Reagents and reactive products for the determination, confirmation and quantification of infection markers in human samples by Enzyme-linked immunosorbent assay (ELISA)* **[NANDO: IVD 0303; IVD 0305]**

1.1. CMV IgM

- CMVM.CE (96 test)

1.2. CMV IgG

- CMVG.CE (96 test)

1.3. Toxo IgM

- TOXOM.CE (96 tests)

1.4. Toxo IgG

- TOXOG.CE (96 tests)

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in accordance with Annex IV (except Section 4) of Directive 98/79/EC
PRÓRROGA/*EXTENSION* — Fecha inicial/ *Initial date*: **10/05/2004**
Fecha de última prórroga/ *Last extension date*: **27/11/2013**

Certificado nº/*Certificate no*	Fecha de validez/*Date of validity*	ON nº/*NB no*
2004 05 0442 CT	**Desde/*From* 26/11/2018 Hasta/*To* 18/11/2023**	**0318**

1.5. RUB IgM

- RUBM.CE (96 tests)

1.6. RUB IgG.

- RUBG.CE (96 tests)
- RUBG.CE.192 (192 tests)
- RUBG.CE.480 (480 tests)

1.7. TORCH IgM

- TORCHM.CE (96 tests)

1.8. *Chlamydia Trachomatis* IgG

- CTG.CE (96 tests)

1.9. *Chlamydia Trachomatis* IgM

- CTM.CE (96 tests)

1.10. *Chlamydia Trachomatis* IgA

- CTA.CE (96 tests)

1.11. *Chlamydia Pneumoniae* IgG

- CPG.CE (96 tests)

1.12. *Chlamydia Pneumoniae* IgM

- CPM.CE (96 tests)

1.13. *Chlamydia Pneumoniae* IgA

- CPA.CE (96 tests)

CORREO ELECTRÓNICO
on0318@aemps.es

ORGANISMO NOTIFICADO 0318

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ANEXO Nº/*ANNEX NO:* I
CERTIFICADO CE DE SISTEMA DE GARANTÍA DE CALIDAD TOTAL
de acuerdo con el Anexo IV (excepto punto 4) de la Directiva 98/79/CE
EC FULL QUALITY ASSURANCE SYSTEM CERTIFICATE
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2. Reactivos y productos reactivos para la determinación, confirmación y cuantificación de marcadores de infección en muestras humanas mediante técnicas de PCR en tiempo real/ *Reagents and reactive products for the determination, confirmation and quantification of infection markers in human samples by Real-Time PCR)* **[NANDO: IVD 0303; IVD 0305]**

2.1. CMV DNA Quantitation (QT) 2nd Generation

- CMVDNAQT.2G.CE (50 tests)
- CMVDNAQT.2G.CE.25 (25 tests)
- CMVDNAQT.2G.CE.100 (100 tests)
- CMVDNAQT.2G.CE.150 (150 tests)

2.2. Dx CMV Assay

- Dx CMV Assay

2.3. *Toxoplasma Gondii* DNA

- TOXODNA.CE (50 tests)
- TOXODNA.CE.25 (25 tests)
- TOXODNA.CE.100 (100tests)
- TOXODNA.CE.150 (150 tests)

2.4. *Chlamydia Trachomatis* DNA

- CTDNA.CE (50 tests)
- CTDNA.CE.25 (25 tests)
- CTDNA.CE.100 (100 tests)
- CTDNA.CE.150 (150 tests)

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3. Reactivos y productos reactivos para la determinación, confirmación y cuantificación de marcadores de infección en muestras humanas mediante ensayos de quimioluminiscencia (CLIA)/ *Reagents and reactive products for the determination, confirmation and quantification of infection markers in human samples by Chemiluminescence Immunoassay (CLIA)* **[NANDO: IVD 0201; IVD 0202; IVD 0203]**

3.1. DIA.CHEMILUX Cytomegalovirus IgM

- RACMVM.CE (100 tests)

3.2. DIA.CHEMILUX Cytomegalovirus IgG

- RACMVG.CE (100 tests)

3.3. DIA.CHEMILUX Toxoplasma IgM

- RATOXOM.CE (100 tests)

3.4. DIA.CHEMILUX Toxoplasma IgG

- RATOXOG.CE (100 tests)

3.5. DIA.CHEMILUX Rubella IgM

- RARUBM.CE (100 tests)

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3.6. DIA.CHEMILUX Rubella IgG

- RARUBG.CE (100 tests)

Este certificado ampara todas las marcas de estos productos incluidas por el fabricante en su declaración de conformidad. / *This certificate covers all trademarks of these products included by the manufacturer in his declaration of conformity*.

Madrid, 23 de noviembre de 2018
DIRECTORA DE LA AGENCIA ESPAÑOLA DE MEDICAMENTOS Y PRODUCTOS SANITARIOS

agencia española de medicamentos y productos sanitarios

Fdo. Mª Jesús Lamas Díaz

Firmado digitalmente por: Agencia Española de Medicamentos y Productos Sanitarios
Fecha de la firma: 23/11/2018
Localizador: YD6VVGJ021

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Ea IgG

Enzyme ImmunoAssay (ELISA) for the quantitative/qualitative determination of IgG antibodies to Epstein Barr Virus Early Antigen in human serum and plasma

- for “in vitro” diagnostic use only -

DIA.PRO
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(Milano) - Italy
Phone +39 02 27007161
Fax +39 02 26007726
e-mail: info@diapro.it

REF EAG.CE
96 Tests

Ea IgG

A. INTENDED USE
Enzyme ImmunoAssay (ELISA) for the quantitative/qualitative determination of IgG antibodies to Epstein Barr Virus Early Antigen in human plasma and sera.
For "in vitro" diagnostic use only.

B. INTRODUCTION
Epstein Barr Virus or EBV is the principal etiological agent of infectious mononucleosis, as well as a contributory factor in the etiology of Burkitt's lymphoma and nasopharyngeal carcinoma, or NPC. A member of the family Herpesviridae, it has a worldwide distribution, such that 80 to 90% of all adults have been infected. Primary infections usually occur during the first decade of life. While childhood infections are mostly asymptomatic, 50 to 70% of young adults undergoing primary EBV infections show mild to severe illness. EBV may cause a persistent, latent infection which can be reactivated under immunosuppression or in AIDS affected patients.
As humoral responses to primary EBV infections are quite rapid, the level and class of antibodies raised in most cases allow classification as to whether the patient is still susceptible, has a current or recent primary infection, had a past infection or may be having reactivated EBV infection. The detection of EBV-specific IgG, IgM and IgA antibodies to its major immunodominant antigens (Nuclear Antigen, Viral Capsid Antigen, Early Antigen) has become therefore an important and useful determination for the monitoring and follow-up of EBV infected patients.

C. PRINCIPLE OF THE TEST
Microplates are coated with EBV-specific affinity purified Early Antigen or EA.
In the 1st incubation, the solid phase is treated with diluted samples and anti-EA IgG are captured, if present, by the antigens.
After washing out all the other components of the sample, in the 2nd incubation bound anti-EA IgG are detected by the addition of anti hIgG antibody, labelled with peroxidase (HRP).
The enzyme captured on the solid phase, acting on the substrate/chromogen mixture, generates an optical signal that is proportional to the amount of anti EA IgG antibodies present in the sample.
IgG in the sample may be quantitated by means of a standard curve calibrated in arbitrary units per milliliter (Uarb/ml) as no international standard is available.

D. COMPONENTS
Each kit contains sufficient reagents to perform 96 tests.

1. Microplate: MICROPLATE
12 strips x 8 microwells coated with **affinity purified EBV Ea**. Plates are sealed into a bag with desiccant.
Allow the microplate to reach room temperature before opening; reseal unused strips in the bag with desiccant and store at 4°C.

2. Calibration Curve: CAL N° …
6 vials. Ready to use and color coded standard curve ranging:
4 ml CAL1 = 0 arbU/ml
4 ml CAL2 = 5 arbU/ml
2 ml CAL3 = 10 arbU/ml
2 ml CAL4 = 20 arbU/ml
2 ml CAL 5 = 50 arbU/ml
4 ml CAL6 = 100 arbU/ml.
Standards are calibrated against an internal Gold Standard or IGS as no international one is defined.
Contains human serum proteins, 2% casein, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.1% Tween 20, 0.09% Na-azide and 0.1% Kathon GC as preservatives. Standards are blue colored.

3. Wash buffer concentrate: WASHBUF 20X
1x60ml/bottle20x concentrated solution. Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2, 0.05% Tween 20 and 0.1% Kathon GC.

4. Enzyme conjugate : CONJ
1x16ml/vial. Ready to use and red colour coded. It contains Horseradish peroxidase conjugated polyclonal antibodies to human IgG, 5% BSA, 10 mM Tris buffer pH 6.8+/-0.1, 0.1% Kathon GC and 0.02% gentamicine sulphate as preservatives.

5. Chromogen/Substrate: SUBS TMB
1x16ml/vial. It contains 50 mM citrate-phosphate buffer pH 3.5-3.8, 4% dimethylsulphoxide, 0.03% tetra-methyl-benzidine (or TMB) and 0.02% hydrogen peroxide (or H_2O_2).
Note: To be stored protected from light as sensitive to strong illumination.

6. Sulphuric Acid: H2SO4 0.3 M
1x15ml/vialIt contains 0.3 M H_2SO_4 solution.
Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

7. Specimen Diluent: DILSPE
2x60ml/vial. It contains 2% casein, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.1% Tween 20, 0.09% Na-azide and 0.1% Kathon GC as preservatives. To be used to dilute the sample.

8. Plate sealing foils n°2

9. Package insert n°1

E. MATERIALS REQUIRED BUT NOT PROVIDED
1. Calibrated Micropipettes (1000, 100 and 10ul) and disposable plastic tips.
2. EIA grade water (bidistilled or deionised, charcoal treated to remove oxidizing chemicals used as disinfectants).
3. Timer with 60 minute range or higher.
4. Absorbent paper tissues.
5. Calibrated ELISA microplate thermostatic incubator (dry or wet) set at +37°C (+/-0.5°C tolerance).
6. Calibrated ELISA microwell reader with 450nm (reading) and with 620-630nm (blanking) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.

F. WARNINGS AND PRECAUTIONS
1. The kit has to be used by skilled and properly trained technical personnel only, under the supervision of a medical doctor responsible of the laboratory.
2. All the personnel involved in performing the assay have to wear protective laboratory clothes, talc-free gloves and glasses. The use of any sharp (needles) or cutting (blades) devices should be avoided. All the personnel involved should be trained in biosafety procedures, as recommended by the Center for Disease Control, Atlanta, U.S. and reported in the National Institute of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
3. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.

4. The laboratory environment should be controlled so as to avoid contaminants such as dust or air-born microbial agents, when opening kit vials and microplates and when performing the test. Protect the Chromogen (TMB) from strong light and avoid vibration of the bench surface where the test is undertaken.
5. Upon receipt, store the kit at 2..8°C into a temperature controlled refrigerator or cold room.
6. Do not interchange components between different lots of the kits. It is recommended that components between two kits of the same lot should not be interchanged.
7. Check that the reagents are clear and do not contain visible heavy particles or aggregates. If not, advise the laboratory supervisor to initiate the necessary procedures for kit replacement.
8. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each sample.
9. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one.
10. Do not use the kit after the expiration date stated on the external container and internal (vials) labels. A study conducted on an opened kit did not pointed out any relevant loss of activity up to six 6 uses of the device and up to 6 months.
11. Treat all specimens as potentially infective. All human serum specimens should be handled at Biosafety Level 2, as recommended by the Center for Disease Control, Atlanta, U.S. in compliance with what reported in the Institutes of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
12. The use of disposable plastic-ware is recommended in the preparation of the liquid components or in transferring components into automated workstations, in order to avoid cross contamination.
13. Waste produced during the use of the kit has to be discarded in compliance with national directives and laws concerning laboratory waste of chemical and biological substances. In particular, liquid waste generated from the washing procedure, from residuals of controls and from samples has to be treated as potentially infective material and inactivated before waste. Suggested procedures of inactivation are treatment with a 10% final concentration of household bleach for 16-18 hrs or heat inactivation by autoclave at 121°C for 20 min..
14. Accidental spills from samples and operations have to be adsorbed with paper tissues soaked with household bleach and then with water. Tissues should then be discarded in proper containers designated for laboratory/hospital waste.
15. The Sulphuric Acid is an irritant. In case of spills, wash the surface with plenty of water
16. Other waste materials generated from the use of the kit (example: tips used for samples and controls, used microplates) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

G. SPECIMEN: PREPARATION AND WARNINGS

1. Blood is drawn aseptically by venepuncture and plasma or serum is prepared using standard techniques of preparation of samples for clinical laboratory analysis. No influence has been observed in the preparation of the sample with citrate, EDTA and heparin.
2. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results. Bar code labeling and electronic reading is strongly recommended.
3. Haemolysed ("red") and visibly hyperlipemic ("milky") samples have to be discarded as they could generate false results. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they could give rise to false results.
4. Sera and plasma can be stored at +2°..8°C for up to five days after collection. For longer storage periods, samples can be stored frozen at –20°C for several months. Any frozen samples should not be freezed/thawed more than once as this may generate particles that could affect the test result.
5. If particles are present, centrifuge at 2.000 rpm for 20 min or filter using 0.2-0.8u filters to clean up the sample for testing.
6. Samples whose anti-Ea IgG antibody concentration is expected to be higher than 100 arbU/ml should be diluted before use, either 1:10 or 1:100 in the Calibrator 0 arbU/ml. Dilutions have to be done in clean disposable tubes by diluting 50 ul of each specimen with 450 ul of Cal 0 (1:10). Then 50 ul of the 1:10 dilution are diluted with 450 ul of the Cal 0 (1:100). Mix tubes thoroughly on vortex and then proceed toward the dilution step reported in section M.

H. PREPARATION OF COMPONENTS AND WARNINGS

Microplate:
Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant is not turned to dark green, indicating a defect of storing.
In this case call Dia.Pro's customer service.
Unused strips have to be placed back inside the aluminum pouch, with the desiccant supplied, firmly zipped and stored at +2°..8°C.
Important Note*: After first opening, remaining strips are stable until the humidity indicator inside the desiccant bag turns from yellow to green.*

Calibration Curve
Ready to use component. Mix carefully on vortex before use.

Wash buffer concentrate:
The whole content of the concentrated solution has to be diluted 20x with bidistilled water and mixed gently end-over-end before use. During preparation avoid foaming as the presence of bubbles could impact on the efficiency of the washing cycles.
Note: *Once diluted, the wash solution is stable for 1 week at +2..8° C.*

Enzyme conjugate:
Ready to use. Mix well on vortex before use.
Be careful not to contaminate the liquid with oxidizing chemicals, air-driven dust or microbes.
If this component has to be transferred use only plastic, possibly sterile disposable containers.

Chromogen/Substrate:
Ready to use. Mix well on vortex before use.
Be careful not to contaminate the liquid with oxidizing chemicals, air-driven dust or microbes.
Do not expose to strong illumination, oxidizing agents and metallic surfaces.
If this component has to be transferred use only plastic, possible sterile disposable container

Sample Diluent
Ready to use component. Mix carefully on vortex before use.

Sulphuric Acid:
Ready to use. Mix well on vortex before use.
Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

Legenda:

Warning **H statements:**
H315 – Causes skin irritation.
H319 – Causes serious eye irritation.

Precautionary **P statements:**
P280 – Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352 – IF ON SKIN: Wash with plenty of soap and water.

P332 + P313 – If skin irritation occurs: Get medical advice/attention.
P305 + P351 + P338 – IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337 + P313 – If eye irritation persists: Get medical advice/attention.
P362 + P363 – Take off contaminated clothing and wash it before reuse.

I. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

1. Micropipettes have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (household alcohol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample. They should also be regularly maintained in order to show a precision of 1% and a trueness of +/-2%. Decontamination of spills or residues of kit components should also be carried out regularly.
2. The ELISA incubator has to be set at +37°C (tolerance of +/- 0.5°C) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water baths are suitable for the incubations, provided that the instrument is validated for the incubation of ELISA tests.
3. The ELISA washer is extremely important to the overall performances of the assay. The washer must be carefully validated and correctly optimised using the kit controls and reference panels, before using the kit for routine laboratory tests. Usually 4-5 washing cycles (aspiration + dispensation of 350ul/well of washing solution = 1 cycle) are sufficient to ensure that the assay performs as expected. A soaking time of 20-30 seconds between cycles is suggested. In order to set correctly their number, it is recommended to run an assay with the kit controls and well characterized negative and positive reference samples, and check to match the values reported below in the section "Internal Quality Control". Regular calibration of the volumes delivered by, and maintenance (decontamination and cleaning of needles) of the washer has to be carried out according to the instructions of the manufacturer.
4. Incubation times have a tolerance of $\pm$5%.
5. The ELISA microplate reader has to be equipped with a reading filter of 450nm and with a second filter (620-630nm, strongly recommended) for blanking purposes. Its standard performances should be (a) bandwidth $\leq$ 10 nm; (b) absorbance range from 0 to $\geq$ 2.0; (c) linearity to $\geq$ 2.0; repeatability $\geq$ 1%. Blanking is carried out on the well identified in the section "Assay Procedure". The optical system of the reader has to be calibrated regularly to ensure that the correct optical density is measured. It should be regularly maintained according to the manufacturer 's instructions.
6. When using an ELISA automated work station, all critical steps (dispensation, incubation, washing, reading, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the section "Internal Quality Control". The assay protocol has to be installed in the operating system of the unit and validated as for the washer and the reader. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set. Particular attention must be paid to avoid carry over by the needles used for dispensing and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells. The use of ELISA automated work stations is recommended when the number of samples to be tested exceed 20-30 units per run.
7. Dia.Pro's customer service offers support to the user in the setting and checking of instruments used in combination with the kit, in order to assure compliance with the requirements described. Support is also provided for the installation of new instruments to be used with the kit.

L. PRE ASSAY CONTROLS AND OPERATIONS

1. Check the expiration date of the kit printed on the external label (primary container). Do not use if expired.
2. Check that the liquid components are not contaminated by visible particles or aggregates.
3. Check that the Chromogen (TMB) is colourless or pale blue by aspirating a small volume of it with a sterile plastic pipette.
4. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box (primary container). Check that the aluminium pouch, containing the microplate, is not punctured or damaged.
5. Dilute all the content of the 20x concentrated Wash Solution as described above.
6. Allow all the other components to reach room temperature (about 1 hr) and then mix gently on vortex all liquid reagents.
7. Set the ELISA incubator at +37°C and prepare the ELISA washer by priming with the diluted washing solution, according to the manufacturers instructions. Set the right number of washing cycles as found in the validation of the instrument for its use with the kit.
8. Check that the ELISA reader is turned on or ensure it will be turned on at least 20 minutes before reading.
9. If using an automated work station, turn on, check settings and be sure to use the right assay protocol.
10. Check that the micropipettes are set to the required volume.
11. Check that all the other equipment is available and ready to use.
12. In case of problems, do not proceed further with the test and advise the supervisor.

M. ASSAY PROCEDURE

The assay has to be carried out according to what reported below, taking care to maintain the same incubation time for all the samples in testing.
The kit may be used for quantitative and qualitative determinations as well.

M1. QUANTITATIVE DETERMINATION:

1. Dilute samples 1:101 into a properly defined dilution tube (example: 1000 µl Sample Diluent + 10 µl sample). Do not dilute the Calibration Set as calibrators are ready to use. Mix carefully all the liquid components on vortex and then proceed as described below.
2. Place the required number of Microwells in the microwell holder. Leave the A1 and B1 empty for the operation of blanking.
3. Dispense 100 µl of Calibrators in duplicate. Then dispense 100 µl of diluted samples in each properly identified well.
4. Incubate the microplate for **60 min at +37°C**.

Important note: *Strips have to be sealed with the adhesive sealing foil, supplied, only when the test is carried out manually. Do not cover strips when using ELISA automatic instruments.*

5. Wash the microplate with an automatic washer as reported previously (section I.3).
6. Pipette 100 µl Enzyme Conjugate into each well, except A1+B1 blanking wells, and cover with the sealer. Check that this red coloured component has been dispensed in all the wells, except A1 and B1.

Important note: *Be careful not to touch the plastic inner surface of the well with the tip filled with the Enzyme Conjugate. Contamination might occur.*

7. Incubate the microplate for **60 min at +37°C**.
8. Wash microwells as in step 5.
9. Pipette 100 µl Chromogen/Substrate mixture into each well, the blank wells A1 and B1 included. Then incubate the microplate at **room temperature (18-24°C) for 20 minutes**.

Important note: *Do not expose to strong direct illumination. High background might be generated.*

10. Pipette 100 µl Sulphuric Acid to stop the enzymatic reaction into all the wells using the same pipetting sequence as in step 9. Addition of acid will turn the positive calibrators and the positive samples from blue to yellow.
11. Measure the colour intensity of the solution in each well, as described in section I.5, at 450nm filter (reading) and at 620-630nm (background subtraction, strongly recommended), blanking the instrument on A1 or B1 or both.

M2. QUALITATIVE DETERMINATION

If only a qualitative determination is required, proceed as described below:

1. Dilute samples 1:101 into a properly defined dilution tube (example: 1000 µl Sample Diluent + 10 µl sample). Do not dilute the Calibration Set as calibrators are ready to use. Mix carefully all the liquid components on vortex and then proceed as described below.
2. Place the required number of Microwells in the microwell holder. Leave A1 well empty for the operation of blanking.
3. Dispense 100 µl of Calibrator 0 arbU/ml and Calibrator 5 arbU/ml in duplicate and Calibrator 100 arbU/ml in single. Then dispense 100 µl of diluted samples in each properly identified well.
4. Incubate the microplate for **60 min at +37°C**.

Important note: *Strips have to be sealed with the adhesive sealing foil, supplied, only when the test is carried out manually. Do not cover strips when using ELISA automatic instruments.*

5. Wash the microplate with an automatic washer as reported previously (section I.3).
6. Pipette 100 µl Enzyme Conjugate into each well, except the A1 well, and cover with the sealer. Check that this red coloured component has been dispensed in all the wells, except A1.

Important note: *Be careful not to touch the plastic inner surface of the well with the tip filled with the Enzyme Conjugate. Contamination might occur.*

7. Incubate the microplate for **60 min at +37°C**.
8. Wash microwells as in step 5.
9. Pipette 100 µl Chromogen/Substrate mixture into each well, the blank well included. Then incubate the microplate at **room temperature (18-24°C) for 20 minutes**.

Important note: *Do not expose to strong direct illumination. High background might be generated.*

10. Pipette 100 µl Sulphuric Acid into all the wells using the same pipetting sequence as in step 9. Addition of acid will turn the positive calibrators, the control serum and the positive samples from yellow to blue.
11. Measure the colour intensity of the solution in each well, as described in section I.5, at 450nm filter (reading) and at 620-630nm (background subtraction, strongly recommended), blanking the instrument on A1.

General Important notes:

1. *If the second filter is not available ensure that no finger prints are present on the bottom of the microwell before reading at 450nm. Finger prints could generate false positive results on reading.*

2. *Reading has to be carried out just after the addition of the Stop Solution and anyway not any longer than 20 minutes after its addition. Some self oxidation of the chromogen can occur leading to high background.*

N. ASSAY SCHEME

Method	**Operations**
Calibrators	100 µl
Samples diluted 1:101	100 µl
1st incubation	**60 min**
Temperature	+37°C
Wash step	4-5 cycles
Enzyme conjugate	100 µl
2nd incubation	**60 min**
Temperature	+37°C
Wash step	4-5 cycles
TMB/H2O2	100 µl
3rd incubation	**20 min**
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm

An example of dispensation scheme for Quantitative Analysis is reported below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	CAL4	S 3									
B	BLK	CAL4	S 4									
C	CAL1	CAL5	S 5									
D	CAL1	CAL5	S 6									
E	CAL2	CAL6	S 7									
F	CAL2	CAL6	S 8									
G	CAL3	S1	S 9									
H	CAL3	S2	S 10									

Legenda: BLK = Blank CAL = Calibrator S = Sample

An example of dispensation scheme in qualitative assays is reported below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S 3	S 11									
B	CAL1	S 4	S 12									
C	CAL1	S 5	S 13									
D	CAL2	S 6	S 14									
E	CAL2	S 7	S 15									
F	CAL6	S 8	S 16									
G	S1	S 9	S 17									
H	S2	S 10	S 18									

Legenda: BLK = Blank CAL = Calibrators S = Sample

O. INTERNAL QUALITY CONTROL

A validation check is carried out on the controls any time the kit is used in order to verify whether the performances of the assay are as qualified.
Control that the following data are matched:

Check	Requirements
Blank well	< 0.100 OD450nm value
CAL 1 0 arbU/ml	< 0.150 mean OD450nm value after blanking coefficient of variation < 30%
CAL 2 5 arbU/ml	OD450nm > OD450nm CAL1 + 0.100
CAL 6 100 arbU/ml	OD450nm > 1.000

If the results of the test match the requirements stated above, proceed to the next section. If they do not, do not proceed any further and operate as follows:

Problem	Check
Blank well > 0.100 OD450nm	1. that the Chromogen/Sustrate solution has not got contaminated during the assay
CAL 1 **0 arbU/ml** > 0.150 OD450nm after blanking coefficient of variation > 30%	1. that the washing procedure and the washer settings are as validated in the pre qualification study; 2. that the proper washing solution has been used and the washer has been primed with it before use; 3. that no mistake has been done in the assay procedure (dispensation of a positive calibrator instead of the negative one; 4. that no contamination of the negative calibrator or of their wells has occurred due spills of positive samples or the enzyme conjugate; 5. that micropipettes haven't got contaminated with positive samples or with the enzyme conjugate 6. that the washer needles are not blocked or partially obstructed.
CAL 2 **5 arbU/ml** OD450nm < OD450nm CAL1 + 0.100	1. that the procedure has been correctly executed; 2. that no mistake has been done in its distribution (ex.: dispensation of a wrong calibrator instead); 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the calibrator has occurred.
CAL 6 **100 arbU/ml** < 1.000 OD450nm	1. that the procedure has been correctly executed; 2. that no mistake has been done in its distribution (dispensation of a wrong calibrator instead) ; 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the positive control has occurred.

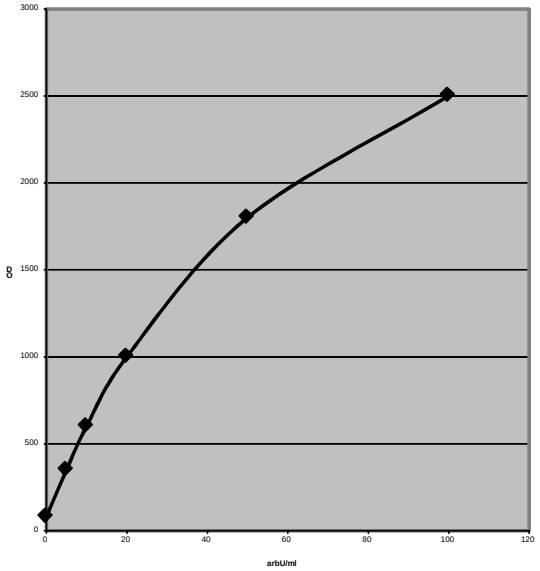
Should one of these problems have happened, after checking, report to the supervisor for further actions.

P. RESULTS

P.1 Quantitative method

If the test turns out to be valid, use for the quantitative method an approved curve fitting program to draw the calibration curve from the values obtained by reading at 450nm (4-parameters interpolation is suggested).
Then on the calibration curve calculate the concentration of anti Ea IgG antibody in samples.

An example of Calibration curve is reported below.



Important Note:
Do not use the calibration curve above to make calculations.

P.2 Qualitative method

In the qualitative method, calculate the mean OD450nm values for the Calibrators 0 and 5 arbU/ml and then check that the assay is valid.

Example of calculation:

Note: *The following data must not be used instead or real figures obtained by the user.*

Calibrator 0 arbU/ml: 0.020 – 0.024 OD450nm
Mean Value: 0.022 OD450nm
Lower than 0.150 – Accepted
Calibrator 5 arbU/ml: 0.250 – 0.270 OD450nm
Mean Value: 0.260 OD450nm
Higher than Cal 0 + 0.100 – Accepted
Calibrator 100 arbU/ml: 2.045 OD450nm
Higher than 1.000 – Accepted

The OD450nm of the Calibrator 5 arbU/ml is considered the cut-off (or Co) of the system.
The ratio between the OD450nm value of the sample and the OD450nm of the Calibrator 5 arbU/ml (or S/Co) can provide a semi-quantitative estimation of the content of specific IgG in the sample.

Q. INTERPRETATION OF RESULTS

Samples with a concentration lower than 5 arbU/ml are considered negative for anti-Ea IgG antibody.
Samples with a concentration higher than 5 arbU/ml are considered positive for anti-Ea IgG antibody.
Ea IgG results alone are not, anyway, enough to provide a clear diagnosis of EBV infection.
At least EBV VCA IgG and EBV VCA IgM results, possibly together with EBNA IgG, are necessary in combination.
A reference range of the minimum essential serological markers of Epstein-Barr infection, derived from Infectious Diseases Handbook, 3rd edition, published by Lexi-Comp Inc., USA, is reported schematically below:

VCA IgM	EBNA (or VCA) IgG	Interpretation
negative	negative	No history of EBV infection
positive	negative	Acute primary infection
negative	**positive**	History of previous infection
positive	**positive**	Reactivation

Important notes:

1. *Interpretation of results should be done under the supervision of the laboratory supervisor to reduce the risk of judgment errors and misinterpretations.*
2. *When test results are transmitted from the laboratory to another facility, attention must be paid to avoid erroneous data transfer.*
3. *Diagnosis has to be done and released to the patient by a suitably qualified medical doctor.*

R. PERFORMANCE CHARACTERISTICS

Evaluation of Performances has been conducted in an external clinical center on negative and positive samples with reference to a FDA approved commercial kit.

1. Limit of detection

No international standard for Ea IgG Antibody detection has been defined so far by the European Community.
In its absence, an Internal Gold Standard (or IGS), derived from a patient with an history of past mononucleosis infection, has been defined in order to provide the device with a constant and excellent sensitivity.

2. Diagnostic Sensitivity and Specificity:

The diagnostic performances were evaluated in a performance evaluation study conducted in an external centre, with excellent experience in the diagnosis of infectious diseases.
The diagnostic sensitivity was studied on samples, pre-tested positive with a different reference kit of European origin in use at the laboratory. Positive samples were collected from patients that experienced mononucleosis infection.
The diagnostic specificity was determined on panels of negative samples from normal individuals and blood donors, classified negative with the reference kit, including potentially interfering specimens.
Both plasma, derived with different standard techniques of preparation (citrate, EDTA and heparin), and sera have been used to determine the specificity. No false reactivity due to the method of specimen preparation has been observed.
Frozen specimens have also been tested to check whether samples freezing interferes with the performance of the test. No interference was observed on clean and particle free samples.
The Performance Evaluation provided the following values :

Sensitivity	≥ 98 %
Specificity	≥ 98 %

3. Reproducibility:

Data obtained from a study conducted on three samples of different Ea IgG reactivity, examined in 16 replicates in three separate runs show CV% values ranging 3-16% depending on OD450nm readings.
The variability shown in the tables did not result in sample misclassification.

S. LIMITATIONS

False positivity has been assessed as less than 2-5% of the normal population depending on the reference kit used.
Frozen samples containing fibrin particles or aggregates may generate false positive results.

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All the IVD Products manufactured by the company are under the control of a certified Quality Management System in compliance with EN ISO 13485 rule. Each lot is submitted to a quality control and released into the market only if conforming with the EC technical specifications and acceptance criteria.

Produced by
Dia.Pro Diagnostic Bioprobes Srl
Via G. Carducci n° 27 – Sesto San Giovanni (MI) – Italy

CE

Dia.Pro
Diagnostic
BioProbes

EC DECLARATION OF CONFORMITY

MANUFACTURER	DIA.PRO DIAGNOSTIC BIOPROBES S.R.L. VIA G. CARDUCCI N° 27 – 20099 SESTO SAN GIOVANNI (MILANO) – ITALY
PRODUCT	**Ea IgG** CODE: **EAG.CE** (96 tests)
CLASSIFICATION	GENERAL IVD
CONFORMITY ASSESSMENT ROUTE	SELF CERTIFICATION

WE HEREBY DECLARE THAT THE ABOVE MENTIONED PRODUCT MEETS THE PROVISIONS OF THE COUNCIL DIRECTIVE 98/79/EC FOR IN VITRO DIAGNOSTIC DEVICES.

ISO CERTIFICATE	UNE EN ISO 13485 N° 2013 11 0039 EN, RELEASED BY AEMPS (AGENCIA ESPAÑOLA DE MEDICAMENTOS Y PRODUCTOS SANITARIOS)

PLACE & DATE OF FIRST ISSUE	MILANO – JUNE 2010
PLACE & DATE OF CURRENT ISSUE	SESTO SAN GIOVANNI (MI) – MARCH 2019
SIGNATURE **Legal Representative** **Dr.ssa Fiorenza Scozzesi**	

Rev:	05/2018

EBNA IgG

Enzyme ImmunoAssay (ELISA) for the quantitative/qualitative determination of IgG antibodies to Epstein Barr Virus Nuclear Antigen in human serum and plasma

- for “in vitro” diagnostic use only -

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REF EBNG.CE
96 Tests

EBNA IgG

A. INTENDED USE
Enzyme ImmunoAssay (ELISA) for the quantitative/qualitative determination of IgG antibodies to Epstein Barr Virus Nuclear Antigen in human plasma and sera.
For "in vitro" diagnostic use only.

B. INTRODUCTION
Epstein Barr Virus or EBV is the principal etiological agent of infectious mononucleosis, as well as a contributory factor in the etiology of Burkitt's lymphoma and nasopharyngeal carcinoma, or NPC. A member of the family Herpesviridae, it has a worldwide distribution, such that 80 to 90% of all adults have been infected. Primary infections usually occur during the first decade of life. While childhood infections are mostly asymptomatic, 50 to 70% of young adults undergoing primary EBV infections show mild to severe illness. EBV may cause a persistent, latent infection which can be reactivated under immunosoppression or in AIDS affected patients. As humoral responses to primary EBV infections are quite rapid, the level and class of antibodies raised in most cases allow classification as to whether the patient is still susceptible, has a current or recent primary infection, had a past infection or may be having reactivated EBV infection. The detection of EBV-specific IgG, IgM and IgA antibodies to its major immunodominant antigens (mainly Nuclear Antigen or EBNA and Viral Capsidic Antigen or VCA) has become therefore an important and useful determination for the monitoring and follow-up of EBV infected patients.

C. PRINCIPLE OF THE TEST
In order to get rid of crossreactions with other viruses of the same family, microplates are coated with affinity purified native EBNA antigen, capable to provide the assay with the highest specificity.
In the 1st incubation, the solid phase is treated with diluted samples and anti-EBNA IgG are captured, if present, by the antigens.
After washing out all the other components of the sample, in the 2nd incubation bound anti-EBNA IgG are detected by the addition of anti hIgG antibody, labeled with peroxidase (HRP). The enzyme captured on the solid phase, acting on the substrate/chromogen mixture, generates an optical signal that is proportional to the amount of anti EBNA IgG antibodies present in the sample.
IgG in the sample may therefore be quantitated by means of a standard curve calibrated in arbitrary units per milliliter (arbU/ml) as no international standard is available.

D. COMPONENTS
Each kit contains sufficient reagents to perform 96 tests.

1. Microplate: MICROPLATE
12 strips x 8 microwells coated with affinity purified native EBNA antigen. Plates are sealed into a bag with desiccant. Allow the microplate to reach room temperature before opening; reseal unused strips in the bag with desiccant and store at 4°C.

2. Calibration Curve: CAL N° …
Ready to use and color coded standard curve ranging:
4 ml CAL1 = 0 arbU/ml
4 ml CAL2 = 5 arbU/ml
2 ml CAL3 = 10 arbU/ml
2 ml CAL4 = 20 arbU/ml
2ml CAL 5 = 50 arbU/ml
4 ml CAL6 = 100 arbU/ml.
Standards are calibrated against an internal Gold Standard or IGS as no international one is defined.
Contains human serum proteins, 2% casein, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.1% Tween 20, 0.09% Na-azide and 0.1% Kathon GC as preservatives. Standards are blue colored.

3. Control Serum: CONTROL …ml
1 vial. Lyophilized.
It contains fetal bovine serum proteins, human IgG antibodies to EBNA at 20 arbU/ml±20%, 0.2 mg/ml gentamicine sulphate and 0.1% Kathon GC as preservatives.

3. Wash buffer concentrate: WASHBUF 20X
1x60ml/bottle20x concentrated solution.
Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2, 0.05% Tween 20 and 0.1% Kathon GC.

4. Enzyme conjugate : CONJ
1x16ml/vial. Ready to use and red colour coded. It contains Horseradish peroxidase conjugated polyclonal antibodies to human IgG, 5% BSA, 10 mM Tris buffer pH 6.8+/-0.1, 0.1% Kathon GC and 0.02% gentamicine sulphate as preservatives.

5. Chromogen/Substrate: SUBS TMB
1x16ml/vial. It contains 50 mM citrate-phosphate buffer pH 3.5-3.8, 4% dimethylsulphoxide, 0.03% tetra-methyl-benzidine (or TMB) and 0.02% hydrogen peroxide (or H_2O_2).
Note: To be stored protected from light as sensitive to strong illumination.

6. Sulphuric Acid: H2SO4 0.3 M
1x15ml/vial it contains 0.3 M H_2SO_4 solution.
Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+ P351+P338, P337+P313, P362+P363).

7. Specimen Diluent: DILSPE
2x60ml/vial. It contains 2% casein, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.1% Tween 20, 0.09% Na-azide and 0.1% Kathon GC as preservatives. To be used to dilute the sample.

8. Plate sealing foils n°2

9. Package insert n°1

E. MATERIALS REQUIRED BUT NOT PROVIDED
1. Calibrated Micropipettes (1000, 100 and 10ul) and disposable plastic tips.
2. EIA grade water (bidistilled or deionised, charcoal treated to remove oxidizing chemicals used as disinfectants).
3. Timer with 60 minute range or higher.
4. Absorbent paper tissues.
5. Calibrated ELISA microplate thermostatic incubator (dry or wet) set at +37°C (+/-0.5°C tolerance).
6. Calibrated ELISA microwell reader with 450nm (reading) and with 620-630nm (blanking) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.

F. WARNINGS AND PRECAUTIONS
1. The kit has to be used by skilled and properly trained technical personnel only, under the supervision of a medical doctor responsible of the laboratory.
2. All the personnel involved in performing the assay have to wear protective laboratory clothes, talc-free gloves and glasses. The use of any sharp (needles) or cutting (blades) devices should be avoided. All the personnel involved should be trained in biosafety procedures, as recommended by the Center for Disease Control, Atlanta, U.S. and reported in the National

Institute of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
3. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.
4. The laboratory environment should be controlled so as to avoid contaminants such as dust or air-born microbial agents, when opening kit vials and microplates and when performing the test. Protect the Chromogen (TMB) from strong light and avoid vibration of the bench surface where the test is undertaken.
5. Upon receipt, store the kit at 2..8°C into a temperature controlled refrigerator or cold room.
6. Do not interchange components between different lots of the kits. It is recommended that components between two kits of the same lot should not be interchanged.
7. Check that the reagents are clear and do not contain visible heavy particles or aggregates. If not, advise the laboratory supervisor to initiate the necessary procedures for kit replacement.
8. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each sample.
9. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one.
10. Do not use the kit after the expiration date stated on the external container and internal (vials) labels. A study conducted on an opened kit did not pointed out any relevant loss of activity up to six 6 uses of the device and up to 3 months.
11. Treat all specimens as potentially infective. All human serum specimens should be handled at Biosafety Level 2, as recommended by the Center for Disease Control, Atlanta, U.S. in compliance with what reported in the Institutes of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
12. The use of disposable plastic-ware is recommended in the preparation of the liquid components or in transferring components into automated workstations, in order to avoid cross contamination.
13. Waste produced during the use of the kit has to be discarded in compliance with national directives and laws concerning laboratory waste of chemical and biological substances. In particular, liquid waste generated from the washing procedure, from residuals of controls and from samples has to be treated as potentially infective material and inactivated before waste. Suggested procedures of inactivation are treatment with a 10% final concentration of household bleach for 16-18 hrs or heat inactivation by autoclave at 121°C for 20 min..
14. Accidental spills from samples and operations have to be adsorbed with paper tissues soaked with household bleach and then with water. Tissues should then be discarded in proper containers designated for laboratory/hospital waste.
15. The Sulphuric Acid is an irritant. In case of spills, wash the surface with plenty of water
16. Other waste materials generated from the use of the kit (example: tips used for samples and controls, used microplates) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

G. SPECIMEN: PREPARATION AND WARNINGS
1. Blood is drawn aseptically by venepuncture and plasma or serum is prepared using standard techniques of preparation of samples for clinical laboratory analysis. No influence has been observed in the preparation of the sample with citrate, EDTA and heparin.
2. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results. Bar code labeling and electronic reading is strongly recommended.
3. Haemolysed ("red") and visibly hyperlipemic ("milky") samples have to be discarded as they could generate false results. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they could give rise to false results.
4. Sera and plasma can be stored at +2°..8°C for up to five days after collection. For longer storage periods, samples can be stored frozen at –20°C for several months. Any frozen samples should not be freezed/thawed more than once as this may generate particles that could affect the test result.
5. If particles are present, centrifuge at 2.000 rpm for 20 min or filter using 0.2-0.8u filters to clean up the sample for testing.
6. Samples whose anti-EBNA IgG antibody concentration is expected to be higher than 100 arbU/ml should be diluted before use, either 1:10 or 1:100 in the Calibrator 0 arbU/ml. Dilutions have to be done in clean disposable tubes by diluting 50 ul of each specimen with 450 ul of Cal 0 (1:10). Then 50 ul of the 1:10 dilution are diluted with 450 ul of the Cal 0 (1:100). Mix tubes thoroughly on vortex and then proceed toward the dilution step reported in section M.

H. PREPARATION OF COMPONENTS AND WARNINGS
Microplate:
Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant is not turned to dark green, indicating a defect of storing.
In this case call Dia.Pro's customer service.
Unused strips have to be placed back inside the aluminum pouch, with the desiccant supplied, firmly zipped and stored at +2°..8°C.
Important Note*: After first opening, remaining strips are stable until the humidity indicator inside the desiccant bag turns from yellow to green.*

Calibration Curve
Ready to use component. Mix carefully on vortex before use.

Control Serum
Add the volume of ELISA grade water, reported on the label, to the lyophilised powder; let fully dissolve and then gently mix on vortex.
Note: *The control after dissolution is not stable. Store frozen in aliquots at –20°C.*

Wash buffer concentrate:
The whole content of the concentrated solution has to be diluted 20x with bidistilled water and mixed gently end-over-end before use. During preparation avoid foaming as the presence of bubbles could impact on the efficiency of the washing cycles.
Note: *Once diluted, the wash solution is stable for 1 week at +2..8° C.*

Enzyme conjugate:
Ready to use. Mix well on vortex before use.
Be careful not to contaminate the liquid with oxidizing chemicals, air-driven dust or microbes.
If this component has to be transferred use only plastic, possibly sterile disposable containers.

Chromogen/Substrate:
Ready to use. Mix well on vortex before use.
Be careful not to contaminate the liquid with oxidizing chemicals, air-driven dust or microbes.
Do not expose to strong illumination, oxidizing agents and metallic surfaces.
If this component has to be transferred use only plastic, possible sterile disposable container

Sample Diluent
Ready to use component. Mix carefully on vortex before use.

Sulphuric Acid:
Ready to use. Mix well on vortex before use.
Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+ P351+P338, P337+P313, P362+P363).

Légende :

Mention de danger, **Phrases H**
H315 – Provoque une irritation cutanée
H319 – Provoque une sévére irritation des yeux.

Conseil de prudence, **Phrases P**
P280 – Porter des gants de protection/des vêtements de protection/un équipement de protection des yeux/ du visage.
P302 + P352 – EN CAS DE CONTACT AVEC LA PEAU: laver abondamment à l'eau et au savon.
P332 + P313 – En cas d'irritation cutanée: consulter un médecin.
P305 + P351 + P338 – EN CAS DE CONTACT AVEC LES YEUX: rincer avec précaution à l'eau pendant plusieurs minutes. Enlever les lentilles de contact si la victime en porte et si elles peuvent être facilement enlevées. Continuer à rincer.
P337 + P313 – Si l'irritation oculaire persiste: consulter un médecin.
P362 + P363 – Enlever les vêtements contaminés et les laver avant reutilization.

I. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

1. Micropipettes have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (household alcohol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample. They should also be regularly maintained in order to show a precision of 1% and a trueness of +/-2%. Decontamination of spills or residues of kit components should also be carried out regularly.
2. The ELISA incubator has to be set at +37°C (tolerance of +/- 0.5°C) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water baths are suitable for the incubations, provided that the instrument is validated for the incubation of ELISA tests.
3. The ELISA washer is extremely important to the overall performances of the assay. The washer must be carefully validated and correctly optimised using the kit controls and reference panels, before using the kit for routine laboratory tests. Usually 4-5 washing cycles (aspiration + dispensation of 350ul/well of washing solution = 1 cycle) are sufficient to ensure that the assay performs as expected. A soaking time of 20-30 seconds between cycles is suggested. In order to set correctly their number, it is recommended to run an assay with the kit controls and well characterized negative and positive reference samples, and check to match the values reported below in the section “Internal Quality Control”. Regular calibration of the volumes delivered by, and maintenance (decontamination and cleaning of needles) of the washer has to be carried out according to the instructions of the manufacturer.
4. Incubation times have a tolerance of $\pm$5%.
5. The ELISA microplate reader has to be equipped with a reading filter of 450nm and with a second filter (620-630nm, strongly recommended) for blanking purposes. Its standard performances should be (a) bandwidth $\leq$ 10 nm; (b) absorbance range from 0 to $\geq$ 2.0; (c) linearity to $\geq$ 2.0; repeatability $\geq$ 1%. Blanking is carried out on the well identified in the section “Assay Procedure”. The optical system of the reader has to be calibrated regularly to ensure that the correct optical density is measured. It should be regularly maintained according to the manufacturer ‘s instructions.
6. When using an ELISA automated work station, all critical steps (dispensation, incubation, washing, reading, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the sections “Validation of Test” and “Assay Performances”. The assay protocol has to be installed in the operating system of the unit and validated as for the washer and the reader. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set. Particular attention must be paid to avoid carry over by the needles used for dispensing and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells. The use of ELISA automated work stations is recommended when the number of samples to be tested exceed 20-30 units per run.
7. Dia.Pro’s customer service offers support to the user in the setting and checking of instruments used in combination with the kit, in order to assure compliance with the requirements described. Support is also provided for the installation of new instruments to be used with the kit.

L. PRE ASSAY CONTROLS AND OPERATIONS

1. Check the expiration date of the kit printed on the external label (primary container). Do not use if expired.
2. Check that the liquid components are not contaminated by visible particles or aggregates.
3. Check that the Chromogen (TMB) is colourless or pale blue by aspirating a small volume of it with a sterile plastic pipette.
4. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box (primary container). Check that the aluminium pouch, containing the microplate, is not punctured or damaged.
5. Dissolve the content of the Control Serum as reported.
6. Dilute all the content of the 20x concentrated Wash Solution as described above.
7. Allow all the other components to reach room temperature (about 1 hr) and then mix gently on vortex all liquid reagents.
8. Set the ELISA incubator at +37°C and prepare the ELISA washer by priming with the diluted washing solution, according to the manufacturers instructions. Set the right number of washing cycles as found in the validation of the instrument for its use with the kit.
9. Check that the ELISA reader is turned on or ensure it will be turned on at least 20 minutes before reading.
10. If using an automated work station, turn on, check settings and be sure to use the right assay protocol.
11. Check that the micropipettes are set to the required volume.
12. Check that all the other equipment is available and ready to use.
13. In case of problems, do not proceed further with the test and advise the supervisor.

M. ASSAY PROCEDURE

The assay has to be carried out according to what reported below, taking care to maintain the same incubation time for all the samples in testing.
The kit may be used for quantitative and qualitative determinations as well.

M1. QUANTITATIVE DETERMINATION:

1. Dilute samples 1:101 into a properly defined dilution tube (example: 1000 µl Sample Diluent + 10 µl sample). Do not dilute the Calibration Set as calibrators are ready to use.

Mix carefully all the liquid components on vortex and then proceed as described below.
2. Place the required number of Microwells in the microwell holder. Leave the A1 and B1 empty for the operation of blanking.
3. Dispense 100 µl of Calibrators and 100 µl Control Serum in duplicate. Then dispense 100 µl of diluted samples in each properly identified well.
4. Incubate the microplate for **60 min at +37°C**.

Important note: *Strips have to be sealed with the adhesive sealing foil, supplied, only when the test is carried out manually. Do not cover strips when using ELISA automatic instruments.*

5. Wash the microplate with an automatic washer as reported previously (section I.3).
6. Pipette 100 µl Enzyme Conjugate into each well, except A1+B1 blanking wells, and cover with the sealer. Check that this red coloured component has been dispensed in all the wells, except A1 and B1.

Important note: *Be careful not to touch the plastic inner surface of the well with the tip filled with the Enzyme Conjugate. Contamination might occur.*

7. Incubate the microplate for **60 min at +37°C**.
8. Wash microwells as in step 5.
9. Pipette 100 µl Chromogen/Substrate mixture into each well, the blank wells A1 and B1 included. Then incubate the microplate at **room temperature (18-24°C) for 20 minutes**.

Important note: *Do not expose to strong direct illumination. High background might be generated.*

10. Pipette 100 µl Sulphuric Acid to stop the enzymatic reaction into all the wells using the same pipetting sequence as in step 9. Addition of acid will turn the positive calibrators, the control serum and the positive samples from blue to yellow.
11. Measure the colour intensity of the solution in each well, as described in section I.5, at 450nm filter (reading) and at 620-630nm (background subtraction, strongly recommended), blanking the instrument on A1 or B1 or both.

M2. QUALITATIVE DETERMINATION

If only a qualitative determination is required, proceed as described below:

1. Dilute samples 1:101 into a properly defined dilution tube (example: 1000 µl Sample Diluent + 10 µl sample). Do not dilute the Calibration Set as calibrators are ready to use. Mix carefully all the liquid components on vortex and then proceed as described below.
2. Place the required number of Microwells in the microwell holder. Leave A1 well empty for the operation of blanking.
3. Dispense 100 µl of Calibrator 0 arbU/ml and Calibrator 10 arbU/ml in duplicate and Calibrator 100 arbU/ml in single. Then dispense 100 µl of diluted samples in each properly identified well.
4. Incubate the microplate for **60 min at +37°C**.

Important note: *Strips have to be sealed with the adhesive sealing foil, supplied, only when the test is carried out manually. Do not cover strips when using ELISA automatic instruments.*

5. Wash the microplate with an automatic washer as reported previously (section I.3).
6. Pipette 100 µl Enzyme Conjugate into each well, except the A1 well, and cover with the sealer. Check that this red coloured component has been dispensed in all the wells, except A1.

Important note: *Be careful not to touch the plastic inner surface of the well with the tip filled with the Enzyme Conjugate. Contamination might occur.*

7. Incubate the microplate for **60 min at +37°C**.
8. Wash microwells as in step 5.
9. Pipette 100 µl Chromogen/Substrate mixture into each well, the blank well included. Then incubate the microplate at **room temperature (18-24°C) for 20 minutes**.

Important note: *Do not expose to strong direct illumination. High background might be generated.*

10. Pipette 100 µl Sulphuric Acid into all the wells using the same pipetting sequence as in step 9. Addition of acid will turn the positive calibrators, the control serum and the positive samples from yellow to blue.
11. Measure the colour intensity of the solution in each well, as described in section I.5, at 450nm filter (reading) and at 620-630nm (background subtraction, strongly recommended), blanking the instrument on A1.

General Important notes:

1. *If the second filter is not available ensure that no finger prints are present on the bottom of the microwell before reading at 450nm. Finger prints could generate false positive results on reading.*
2. *Reading has to be carried out just after the addition of the Stop Solution and anyway not any longer than 20 minutes after its addition. Some self oxidation of the chromogen can occur leading to high background.*

N. ASSAY SCHEME

Method	**Operations**
Calibrators & Control(*) Samples diluted 1:101	100 µl 100 µl
1st incubation	**60 min**
Temperature	+37°C
Wash step	4-5 cycles
Enzyme conjugate	100 µl
2nd incubation	**60 min**
Temperature	+37°C
Wash step	4-5 cycles
TMB/H2O2	100 µl
3rd incubation	**20 min**
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm

(*) Important Notes:

- *The Control Serum (CS) it does not affect the test's results calculation.*
- *The Control Serum (CS) used only if a laboratory internal quality control is required by the Management.*

An example of dispensation scheme for Quantitative Analysis is reported below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	CAL4	S 1									
B	BLK	CAL4	S 2									
C	CAL1	CAL5	S 3									
D	CAL1	CAL5	S 4									
E	CAL2	CAL6	S 5									
F	CAL2	CAL6	S 6									
G	CAL3	CS(*)	S 7									
H	CAL3	CS(*)	S 8									

Legenda: BLK = Blank CAL = Calibrator
S = Sample CS(*)= Control Serum - Not mandatory

An example of dispensation scheme in qualitative assays is reported below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S3	S11									
B	CAL1	S4	S12									
C	CAL1	S5	S13									
D	CAL3	S6	S14									
E	CAL3	S7	S15									
F	CAL6	S8	S16									
G	S1	S9	S17									
H	S2	S10	S18									

Legenda: BLK = Blank CAL = Calibrators
S = Sample

O. INTERNAL QUALITY CONTROL

A validation check is carried out on the calibrators any time the kit is used in order to verify whether the performances of the assay are as qualified.

Control that the following data are matched:

Check	Requirements
Blank well	< 0.100 OD450nm value
CAL 1 0 arbU/ml	< 0.150 mean OD450nm value after blanking coefficient of variation < 30%
CAL 2 5 arbU/ml	OD450nm > OD450nm CAL1 + 0.100
CAL 3 10 arbU/ml	OD450nm > OD450nm CAL1 + 0.200
CAL 6 100 arbU/ml	OD450nm > 1.000

If the results of the test match the requirements stated above, proceed to the next section.

If they do not, do not proceed any further and operate as follows:

Problem	Check
Blank well > 0.100 OD450nm	1. that the Chromogen/Sustrate solution has not got contaminated during the assay
CAL 1 **0 arbU/ml** > 0.150 OD450nm after blanking coefficient of variation > 30%	1. that the washing procedure and the washer settings are as validated in the pre qualification study; 2. that the proper washing solution has been used and the washer has been primed with it before use; 3. that no mistake has been done in the assay procedure (dispensation of a positive calibrator instead of the negative one; 4. that no contamination of the negative calibrator or of their wells has occurred due spills of positive samples or the enzyme conjugate; 5. that micropipettes haven't got contaminated with positive samples or with the enzyme conjugate 6. that the washer needles are not blocked or partially obstructed.
CAL 2 **5 arbU/ml** OD450nm < OD450nm CAL1 + 0.100	1. that the procedure has been correctly executed; 2. that no mistake has been done in its distribution (ex.: dispensation of a wrong calibrator instead); 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the calibrator has occurred.
CAL 3 **10 arbU/ml** OD450nm < OD450nm CAL1 + 0.200	1. that the procedure has been correctly executed; 2. that no mistake has been done in its distribution (ex.: dispensation of a wrong calibrator instead); 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the calibrator has occurred.
CAL 6 **100 arbU/ml** < 1.000 OD450nm	1. that the procedure has been correctly executed; 2. that no mistake has been done in its distribution (dispensation of a wrong calibrator instead) ; 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the positive control has occurred.

Should one of these problems have happened, after checking, report to the supervisor for further actions.

**** Note:**

If Control Serum has used, verify the following data:

Check	Requirements
Control Serum	Mean OD450nm CAL4 +/-20%

If the results of the test doesn't match the requirements stated above, operate as follows:

Problem	Check
Control Serum Different from Expected value	1. that the procedure has been correctly executed; 2. that no mistake has been done in its distribution (dispensation of a wrong calibrator instead); 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the control has occurred.

Anyway, if all other parameters (Blank, CAL1, CAL2, CAL 6), match the established requirements, the test may be considered valid.

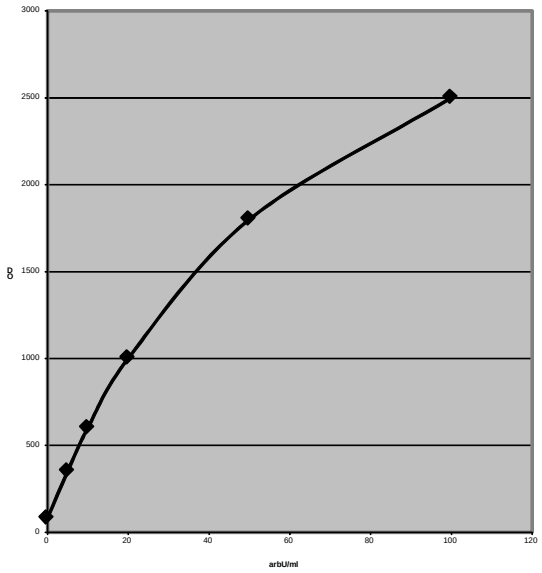
P. RESULTS

P.1 Quantitative method

If the test turns out to be valid, use for the quantitative method an approved curve fitting program to draw the calibration curve from the values obtained by reading at 450nm (4-parameters interpolation is suggested).

Then on the calibration curve calculate the concentration of anti EBNA IgG antibody in samples.

An example of Calibration curve is reported below.



Important Note:
Do not use the calibration curve above to make calculations.

P.2 Qualitative method
In the qualitative method, calculate the mean OD450nm values for the Calibrators 0 and 10 arbU/ml and then check that the assay is valid.

Example of calculation:

Note: *The following data must not be used instead or real figures obtained by the user.*

Calibrator 0 arbU/ml: 0.020 – 0.024 OD450nm
Mean Value: 0.022 OD450nm
Lower than 0.150 – Accepted

Calibrator 10 arbU/ml: 0.450 – 0.470 OD450nm
Mean Value: 0.460 OD450nm
Higher than Cal 0 + 0.200 – Accepted

Calibrator 100 arbU/ml: 2.045 OD450nm
Higher than 1.000 – Accepted

The OD450nm of the Calibrator 10 arbU/ml is considered the cut-off (or Co) of the system.
The ratio between the OD450nm value of the sample and the OD450nm of the Calibrator 10 arbU/ml (or S/Co) can provide a semi-quantitative estimation of the content of specific IgG in the sample.

Q. INTERPRETATION OF RESULTS
Samples with a concentration lower than 5 arbU/ml are considered negative for anti EBNA IgG antibody.
Samples with a concentration ranging 5-10 arbU/ml are considered in the gray-zone. Samples with a concentration higher than 10 arbU/ml are considered positive for anti EBNA IgG antibody.
EBNA IgG results alone are not, anyway, enough to provide a clear diagnosis of EBV infection. At least EBV VCA IgM results are necessary in combination.
A reference range of the minimum essential serological markers of Epstein-Barr infection, derived from Infectious Diseases Handbook, 3rd edition, published by Lexi-Comp Inc., USA, is reported schematically below:

VCA IgM	EBNA IgG	Interpretation
negative	negative	No history of EBV infection
positive	negative	Acute primary infection
negative	**positive**	History of previous infection
positive	**positive**	Reactivation

Important notes:
1. *Interpretation of results should be done under the supervision of the laboratory supervisor to reduce the risk of judgment errors and misinterpretations.*
2. *When test results are transmitted from the laboratory to another facility, attention must be paid to avoid erroneous data transfer.*
3. *Diagnosis has to be done and released to the patient by a suitably qualified medical doctor.*

R. PERFORMANCE CHARACTERISTICS
Evaluation of Performances has been conducted in an external clinical center on negative and positive samples with reference to a FDA approved commercial kit.

1. Limit of detection
No international standard for EBNA IgG Antibody detection has been defined so far by the European Community.
In its absence, an Internal Gold Standard (or IGS), derived from a patient with an history of past mononucleosis infection, has been defined in order to provide the device with a constant and excellent sensitivity.

2. Diagnostic Sensitivity and Specificity:
The method is based on the use of an affinity purified native EBNA antigen to provide the assay with the highest specifity to EBV.
The diagnostic performances were evaluated in a performance evaluation study conducted in an external centre, with excellent experience in the diagnosis of infectious diseases and in particular in EBV infection.
The Diagnostic Sensitivity was studied on more than 50 samples, pre-tested positive with two reference kits of European origin in use at the laboratory. Positive samples were collected from patients that experienced mononucleosis infection.
The diagnostic specificity was determined on panels of more than 50 negative samples from normal individuals and blood donors, classified negative with the reference kit, including potentially interfering specimens.
Both plasma, derived with different standard techniques of preparation (citrate, EDTA and heparin), and sera have been used to determine the specificity.
No false reactivity due to the method of specimen preparation has been observed.
Frozen specimens have also been tested to check whether samples freezing interferes with the performance of the test. No interference was observed on clean and particle free samples.
The Performance Evaluation provided the following values :

Sensitivity	≥ 98 %
Specificity	≥ 98 %

3. Reproducibility:
Data obtained from a study conducted on three samples of different EBNA IgG reactivity, examined in 16 replicates in three separate runs show CV% values ranging 5-20% depending on OD450nm readings.
The variability shown in the tables did not result in sample misclassification.

S. LIMITATIONS
Frozen samples containing fibrin particles or aggregates may generate false positive results.
Depending on the reference kit in use, due to some heterogeneity among different devices, the presence of 2-5% false reactivity may be seen.

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All the IVD Products manufactured by the company are under the control of a certified Quality Management System in compliance with ISO 13485 rule. Each lot is submitted to a quality control and released into the market only if conforming with the EC technical specifications and acceptance criteria.

Produced by
Dia.Pro Diagnostic Bioprobes Srl
Via G. Carducci n° 27 – Sesto San Giovanni (MI) – Italy

CE

Dia.Pro
Diagnostic
BioProbes

EC DECLARATION OF CONFORMITY

MANUFACTURER	DIA.PRO DIAGNOSTIC BIOPROBES S.R.L. VIA G. CARDUCCI N° 27 – 20099 SESTO SAN GIOVANNI (MILANO) – ITALY
PRODUCT	**EBNA IgG** CODE: **EBNG.CE** (96 tests)
CLASSIFICATION	GENERAL IVD
CONFORMITY ASSESSMENT ROUTE	SELF CERTIFICATION

WE HEREBY DECLARE THAT THE ABOVE MENTIONED PRODUCT MEETS THE PROVISIONS OF THE COUNCIL DIRECTIVE 98/79/EC FOR IN VITRO DIAGNOSTIC DEVICES.

ISO CERTIFICATE	UNE EN ISO 13485 N° 2013 11 0039 EN, RELEASED BY AEMPS (AGENCIA ESPAÑOLA DE MEDICAMENTOS Y PRODUCTOS SANITARIOS)

PLACE & DATE OF FIRST ISSUE	MILANO – SEPTEMBER 2004
PLACE & DATE OF CURRENT ISSUE	SESTO SAN GIOVANNI (MI) – MARCH 2019
SIGNATURE **Legal Representative** **Dr.ssa Fiorenza Scozzesi**	

Rev:	05/2018

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EC DECLARATION OF CONFORMITY

MANUFACTURER	DIA.PRO DIAGNOSTIC BIOPROBES S.R.L. VIA G. CARDUCCI N° 27 – 20099 SESTO SAN GIOVANNI (MILANO) – ITALY
PRODUCT	**VCA IgG** CODE: **VCAG.CE** (96 tests)
CLASSIFICATION	GENERAL IVD
CONFORMITY ASSESSMENT ROUTE	SELF CERTIFICATION

WE HEREBY DECLARE THAT THE ABOVE MENTIONED PRODUCT MEETS THE PROVISIONS OF THE COUNCIL DIRECTIVE 98/79/EC FOR IN VITRO DIAGNOSTIC DEVICES.

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Rev:	05/2018

VCA IgG

Enzyme ImmunoAssay (ELISA) for the quantitative/qualitative determination of IgG antibodies to Epstein Barr Virus Capsidic Antigen in human serum and plasma

- for "in vitro" diagnostic use only -

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REF VCAG.CE
96 Tests

VCA IgG

A. INTENDED USE
Enzyme ImmunoAssay (ELISA) for the quantitative/qualitative determination of IgG antibodies to Epstein Barr Virus Capsidic Antigen in human plasma and sera.
For "in vitro" diagnostic use only.

B. INTRODUCTION
Epstein Barr Virus or EBV is the principal etiological agent of infectious mononucleosis, as well as a contributory factor in the etiology of Burkitt's lymphoma and nasopharyngeal carcinoma, or NPC. A member of the family Herpesviridae, it has a worldwide distribution, such that 80 to 90% of all adults have been infected. Primary infections usually occur during the first decade of life. While childhood infections are mostly asymptomatic, 50 to 70% of young adults undergoing primary EBV infections show mild to severe illness. EBV may cause a persistent, latent infection which can be reactivated under immunosoppression or in AIDS affected patients. As humoral responses to primary EBV infections are quite rapid, the level and class of antibodies raised in most cases allow classification as to whether the patient is still susceptible, has a current or recent primary infection, had a past infection or may be having reactivated EBV infection. The detection of EBV-specific IgG, IgM and IgA antibodies to its major immunodominant antigens (mainly Nuclear Antigen or EBNA and Viral Capsidic Antigen or VCA) has become therefore an important and useful determination for the monitoring and follow-up of EBV infected patients.

C. PRINCIPLE OF THE TEST
In order to get rid of crossreactions with other viruses of the same family, microplates are coated with affinity purified native VCA antigen, to provide the assay with the highest specificity and sensitivity.
In the 1st incubation, the solid phase is treated with diluted samples and anti-VCA IgG are captured, if present, by the antigens.
After washing out all the other components of the sample, in the 2nd incubation bound anti-VCA IgG are detected by the addition of anti hIgG antibody, labeled with peroxidase (HRP). The enzyme captured on the solid phase, acting on the substrate/chromogen mixture, generates an optical signal that is proportional to the amount of anti-VCA IgG antibodies present in the sample.
IgG in the sample may therefore be quantitated by means of a standard curve calibrated in arbitrary units per milliliter (arbU/ml) as no international standard is available.

D. COMPONENTS
Each kit contains sufficient reagents to perform 96 tests.

1. Microplate: MICROPLATE
12 strips x 8 microwells coated with affinity purified native VCA antigen. Plates are sealed into a bag with desiccant.
Allow the microplate to reach room temperature before opening; reseal unused strips in the bag with desiccant and store at 4°C.

2. Calibration Curve: CAL N° …
Ready to use and color coded standard curve ranging:
4 ml CAL1 = 0 arbU/ml
4 ml CAL2 = 5 arbU/ml
2 ml CAL3 =10 arbU/ml
2 ml CAL4 = 20 arbU/ml
2 ml CAL 5 = 50 arbU/ml
4 ml CAL6 = 100 arbU/ml.
Standards are calibrated against an internal Gold Standard or IGS as no international one is defined.
Contains human serum proteins, 2% casein, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.1% Tween 20, 0.09% Na-azide and 0.1% Kathon GC as preservatives. Standards are blue colored.

3. Control Serum: CONTROL …ml
1 vial. Lyophilized. It contains bovine serum proteins, human IgG antibodies to VCA at 20 arbU/ml±20%, 0.2 mg/ml gentamicine sulphate and 0.1% Kathon GC as preservatives.

3. Wash buffer concentrate: WASHBUF 20X
1x60ml/bottle20x concentrated solution. Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2, 0.05% Tween 20 and 0.1% Kathon GC.

4. Enzyme conjugate : CONJ
1x16ml/vial. Ready to use and red colour coded. It contains Horseradish peroxidase conjugated polyclonal antibodies to human IgG, 5% BSA, 10 mM Tris buffer pH 6.8+/-0.1, 0.1% Kathon GC and 0.02% gentamicine sulphate as preservatives.

5. Chromogen/Substrate: SUBS TMB
1x16ml/vial. It contains 50 mM citrate-phosphate buffer pH 3.5-3.8, 4% dimethylsulphoxide, 0.03% tetra-methyl-benzidine (or TMB) and 0.02% hydrogen peroxide (or H_2O_2).
Note: To be stored protected from light as sensitive to strong illumination.

6. Sulphuric Acid: H2SO4 0.3 M
1x15ml/vialIt contains 0.3 M H_2SO_4 solution.
Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

7. Specimen Diluent: DILSPE
2x60ml/vial. It contains 2% casein, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.1% Tween 20, 0.09% Na-azide and 0.1% Kathon GC as preservatives. To be used to dilute the sample.

8. Plate sealing foils n°2

9. Package insert n°1

E. MATERIALS REQUIRED BUT NOT PROVIDED
1. Calibrated Micropipettes (1000, 100 and 10ul) and disposable plastic tips.
2. EIA grade water (bidistilled or deionised, charcoal treated to remove oxidizing chemicals used as disinfectants).
3. Timer with 60 minute range or higher.
4. Absorbent paper tissues.
5. Calibrated ELISA microplate thermostatic incubator (dry or wet) set at +37°C (+/-0.5°C tolerance).
6. Calibrated ELISA microwell reader with 450nm (reading) and with 620-630nm (blanking) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.

F. WARNINGS AND PRECAUTIONS
1. The kit has to be used by skilled and properly trained technical personnel only, under the supervision of a medical doctor responsible of the laboratory.
2. All the personnel involved in performing the assay have to wear protective laboratory clothes, talc-free gloves and glasses. The use of any sharp (needles) or cutting (blades) devices should be avoided. All the personnel involved should be trained in biosafety procedures, as recommended by the Center for Disease Control, Atlanta, U.S. and reported in the National Institute of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.

3. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.
4. The laboratory environment should be controlled so as to avoid contaminants such as dust or air-born microbial agents, when opening kit vials and microplates and when performing the test. Protect the Chromogen (TMB) from strong light and avoid vibration of the bench surface where the test is undertaken.
5. Upon receipt, store the kit at 2..8°C into a temperature controlled refrigerator or cold room.
6. Do not interchange components between different lots of the kits. It is recommended that components between two kits of the same lot should not be interchanged.
7. Check that the reagents are clear and do not contain visible heavy particles or aggregates. If not, advise the laboratory supervisor to initiate the necessary procedures for kit replacement.
8. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each sample.
9. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one.
10. Do not use the kit after the expiration date stated on the external container and internal (vials) labels. A study conducted on an opened kit did not pointed out any relevant loss of activity up to six 6 uses of the device and up to 6 months.
11. Treat all specimens as potentially infective. All human serum specimens should be handled at Biosafety Level 2, as recommended by the Center for Disease Control, Atlanta, U.S. in compliance with what reported in the Institutes of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
12. The use of disposable plastic-ware is recommended in the preparation of the liquid components or in transferring components into automated workstations, in order to avoid cross contamination.
13. Waste produced during the use of the kit has to be discarded in compliance with national directives and laws concerning laboratory waste of chemical and biological substances. In particular, liquid waste generated from the washing procedure, from residuals of controls and from samples has to be treated as potentially infective material and inactivated before waste. Suggested procedures of inactivation are treatment with a 10% final concentration of household bleach for 16-18 hrs or heat inactivation by autoclave at 121°C for 20 min..
14. Accidental spills from samples and operations have to be adsorbed with paper tissues soaked with household bleach and then with water. Tissues should then be discarded in proper containers designated for laboratory/hospital waste.
15. The Sulphuric Acid is an irritant. In case of spills, wash the surface with plenty of water
16. Other waste materials generated from the use of the kit (example: tips used for samples and controls, used microplates) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

G. SPECIMEN: PREPARATION AND WARNINGS

1. Blood is drawn aseptically by venipuncture and plasma or serum is prepared using standard techniques of preparation of samples for clinical laboratory analysis. No influence has been observed in the preparation of the sample with citrate, EDTA and heparin.
2. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results. Bar code labeling and electronic reading is strongly recommended.
3. Haemolysed ("red") and visibly hyperlipemic ("milky") samples have to be discarded as they could generate false results. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they could give rise to false results.
4. Sera and plasma can be stored at +2°..8°C for up to five days after collection. For longer storage periods, samples can be stored frozen at –20°C for several months. Any frozen samples should not be freezed/thawed more than once as this may generate particles that could affect the test result.
5. If particles are present, centrifuge at 2.000 rpm for 20 min or filter using 0.2-0.8u filters to clean up the sample for testing.
6. Samples whose anti-VCA IgG antibody concentration is expected to be higher than 100 arbU/ml should be diluted before use, either 1:10 or 1:100 in the Calibrator 0 arbU/ml. Dilutions have to be done in clean disposable tubes by diluting 50 ul of each specimen with 450 ul of Cal 0 (1:10). Then 50 ul of the 1:10 dilution are diluted with 450 ul of the Cal 0 (1:100). Mix tubes thoroughly on vortex and then proceed toward the dilution step reported in section M.

H. PREPARATION OF COMPONENTS AND WARNINGS

Microplate:
Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant is not turned to dark green, indicating a defect of storing.
In this case call Dia.Pro's customer service.
Unused strips have to be placed back inside the aluminum pouch, with the desiccant supplied, firmly zipped and stored at +2°..8°C.
***Important Note**: After first opening, remaining strips are stable until the humidity indicator inside the desiccant bag turns from yellow to green.*

Calibration Curve
Ready to use component. Mix carefully on vortex before use.

Control Serum
Add the volume of ELISA grade water, reported on the label, to the lyophilised powder; let fully dissolve and then gently mix on vortex.
***Note:** The control after dissolution is not stable. Store frozen in aliquots at –20°C.*

Wash buffer concentrate:
The whole content of the concentrated solution has to be diluted 20x with bidistilled water and mixed gently end-over-end before use. During preparation avoid foaming as the presence of bubbles could impact on the efficiency of the washing cycles.
***Note:** Once diluted, the wash solution is stable for 1 week at +2..8° C.*

Enzyme conjugate:
Ready to use. Mix well on vortex before use.
Be careful not to contaminate the liquid with oxidizing chemicals, air-driven dust or microbes.
If this component has to be transferred use only plastic, possibly sterile disposable containers.

Chromogen/Substrate:
Ready to use. Mix well on vortex before use.
Be careful not to contaminate the liquid with oxidizing chemicals, air-driven dust or microbes.
Do not expose to strong illumination, oxidizing agents and metallic surfaces.
If this component has to be transferred use only plastic, possible sterile disposable container

Sample Diluent
Ready to use component. Mix carefully on vortex before use.

Sulphuric Acid:
Ready to use. Mix well on vortex before use.
Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

Legenda:

Warning **H statements:**
H315 – Causes skin irritation.
H319 – Causes serious eye irritation.

Precautionary **P statements:**
P280 – Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352 – IF ON SKIN: Wash with plenty of soap and water.
P332 + P313 – If skin irritation occurs: Get medical advice/attention.
P305 + P351 + P338 – IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337 + P313 – If eye irritation persists: Get medical advice/attention.
P362 + P363 – Take off contaminated clothing and wash it before reuse.

I. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

1. Micropipettes have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (household alcohol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample. They should also be regularly maintained in order to show a precision of 1% and a trueness of +/-2%. Decontamination of spills or residues of kit components should also be carried out regularly.
2. The ELISA incubator has to be set at +37°C (tolerance of +/- 0.5°C) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water baths are suitable for the incubations, provided that the instrument is validated for the incubation of ELISA tests.
3. The ELISA washer is extremely important to the overall performances of the assay. The washer must be carefully validated and correctly optimised using the kit controls and reference panels, before using the kit for routine laboratory tests. Usually 4-5 washing cycles (aspiration + dispensation of 350ul/well of washing solution = 1 cycle) are sufficient to ensure that the assay performs as expected. A soaking time of 20-30 seconds between cycles is suggested. In order to set correctly their number, it is recommended to run an assay with the kit controls and well characterized negative and positive reference samples, and check to match the values reported below in the section “Internal Quality Control”. Regular calibration of the volumes delivered by, and maintenance (decontamination and cleaning of needles) of the washer has to be carried out according to the instructions of the manufacturer.
4. Incubation times have a tolerance of $\pm$5%.
5. The ELISA microplate reader has to be equipped with a reading filter of 450nm and with a second filter (620-630nm, strongly recommended) for blanking purposes. Its standard performances should be (a) bandwidth $\leq$ 10 nm; (b) absorbance range from 0 to $\geq$ 2.0; (c) linearity to $\geq$ 2.0; repeatability $\geq$ 1%. Blanking is carried out on the well identified in the section “Assay Procedure”. The optical system of the reader has to be calibrated regularly to ensure that the correct optical density is measured. It should be regularly maintained according to the manufacturer ‘s instructions.
6. When using an ELISA automated work station, all critical steps (dispensation, incubation, washing, reading, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the section “Internal Quality Control”. The assay protocol has to be installed in the operating system of the unit and validated as for the washer and the reader. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set. Particular attention must be paid to avoid carry over by the needles used for dispensing and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells. The use of ELISA automated work stations is recommended when the number of samples to be tested exceed 20-30 units per run.
7. Dia.Pro’s customer service offers support to the user in the setting and checking of instruments used in combination with the kit, in order to assure compliance with the requirements described. Support is also provided for the installation of new instruments to be used with the kit.

L. PRE ASSAY CONTROLS AND OPERATIONS

1. Check the expiration date of the kit printed on the external label (primary container). Do not use if expired.
2. Check that the liquid components are not contaminated by visible particles or aggregates.
3. Check that the Chromogen (TMB) is colourless or pale blue by aspirating a small volume of it with a sterile plastic pipette.
4. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box (primary container). Check that the aluminium pouch, containing the microplate, is not punctured or damaged.
5. Dissolve the content of the Control Serum as reported.
6. Dilute all the content of the 20x concentrated Wash Solution as described above.
7. Allow all the other components to reach room temperature (about 1 hr) and then mix gently on vortex all liquid reagents.
8. Set the ELISA incubator at +37°C and prepare the ELISA washer by priming with the diluted washing solution, according to the manufacturers instructions. Set the right number of washing cycles as found in the validation of the instrument for its use with the kit.
9. Check that the ELISA reader is turned on or ensure it will be turned on at least 20 minutes before reading.
10. If using an automated work station, turn on, check settings and be sure to use the right assay protocol.
11. Check that the micropipettes are set to the required volume.
12. Check that all the other equipment is available and ready to use.
13. In case of problems, do not proceed further with the test and advise the supervisor.

M. ASSAY PROCEDURE

The assay has to be carried out according to what reported below, taking care to maintain the same incubation time for all the samples in testing.
The kit may be used for quantitative and qualitative determinations as well.

M1. QUANTITATIVE DETERMINATION:

1. Dilute samples 1:101 into a properly defined dilution tube (example: 1000 µl Sample Diluent + 10 µl sample). Do not dilute the Calibration Set as calibrators are ready to use. Mix carefully all the liquid components on vortex and then proceed as described below.
2. Place the required number of Microwells in the microwell holder. Leave the A1 and B1 empty for the operation of blanking.
3. Dispense 100 µl of Calibrators and 100 µl Control Serum in duplicate. Then dispense 100 µl of diluted samples in each properly identified well.
4. Incubate the microplate for **60 min at +37°C**.

Important note: *Strips have to be sealed with the adhesive sealing foil, supplied, only when the test is carried out manually. Do not cover strips when using ELISA automatic instruments.*

5. Wash the microplate with an automatic washer as reported previously (section I.3).
6. Pipette 100 µl Enzyme Conjugate into each well, except A1+B1 blanking wells, and cover with the sealer. Check that this red coloured component has been dispensed in all the wells, except A1 and B1.

Important note: *Be careful not to touch the plastic inner surface of the well with the tip filled with the Enzyme Conjugate. Contamination might occur.*

7. Incubate the microplate for **60 min at +37°C**.
8. Wash microwells as in step 5.
9. Pipette 100 µl Chromogen/Substrate mixture into each well, the blank wells A1 and B1 included. Then incubate the microplate at **room temperature (18-24°C) for 20 minutes**.

Important note: *Do not expose to strong direct illumination. High background might be generated.*

10. Pipette 100 µl Sulphuric Acid to stop the enzymatic reaction into all the wells using the same pipetting sequence as in step 9. Addition of acid will turn the positive calibrators, the control serum and the positive samples from blue to yellow.
11. Measure the colour intensity of the solution in each well, as described in section I.5, at 450nm filter (reading) and at 620-630nm (background subtraction, strongly recommended), blanking the instrument on A1 or B1 or both.

M2. QUALITATIVE DETERMINATION

If only a qualitative determination is required, proceed as described below:

1. Dilute samples 1:101 into a properly defined dilution tube (example: 1000 µl Sample Diluent + 10 µl sample). Do not dilute the Calibration Set as calibrators are ready to use. Mix carefully all the liquid components on vortex and then proceed as described below.
2. Place the required number of Microwells in the microwell holder. Leave A1 well empty for the operation of blanking.
3. Dispense 100 µl of Calibrator 0 arbU/ml and Calibrator 5 arbU/ml in duplicate and Calibrator 100 arbU/ml in single. Then dispense 100 µl of diluted samples in each properly identified well.
4. Incubate the microplate for **60 min at +37°C**.

Important note: *Strips have to be sealed with the adhesive sealing foil, supplied, only when the test is carried out manually. Do not cover strips when using ELISA automatic instruments.*

5. Wash the microplate with an automatic washer as reported previously (section I.3).
6. Pipette 100 µl Enzyme Conjugate into each well, except the A1 well, and cover with the sealer. Check that this red coloured component has been dispensed in all the wells, except A1.

Important note: *Be careful not to touch the plastic inner surface of the well with the tip filled with the Enzyme Conjugate. Contamination might occur.*

7. Incubate the microplate for **60 min at +37°C**.
8. Wash microwells as in step 5.
9. Pipette 100 µl Chromogen/Substrate mixture into each well, the blank well included. Then incubate the microplate at **room temperature (18-24°C) for 20 minutes**.

Important note: *Do not expose to strong direct illumination. High background might be generated.*

10. Pipette 100 µl Sulphuric Acid into all the wells using the same pipetting sequence as in step 9. Addition of acid will turn the positive calibrators, the control serum and the positive samples from yellow to blue.
11. Measure the colour intensity of the solution in each well, as described in section I.5, at 450nm filter (reading) and at 620-630nm (background subtraction, strongly recommended), blanking the instrument on A1.

General Important notes:

1. *If the second filter is not available ensure that no finger prints are present on the bottom of the microwell before reading at 450nm. Finger prints could generate false positive results on reading.*
2. *Reading has to be carried out just after the addition of the Stop Solution and anyway not any longer than 20 minutes after its addition. Some self oxidation of the chromogen can occur leading to high background.*

N. ASSAY SCHEME

Method	**Operations**
Calibrators	100 µl
Control Serum (*)	100 µl
Samples diluted 1:101	100 µl
1st incubation	**60 min**
Temperature	+37°C
Wash step	4-5 cycles
Enzyme conjugate	100 µl
2nd incubation	**60 min**
Temperature	+37°C
Wash step	4-5 cycles
TMB/H2O2	100 µl
3rd incubation	**20 min**
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm

(*) Important Notes:

- *The Control Serum (CS) it does not affect the test's results calculation.*
- *The Control Serum (CS) used only if a laboratory internal quality control is required by the Management.*

An example of dispensation scheme for Quantitative Analysis is reported below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	CAL4	S1									
B	BLK	CAL4	S2									
C	CAL1	CAL5	S3									
D	CAL1	CAL5	S4									
E	CAL2	CAL6	S5									
F	CAL2	CAL6	S6									
G	CAL3	CS**(*)**	S7									
H	CAL3	CS**(*)**	S8									

Legenda: BLK = Blank CAL = Calibrator S = Sample
CS(*)= Control Serum - Not mandatory

An example of dispensation scheme in qualitative assays is reported below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S 3	S 11									
B	CAL1	S 4	S 12									
C	CAL1	S 5	S 13									
D	CAL2	S 6	S 14									
E	CAL2	S 7	S 15									
F	CAL6	S 8	S 16									
G	S1	S 9	S 17									
H	S2	S 10	S 18									

Legenda: BLK = Blank CAL = Calibrators
S = Sample

O. INTERNAL QUALITY CONTROL

A validation check is carried out on the controls any time the kit is used in order to verify whether the performances of the assay are as qualified.
Control that the following data are matched:

Check	Requirements
Blank well	< 0.100 OD450nm value
CAL 1 0 arbU/ml	< 0.150 mean OD450nm value after blanking coefficient of variation < 30%
CAL 2 5 arbU/ml	OD450nm > OD450nm CAL1 + 0.100
CAL 6 100 arbU/ml	OD450nm > 1.000

If the results of the test match the requirements stated above, proceed to the next section. If they do not, do not proceed any further and operate as follows:

Problem	Check
Blank well > 0.100 OD450nm	1. that the Chromogen/Sustrate solution has not got contaminated during the assay
CAL 1 **0 arbU/ml** > 0.150 OD450nm after blanking coefficient of variation > 30%	1. that the washing procedure and the washer settings are as validated in the pre qualification study; 2. that the proper washing solution has been used and the washer has been primed with it before use; 3. that no mistake has been done in the assay procedure (dispensation of a positive calibrator instead of the negative one; 4. that no contamination of the negative calibrator or of their wells has occurred due spills of positive samples or the enzyme conjugate; 5. that micropipettes haven't got contaminated with positive samples or with the enzyme conjugate 6. that the washer needles are not blocked or partially obstructed.
CAL 2 **5 arbU/ml** OD450nm < OD450nm CAL1 + 0.100	1. that the procedure has been correctly executed; 2. that no mistake has been done in its distribution (ex.: dispensation of a wrong calibrator instead); 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the calibrator has occurred.
CAL 6 **100 arbU/ml** < 1.000 OD450nm	1. that the procedure has been correctly executed; 2. that no mistake has been done in its distribution (dispensation of a wrong calibrator instead) ; 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the positive control has occurred.

Should one of these problems have happened, after checking, report to the supervisor for further actions.

**** Note:**

If Control Serum has used, verify the following data:

Check	Requirements
Control Serum	Mean OD450nm CAL4 ±20%

If the results of the test doesn't match the requirements stated above, operate as follows:

Problem	Check
Control Serum Different from Expected value	1. that the procedure has been correctly executed; 2. that no mistake has been done in its distribution (dispensation of a wrong calibrator instead); 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the control serum has occurred.

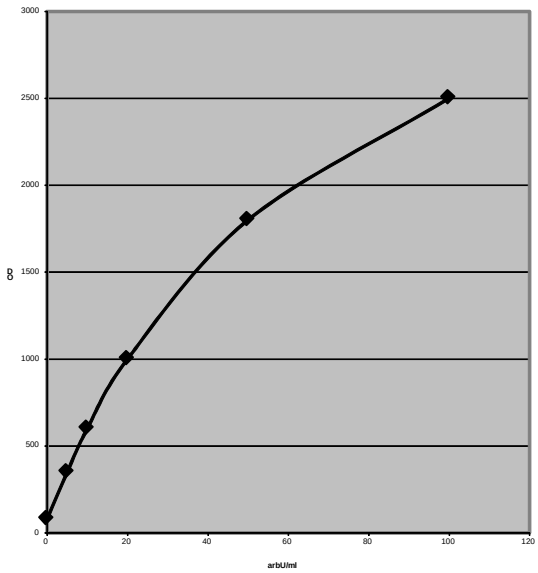
Anyway, if all other parameters (Blank, CAL1, CAL2, CAL 6), match the established requirements, the test may be considered valid.

P. RESULTS

P.1 Quantitative method

If the test turns out to be valid, use for the quantitative method an approved curve fitting program to draw the calibration curve from the values obtained by reading at 450nm (4-parameters interpolation is suggested).
Then on the calibration curve calculate the concentration of anti VCA IgG antibody in samples.

An example of Calibration curve is reported below.



Important Note:
Do not use the calibration curve above to make calculations.

P.2 Qualitative method

In the qualitative method, calculate the mean OD450nm values for the Calibrators 0 and 5 arbU/ml and then check that the assay is valid.

Example of calculation:

Note: *The following data must not be used instead or real figures obtained by the user.*

Calibrator 0 arbU/ml: 0.020 – 0.024 OD450nm
Mean Value: 0.022 OD450nm
Lower than 0.150 – Accepted

Calibrator 5 arbU/ml: 0.250 – 0.270 OD450nm
Mean Value: 0.260 OD450nm
Higher than Cal 0 + 0.100 – Accepted

Calibrator 100 arbU/ml: 2.045 OD450nm
Higher than 1.000 – Accepted

The OD450nm of the Calibrator 5 arbU/ml is considered the cut-off (or Co) of the system.
The ratio between the OD450nm value of the sample and the OD450nm of the Calibrator 5 arbU/ml (or S/Co) can provide a semi-quantitative estimation of the content of specific IgG in the sample.

Q. INTERPRETATION OF RESULTS

Samples with a concentration lower than 5 arbU/ml are considered negative for anti-VCA IgG antibody.
Samples with a concentration higher than 5 arbU/ml are considered positive for anti-VCA IgG antibody.
VCA IgG results alone are not, anyway, enough to provide a clear diagnosis of EBV infection. At least EBV VCA IgM results, possibly together with EBNA IgG, are necessary in combination.
A reference range of the minimum essential serological markers of Epstein-Barr infection, derived from Infectious Diseases Handbook, 3rd edition, published by Lexi-Comp Inc., USA, is reported schematically below:

VCA IgM	EBNA (or VCA) IgG	Interpretation
negative	negative	No history of EBV infection
positive	negative	Acute primary infection
negative	**positive**	History of previous infection
positive	**positive**	Reactivation

Important notes:

1. *Interpretation of results should be done under the supervision of the laboratory supervisor to reduce the risk of judgment errors and misinterpretations.*
2. *When test results are transmitted from the laboratory to another facility, attention must be paid to avoid erroneous data transfer.*
3. *Diagnosis has to be done and released to the patient by a suitably qualified medical doctor.*

R. PERFORMANCE CHARACTERISTICS

Evaluation of Performances has been conducted in an external clinical center on negative and positive samples with reference to a FDA approved commercial kit.

1. Limit of detection

No international standard for VCA IgG Antibody detection has been defined so far by the European Community.
In its absence, an Internal Gold Standard (or IGS), derived from a patient with an history of past mononucleosis infection, has been defined in order to provide the device with a constant and excellent sensitivity.

2. Diagnostic Sensitivity and Specificity:

Microplates are coated with with affinity purified native VCA antigen capable to provide the assay with the highest specificity and sensitivity.
The diagnostic performances were evaluated in a performance evaluation study conducted in an external centre, with excellent experience in the diagnosis of infectious diseases.
The diagnostic sensitivity was studied on more than 50 samples, pre-tested positive with a different reference kit of European origin in use at the laboratory. Positive samples were collected from patients that experienced mononucleosis infection.
The diagnostic specificity was determined on panels of more than 50 negative samples from normal individuals and blood donors, classified negative with the reference kit, including potentially interfering specimens.
Both plasma, derived with different standard techniques of preparation (citrate, EDTA and heparin), and sera have been used to determine the specificity. No false reactivity due to the method of specimen preparation has been observed.
Frozen specimens have also been tested to check whether samples freezing interferes with the performance of the test. No interference was observed on clean and particle free samples.
The Performance Evaluation provided the following values :

Sensitivity	≥ 98 %
Specificity	≥ 98 %

3. Reproducibility:

Data obtained from a study conducted on three samples of different VCA IgG reactivity, examined in 16 replicates in three separate runs show CV% values ranging 3-16% depending on OD450nm readings.
The variability shown in the tables did not result in sample misclassification.

S. LIMITATIONS

False positivity has been assessed as less than 2-5% of the normal population depending on the reference kit used.
Frozen samples containing fibrin particles or aggregates may generate false positive results.

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All the IVD Products manufactured by the company are under the control of a certified Quality Management System in compliance with ISO 13485 rule. Each lot is submitted to a quality control and released into the market only if conforming with the EC technical specifications and acceptance criteria.

Produced by
Dia.Pro Diagnostic Bioprobes Srl
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CE

VCA IgM

"Capture" Enzyme ImmunoAssay (ELISA) for the quantitative/qualitative determination of IgM class antibodies to Epstein Barr Virus Capsidic Antigen in human plasma and sera

- for "in vitro" diagnostic use only -

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REF VCAM.CE
96 Tests

VCA IgM

A. INTENDED USE

Enzyme ImmunoAssay (ELISA) for the quantitative or qualitative determination of IgM class antibodies to Epstein Barr Virus (EBV) Capsidic Antigen in human plasma and sera with the "capture" system.
The kit is intended for the classification of the viral infective agent and the follow-up of EBV infected patients.
For “in vitro” diagnostic use only.

B. INTRODUCTION

Epstein Barr Virus or EBV is the principal etiological agent of infectious mononucleosis, as well as a contributory factor in the etiology of Burkitt’s lymphoma and nasopharyngeal carcinoma, or NPC.
A member of the family Herpesviridae, it has a worldwide distribution, such that 80 to 90% of all adults have been infected. Primary infections usually occur during the first decade of life. While childhood infections are mostly asymptomatic, 50 to 70% of young adults undergoing primary EBV infections show mild to severe illness.
EBV may cause a persistent, latent infection which can be reactivated under immunosoppression or in AIDS affected patients. As humoral responses to primary EBV infections are quite rapid, the level and class of antibodies raised in most cases allow classification as to whether the patient is still susceptible, has a current or recent primary infection, had a past infection or may be having reactivated EBV infection.
The detection of EBV-specific IgG, IgM and IgA antibodies to its major immunodominant antigens has become therefore an important and useful determination for the monitoring and follow-up of EBV infected patients.

C. PRINCIPLE OF THE TEST

The assay is based on the “IgM Capture” method and on affinity purified native VCA antigen.
Microplates are coated with a polyclonal anti-hIgM antibody that in the 1st incubation “captures” specifically this class of antibodies.
After washing out all the other components of the sample, in the 2nd incubation bound anti EBV-VCA IgM are detected by the addition of a complex formed by biotinilated affinity purified native VCA antigen and Streptavidine, labelled with peroxidase (HRP).
The enzyme captured on the solid phase, acting on the substrate/chromogen mixture, generates an optical signal that is proportional to the amount of IgM antibodies present in the sample and can be detected by an ELISA reader.
Quantification of IgM is made possible by a standard curve calibrated in arbitrary units, in absence of an international standard to refer to.

D. COMPONENTS

Each kit contains sufficient reagents to carry out 96 tests.

1. Microplate: MICROPLATE
12 strips x 8 breakable wells coated with affinity-purified anti human IgM specific (u-chain) goat polyclonal antibody and sealed into a bag with desiccant. Allow the microplate to reach room temperature before opening; reseal unused strips in the bag with desiccant and store at 4°C.

2. Calibration Curve: CAL N° …
Ready to use and color coded standard curve ranging:
4 ml CAL1 = 0 arbU/ml
4 ml CAL2 = 10 arbU/ml
2 ml CAL3 =20 arbU/ml
2 ml CAL4 = 50 arbU/ml
4 ml CAL5 = 100 arbU/ml.
Standards are calibrated against an internal Gold Standard or IGS as no international one is defined.
Contains human serum proteins, 2% casein, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.1% Tween 20, 0.09% Na-azide and 0.1% Kathon GC as preservatives. Standards are blue colored.

3. Control Serum: CONTROL …ml
1 vial. Lyophilized. Contains fetal bovine serum proteins, human anti EBV VCA IgM antibodies at 20 ± 20% arbU/ml, 0.2 mg/ml gentamicine sulphate and 0.1% Kathon GC as preservatives.
Important Note: The volume necessary to dissolve the content of the vial may vary from lot to lot. Please use the right volume reported on the label .

3. Wash buffer concentrate: WASHBUF 20X
1x60ml/bottle. 20x concentrated solution.
Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2, 0.05% Tween 20 and 0.1% Kathon GC.

4. Enzyme conjugate: CONJ 20X
1x0.8 ml/vial. 20x concentrated solution. It contains peroxidase (HRP) labeled Streptavidine, dissolved into a buffered solution of 10 mM Tris buffer pH 6.8+/-0.1, 5% BSA, 0.1% Kathon GC and 0.02% gentamicine sulphate as preservatives.

5.Antigen Diluent : AG DIL
n° 1 vial of 16 ml. Protein buffer solution for the preparation of the working EBV VC antigen. The solution contains 10 mM Tris buffer pH 6.8+/-0.1, 2% BSA, 0.1% Kathon GC and 0.2 mg/ml gentamicine sulphate as preservatives. The reagent is code coloured with 0.01% red alimentary dye

6. EBV VCA Antigen : Ag VCA
1x6 vials. Lyophilized reagent to be dissolved with 1.9 ml of Antigen Diluent as reported in the proper section. It contains biotinilated affinity purified native VCA antigen, 25 mM Tris buffer pH 7.8+/-0.1 and 5% BSA as proteic carrier.

7. Specimen Diluent: DILSPE
2x60.0 ml/vial. Buffered solution for the dilution of samples. It contains 2% casein, 0.2 M Tris buffer pH 6.0+/-0.1, 0.2% Tween 20, 0.1% Kathon GC and 0.09% sodium azide as preservatives. The component is blue color coded.

8. Chromogen/Substrate: SUBS TMB
1x16ml/vial. Contains a 50 mM citrate-phosphate buffered solution at pH 3.5-3.8, 0.03% tetra-methyl-benzidine or TMB and 0.02% hydrogen peroxide of H_2O_2.
Note: To be stored protected from light as sensitive to strong illumination.

9. Sulphuric Acid: H_2SO_4 0.3 M
1x15ml/vial. Contains 0.3 M H_2SO_4 solution.
Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

10. Plate sealing foils n° 2

11. Package insert n° 1

E. MATERIALS REQUIRED BUT NOT PROVIDED

1. Calibrated Micropipettes in the range 10-1000 ul and disposable plastic tips.
2. EIA grade water (double distilled or deionised, charcoal treated to remove oxidizing chemicals used as disinfectants).
3. Timer with 60 minute range or higher.

4. Absorbent paper tissues.
5. Calibrated ELISA microplate thermostatic incubator (dry or wet) set at +37°C.
6. Calibrated ELISA microwell reader with 450nm (reading) and if with 620-630nm (blanking) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.

F. WARNINGS AND PRECAUTIONS

1. The kit has to be used by skilled and properly trained technical personnel only, under the supervision of a medical doctor responsible of the laboratory.
2. All the personnel involved in performing the assay have to wear protective laboratory clothes, talc-free gloves and glasses. The use of any sharp (needles) or cutting (blades) devices should be avoided. All the personnel involved should be trained in biosafety procedures, as recommended by the Center for Disease Control, Atlanta, U.S. and reported in the National Institute of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
3. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.
4. The laboratory environment should be controlled so as to avoid contaminants such as dust or air-born microbial agents, when opening kit vials and microplates and when performing the test. Protect the Chromogen/Substrate (TMB/H_2O_2) from strong light and avoid vibration of the bench surface where the test is undertaken.
5. Upon receipt, store the kit at +2..8°C into a temperature controlled refrigerator or cold room.
6. Do not interchange components between different lots of the kits. It is recommended that components between two kits of the same lot should not be interchanged.
7. Check that the reagents are clear and do not contain visible heavy particles or aggregates. If not, advise the laboratory supervisor to initiate the necessary procedures.
8. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each sample.
9. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one.
10. Do not use the kit after the expiration date stated on external (primary container) and internal (vials) labels.
11. Treat all specimens as potentially infective. All human serum specimens should be handled at Biosafety Level 2, as recommended by the Center for Disease Control, Atlanta, U.S. in compliance with what reported in the Institutes of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
12. The use of disposable plastic labware is recommended in the preparation of the washing solution or in transferring components into other containers of automated workstations, in order to avoid contamination.
13. Waste produced during the use of the kit has to be discarded in compliance with national directives and laws concerning laboratory waste of chemical and biological substances. In particular, liquid waste generated from the washing procedure, from residuals of controls and from samples has to be treated as potentially infective material and inactivated. Suggested procedures of inactivation are treatment with a 10% final concentration of household bleach for 16-18 hrs or heat inactivation by autoclave at 121°C for 20 min..
14. Accidental spills have to be adsorbed with paper tissues soaked with household bleach and then with water. Tissues should then be discarded in proper containers designated for laboratory/hospital waste.
15. The Sulphuric Acid is an irritant. In case of spills, wash the surface with plenty of water.
16. Other waste materials generated from the use of the kit (example: tips used for samples and controls, used microplates) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

G. SPECIMEN: PREPARATION AND RECOMMANDATIONS

1. Blood is drawn aseptically by venepuncture and plasma or serum is prepared using standard techniques of preparation of samples for clinical laboratory analysis. No influence has been observed in the preparation of the sample with citrate, EDTA and heparin.
2. Avoid any addition of preservatives to samples; especially sodium azide as this chemical would affect the enzymatic activity of the conjugate.
3. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results. When the kit is used for the screening of blood units, bar code labeling and electronic reading is strongly recommended.
4. Haemolysed (red) and visibly hyperlipemic ("milky") samples have to be discarded as they could generate false results. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they could give rise to false results.
5. Sera and plasma can be stored at +2°..8°C for up to five days after collection. For longer storage periods, samples can be stored frozen at –20°C for several months. Any frozen samples should not be frozen/thawed more than once as this may generate particles that could affect the test result.
6. If particles are present, centrifuge at 2.000 rpm for 20 min or filter using 0.2-0.8u filters to clean up the sample for testing.

H. PREPARATION OF COMPONENTS AND WARNINGS

A study conducted on an opened kit has not pointed out any relevant loss of activity up to 6 re-uses of the device and up to 3 months.

Microplate:
Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant has not turned dark green, indicating a defect in storing.
In this case, call Dia.Pro's customer service.
Unused strips have to be placed back inside the aluminum pouch, with the desiccant supplied, firmly zipped and stored at +2°..8°C.
***Important Note**: After first opening, remaining strips are stable until the humidity indicator inside the desiccant bag turns from yellow to green.*

Calibration Curve
Ready to use. Mix well on vortex before use.

Control Serum:
Lyophilized reagent to be dissolved with EIA grade water as reported in the label.
***Note:** In order to maintain its reactivity fully preserved, upon dissolution keep the excess frozen in aliquots at –20°C and use just once. Do not freeze again.*

Wash buffer concentrate:
The whole content of the 20x concentrated solution has to be diluted with bidistilled water up to 1200 ml and mixed gently end-over-end before use. During preparation avoid foaming as the presence of bubbles could impact on the efficiency of the washing cycles.

Note: *Once diluted, the wash solution is stable for 1 week at +2..8° C.*

Antigen-Conjugate Complex:
Proceed carefully as follows:
1. Dissolve the content of a lyophilized vial with 1.9 ml of Antigen Diluent. Let fully dissolved the lyophilized content and then gently mix on vortex.
2. Gently mix the concentrated Enzyme Conjugate on vortex. Then add 0.1 ml of it to the vial of the dissolved EBV VC Ag and mix gently on vortex.

Important Notes*:*
1. *Dissolve and prepare only the number of vials necessary to the test. The complex obtained is not stable. Store any residual solution frozen in aliquots at –20°C.*
2. *The preparation of the complex has to be done* ***right before*** *the dispensation of samples and controls into the plate. Mix again on vortex gently just before its use.*

Specimen Diluent
Ready to use. Mix on vortex before use.

Chromogen/Substrate:
Ready to use. Mix well on vortex before use.
Avoid contamination of the liquid with oxidizing chemicals, air-driven dust or microbes. Do not expose to strong light, oxidizing agents and metallic surfaces.
If this component has to be transferred use only plastic, and if possible, sterile disposable container.

Sulphuric Acid:
Ready to use. Mix well on vortex before use.
Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

Legenda:

Warning **H statements:**
H315 – Causes skin irritation.
H319 – Causes serious eye irritation.

Precautionary **P statements:**
P280 – Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352 – IF ON SKIN: Wash with plenty of soap and water.
P332 + P313 – If skin irritation occurs: Get medical advice/attention.
P305 + P351 + P338 – IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337 + P313 – If eye irritation persists: Get medical advice/attention.
P362 + P363 – Take off contaminated clothing and wash it before reuse.

I. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

1. Micropipettes have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (70% ethanol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample or the components of the kit. They should also be regularly maintained in order to show a precision of 1% and a trueness of $\pm$2%.
2. The ELISA incubator has to be set at +37°C (tolerance of $\pm$0.5°C) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water baths are suitable for the incubations, provided that the instrument is validated for the incubation of ELISA tests.
3. The ELISA washer is extremely important to the overall performances of the assay. The washer must be carefully validated and correctly optimized using the kit controls/calibrator and reference panels, before using the kit for routine laboratory tests. Usually 4-5 washing cycles (aspiration + dispensation of 350ul/well of washing solution = 1 cycle) are sufficient to ensure that the assay performs as expected. A soaking time of 20-30 seconds between cycles is suggested. In order to set correctly their number, it is recommended to run an assay with the kit controls/calibrator and well characterized negative and positive reference samples, and check to match the values reported below in the section “Internal Quality Control”. Regular calibration of the volumes delivered and maintenance (decontamination and cleaning of needles) of the washer has to be carried out according to the instructions of the manufacturer.
4. Incubation times have a tolerance of $\pm$5%.
5. The ELISA microplate reader has to be equipped with a reading filter of 450nm and with a second filter (620-630nm, strongly recommended) for blanking purposes. Its standard performances should be (a) bandwidth $\leq$ 10 nm; (b) absorbance range from 0 to $\geq$ 2.0; (c) linearity to $\geq$ 2.0; repeatability $\geq$ 1%. Blanking is carried out on the well identified in the section “Assay Procedure”. The optical system of the reader has to be calibrated regularly to ensure that the correct optical density is measured. It should be regularly maintained according to the manufacturer ‘s instructions.
6. When using an ELISA automated work station, all critical steps (dispensation, incubation, washing, reading, shaking, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the section “Internal Quality Control”. The assay protocol has to be installed in the operating system of the unit and validated as for the washer and the reader. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set. Particular attention must be paid to avoid carry over by the needles used for dispensing samples and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells due to strongly reactive samples, leading to false positive results. The use of ELISA automated work stations is recommended for blood screening and when the number of samples to be tested exceed 20-30 units per run.
7. Dia.Pro’s customer service offers support to the user in the setting and checking of instruments used in combination with the kit, in order to assure full compliance with the requirements described. Support is also provided for the installation of new instruments to be used with the kit.

L. PRE ASSAY CONTROLS AND OPERATIONS

1. Check the expiration date of the kit printed on the external label (primary container). Do not use if expired.
2. Check that the liquid components are not contaminated by visible particles or aggregates.
3. Check that the Chromogen/Substrate is colorless or pale blue by aspirating a small volume of it with a sterile plastic pipette.
4. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box (primary container).
5. Check that the aluminum pouch, containing the microplate, is not punctured or damaged.
6. Dilute all the content of the 20x concentrated Wash Solution as described above.
7. Dissolve the Control Serum as described above and gently mix.

8. Prepare the Antigen/Conjugate complex as reported before.
9. Allow all the other components to reach room temperature (about 1 hr) and then mix gently on vortex all liquid reagents.
10. Set the ELISA incubator at +37°C and prepare the ELISA washer by priming with the diluted washing solution, according to the manufacturers instructions. Set the right number of washing cycles as found in the validation of the instrument for its use with the kit.
11. Check that the ELISA reader is turned on or ensure it will be turned on at least 20 minutes before reading.
12. If using an automated work station, turn on, check settings and be sure to use the right assay protocol.
13. Check that the micropipettes are set to the required volume.
14. Check that all the other equipment is available and ready to use.
15. In case of problems, do not proceed further with the test and advise the supervisor.

M. ASSAY PROCEDURE

The assay has to be carried out according to what reported below, taking care to maintain the same incubation time for all the samples in testing.
Two procedures can be carried out with the device according to the request of the clinician.

M.1 Quantitative analysis

1. Place the required number of strips in the microplate holder. Leave A1 and B1 wells empty for the operation of blanking.
Store the other strips into the bag in presence of the desiccant at 2..8°C, sealed.
2. Dilute samples 1:101 dispensing 1 ml Specimen Diluent into a disposable tube and then 10 ul sample; mix on vortex before use. Do not dilute the calibrators and the control serum as they are ready-to-use.
3. Prepare the Antigen/Conjugate complex as reported in Section H.
4. Pipette 100 µl of all the Calibrators and 100 µl of Control Serum in duplicate; then dispense 100 µl of samples. The Control Serum is used to verify that the whole analytical system works as expected. Check that Calibrators Control Serum and samples have been correctly added. Then incubate the microplate at **+37°C for 60 min**.

Important note: *Strips have to be sealed with the adhesive sealing foil only when the test is performed manually. Do not cover strips when using ELISA automatic instruments.*

5. Wash the microplate as reported in section I.3.
6. In all the wells, except A1 and B1, pipette 100 µl Antigen/ Conjugate Complex. Check that the reagent has been correctly added. Incubate the microplate at **+37°C for 60 minutes.**

Important note: *Be careful not to touch the inner surface of the well with the pipette tip when dispensing the Complex. Contamination might occur.*

7. Wash the microplate as described in section I.3.
8. Pipette 100 µl TMB/H_2O_2 mixture in each well, the blank wells A1+B1 included. Check that the reagent has been correctly added. Then incubate the microplate at **room temperature for 20 minutes**.

Important note: *Do not expose to strong direct light as a high background might be generated.*

9. Stop the enzymatic reaction by pipette 100 µl Sulphuric Acid into each well and using the same pipetting sequence as in step 8. Then measure the color intensity with a microplate reader at 450nm (reading) and at 620-630nm (blanking, strongly recommended), blanking the instrument on A1, or B1 or both wells.

M.2 Qualitative analysis

1. Place the required number of strips in the microplate holder. Leave A1 well empty for the operation of blanking.
Store the other strips into the bag in presence of the desiccant at 2..8°C, sealed.
2. Dilute samples 1:101 dispensing 1 ml Specimen Diluent into a disposable tube and then 10 ul sample; mix on vortex before use. Do not dilute the calibrators as they are ready-to-use. Then prepare the Antigen/Conjugate complex as reported in Section H.
3. Pipette 100 µl CAL 1 in duplicate, 100 µl CAL 2 in duplicate, 100 µl CAL 5 in single. Then dispense 100 µl of samples. Check that Calibrators and samples have been correctly added. Then incubate the microplate at **+37°C for 60 min**.

Important note: *Strips have to be sealed with the adhesive sealing foil only when the test is performed manually. Do not cover strips when using ELISA automatic instruments.*
4. Wash the microplate as reported in section I.3.
5. In all the wells, except A, pipette 100 µl Antigen/ Conjugate Complex. Check that the reagent has been correctly added. Incubate the microplate at **+37°C for 60 minutes.**

Important note: *Be careful not to touch the inner surface of the well with the pipette tip when dispensing the Complex. Contamination might occur.*

6. Wash the microplate as described in section I.3.
7. Pipette 100 µl TMB/H_2O_2 mixture in each well, the blank well A1 included. Check that the reagent has been correctly added. Then incubate the microplate at **room temperature for 20 minutes**.

Important note: *Do not expose to strong direct light as a high background might be generated.*

8. Stop the enzymatic reaction by pipette 100 µl Sulphuric Acid into each well and using the same pipetting sequence as in step 7. Then measure the color intensity with a microplate reader at 450nm (reading) and at 620-630nm (blanking, strongly recommended), blanking the instrument on A1.

Important general notes:

1. *If the second filter is not available, ensure that no finger prints are present on the bottom of the microwell before reading at 450nm. Finger prints could generate false positive results on reading.*
2. *Reading has should ideally be performed immediately after the addition of the Stop Solution but definitely no longer than 20 minutes afterwards. Some self oxidation of the chromogen can occur leading to a higher background.*

N. ASSAY SCHEME

Calibrators Control Serum (*) Samples diluted 1:101	100 ul 100 ul 100 ul
1st incubation	**60 min**
Temperature	+37°C
Enzyme Conjugate	100 ul
2nd incubation	**60 min**
Temperature	+37°C
TMB/H2O2 mix	100 ul
3rd incubation	**20 min**
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm & 620nm

(*) Important Notes:

- *The Control Serum (CS) it does not affect the test's results calculation.*
- *The Control Serum (CS) used only if a laboratory internal quality control is required by the Management.*

An example of dispensation scheme in quantitative assays is reported below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	CAL4	S3									
B	BLK	CAL4	S4									
C	CAL1	CAL5	S5									
D	CAL1	CAL5	S6									
E	CAL2	CS**(*)**	S7									
F	CAL2	CS**(*)**	S8									
G	CAL3	S1	S9									
H	CAL3	S2	S10									

Legenda: BLK = Blank // CAL = Calibrators // // S = Sample//
CS = Control Serum - Not mandatory

An example of dispensation scheme in qualitative assays is reported below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S 2	S 10									
B	CAL1	S 3	S 11									
C	CAL1	S 4	S 12									
D	CAL2	S 5	S 13									
E	CAL2	S 6	S 14									
F	CAL5	S 7	S 15									
G	S1	S 8	S 16									
H	S2	S 9	S 17									

Legenda: BLK = Blank // CAL = Calibrators // S = Sample

O. INTERNAL QUALITY CONTROL

A validation check is carried out on the calibrators any time the kit is used in order to verify whether the performances of the assay are as qualified.

Control that the following data are matched:

Parameters	Requirements
Blank well	< 0.100 OD450nm
Calibrator 1 0 arbU/ml	< 0.200 OD450nm after blanking
Calibrator 2 10 arbU/ml	OD450nm higher than the OD450nm of CAL 1 + 0.100
Calibrator 5 100 arbU/ml	> 1.000 OD450nm
Coefficient of variation	< 30% for the Calibrator 1

If the results of the test match the requirements stated above, proceed to the next section.

If they do not, do not proceed any further and perform the following checks:

Problem	Check
Blank well > 0.100 OD450nm	1. that the Chromogen/Substrate solution has not become contaminated during the assay
CAL 1 OD450nm > 0.200 coefficient of variation > 30%	1. that the washing procedure and the washer settings are as validated in the pre qualification study; 2. that the proper washing solution has been used and the washer has been primed with it before use; 3. that no mistake has been done in the assay procedure when the dispensation of calibrators is carried out; 4. that no contamination of the Cal 1 or of the wells where it was dispensed has occurred due to spills of positive samples or Antigen/Conjugate complex; 5. that micropipettes have not become contaminated with positive samples or with the Antigen/Conjugate complex 6. that the washer needles are not blocked or partially obstructed.
CAL 2 OD450nm < Cal 1 + 0.100	1. that the procedure has been correctly performed; 2. that no mistake has occurred during its distribution (ex.: dispensation of a wrong calibrator); 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the calibrator has occurred.
CAL 5 OD450nm < 1.000	1. that the procedure has been correctly performed; 2. that no mistake has occurred during its distribution; 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the calibration has occurred.

**** Note:**

If Control Serum has used, verify the following data:

Check	Requirements
Control Serum	OD450nm = OD450nm CAL 20 arbU/ml +/-20%

If the results of the test doesn't match the requirements stated above, operate as follows:

Problem	Check
Control Serum Different from Expected value	1. that the procedure has been correctly performed; 2. that no mistake has occurred during its distribution (e.g.: dispensation of a wrong calibrator); 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the control has occurred.

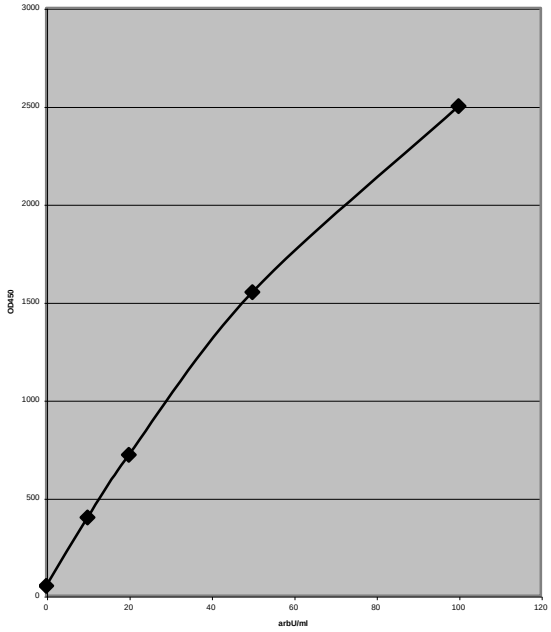
Anyway, if all other parameters (Blank, CAL1, CAL2, CAL 5), match the established requirements, the test may be considered valid.

P. RESULTS

P.1 Quantitative method

If the test turns out to be valid, use for the quantitative method an approved curve fitting program to draw the calibration curve from the values obtained by reading at 450nm (4-parameters interpolation is suggested).

Then on the calibration curve calculate the concentration of anti EBV VCA IgM antibody in samples.
An example of Calibration curve is reported below.



Note: *Do not use these data to calculate the real assay results. The figures above are reported only as an example.*

P.2 Qualitative method
Check that the assay is valid.
An example is provided below:

Note: *The following data must not be used instead or real figures obtained by the user.*

Calibrator 0 arbU/ml: 0.020 – 0.024 OD450nm
Mean Value: 0.022 OD450nm
Lower than 0.200 – Accepted
Calibrator 10 arbIU/ml: 0.250 – 0.270 OD450nm
Mean Value: 0.260 OD450nm
Higher than CAL 1 + 0.100 – Accepted
Calibrator 100 arbU/ml: 2.045 OD450nm
Higher than 1.000 – Accepted

The OD450nm of the Calibrator 10 arbU/ml is considered the cut-off (or Co) of the system.
The ratio between the OD450nm value of the sample and the OD450nm of the Calibrator 10 arbU/ml (or S/Co) can provide a semi-quantitative estimation of the content of specific IgM in the sample.

Q. INTERPRETATION OF RESULTS
Samples with a concentration lower than 10 arbU/ml are considered negative for anti EBV VCA IgM antibody.
Samples with a concentration higher than 10 arbU/ml are considered positive for anti EBV VCA IgM antibody. The patient is likely to be in the acute phase of infection (mononucleosis).

VCA IgM results alone are not, anyway, enough to provide a clear diagnosis of EBV infection. At least EBNA IgG results are necessary in combination.
A reference range of the minimum essential serological markers of Eptein-Barr infection, derived from Infectious Diseases Handbook, 3rd edition, published by Lexi-Comp Inc., USA, is reported schematically below:

VCA IgM	EBNA IgG	Interpretation
negative	negative	No history of EBV infection
positive	negative	Acute primary infection
negative	**positive**	History of previous infection
positive	**positive**	Reactivation

Important notes:
1. *Interpretation of results should be done under the supervision of the laboratory supervisor to reduce the risk of judgment errors and misinterpretations.*
2. *When test results are transmitted from the laboratory to another facility, attention must be paid to avoid erroneous data transfer.*
3. *Diagnosis has to be done and released to the patient by a suitably qualified medical doctor.*

R. PERFORMANCE CHARACTERISTICS
Evaluation of Performances has been conducted in an external clinical center on panels of negative and positive samples with reference to a commercial kit.

1. Limit of detection
No international standard for EBV VCA IgM Antibody detection has been defined so far by the European Comunity.
In its absence, an Internal Gold Standard (or IGS), derived from a patient in the acute phase of mononucleosis infection, has been defined in order to provide the device with a constant and excellent sensitivity.

2. Diagnostic Sensitivity and Specificity:
The assay is based on the "IgM Capture" method and on affinity purified native VCA antigen in order to provide the highest specificity and sensitivity.
The diagnostic sensitivity was studied on more than 50 samples, pre-tested positive with the reference kit of European origin in use at the laboratory. Positive samples were collected from patients undergoing acute mononucleosis infection.
The diagnostic specificity was determined on panels of more than 250 negative samples from normal individuals and blood donors, classified negative with the reference kit, including potentially interfering specimens.
Both plasma, derived with different standard techniques of preparation (citrate, EDTA and heparin), and sera have been used to determine the specificity. No false reactivity due to the method of specimen preparation has been observed.
Frozen specimens have also been tested to check whether samples freezing interferes with the performance of the test. No interference was observed on clean and particle free samples.
The Performance Evaluation provided the following values :

Sensitivity	> 98 %
Specificity	> 98 %

3. Reproducibility:
Data obtained from a study conducted on three samples of different VCA IgM reactivity, examined in 16 replicates in three separate runs showed CV% results ranging 2-8%, depending on the OD450nm readings.
The variability shown in the tables did not result in sample misclassification.

S. LIMITATIONS
False positivity has been assessed as less than 2 % of the normal population, mostly due to high titers of Rheumatoid Factor. IgM capture systems, even if acknowledged to be more specific than sandwich assays, may in fact be influenced by this kind of interfering substance..
Frozen samples containing fibrin particles or aggregates may generate false positive results.

T. CONFIRMATION TEST

In order to provide the medical doctor with the best accuracy in testing for EBV infection, a confirmation assay is reported.
The confirmation test has to be carried out on any positive sample before a diagnosis of primary infection of EBV is released to the doctor.

Proceed for confirmation as follows:

1. Prepare the Antigen/Conjugate Complex as described in the proper section.
2. The well A1 of the strip is left empty for blanking.
3. CAL 2 (10 arbU/ml) is dispensed in the strip in positions B1+C1.
4. The positive sample to be confirmed, diluted 1:101, is dispensed in the strip in position D1+E1.
5. The strip is incubated for 60 min at +37°C.
6. After washing, the blank well A1 is left empty.
7. 100 µl of Antigen/Conjugate Complex are dispensed in wells B1+C1+D1.
8. Then 100 µl of Enzyme Conjugate (CONJ) alone are added to well E1. ***Note:*** *This material does not contain any VCA antigen, only the conjugate*
9. The strip is incubated for 60 min at +37°C.
10. After washing, 100 µl Chromogen/Substrate are added to all the wells and the strip is incubated for 20 min at r.t.
11. 100 µl Sulphuric Acid are added to all the wells and then their color intensity is measured at 450nm (reading filter) and at 620-630nm (background subtraction, strongly recommended), blanking the instrument on A1.

Interpretation of results is carried out as follows:

1. If the sample in position D1 shows an OD450nm lower than the one of CAL 2, a problem of dispensation or contamination in the first test is likely to be occurred. The Assay Procedure in Section M has to be repeated to double check the analysis.
2. If the sample in position D1 shows an OD450nm value higher than the one of CAL 2 and in position E1 shows an OD450nm value still higher than the one of CAL 2, the sample is considered a **false positive**. The reactivity of the sample is in fact not dependent on the specific presence of EBV VCA antigens and a crossreaction with the enzyme conjugate has occurred.
3. If the sample in position D1 shows an OD450nm value higher than the one of CAL 2 and in position E1 shows an OD450nm value lower the one of CAL 2, the sample is considered a **true positive**. The reactivity of the sample is in fact dependent on the specific presence of EBV VCA antigens and not due to any crossreaction with the conjugate alone.

The following table is reported for the interpretation of results

Well	OD450nm		
D1	< CAL 2	> CAL 2	> CAL 2
E1	< CAL 2	> CAL 2	< CAL 2
Interpretation	Problem of contam.	False positive	True positive

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All the IVD Products manufactured by the company are under the control of a certified Quality Management System in compliance with ISO 13485 rule. Each lot is submitted to a quality control and released into the market only if conforming with the EC technical specifications and acceptance criteria.

Produced by
Dia.Pro Diagnostic Bioprobes Srl
Via G. Carducci n° 27 – Sesto San Giovanni (MI) – Italy

CE

Dia.Pro
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BioProbes

EC DECLARATION OF CONFORMITY

MANUFACTURER	DIA.PRO DIAGNOSTIC BIOPROBES S.R.L. VIA G. CARDUCCI N° 27 – 20099 SESTO SAN GIOVANNI (MILANO) – ITALY
PRODUCT	**VCA IgM** CODE: **VCAM.CE** (96 tests)
CLASSIFICATION	GENERAL IVD
CONFORMITY ASSESSMENT ROUTE	SELF CERTIFICATION

WE HEREBY DECLARE THAT THE ABOVE MENTIONED PRODUCT MEETS THE PROVISIONS OF THE COUNCIL DIRECTIVE 98/79/EC FOR IN VITRO DIAGNOSTIC DEVICES.

ISO CERTIFICATE	UNE EN ISO 13485 N° 2013 11 0039 EN, RELEASED BY AEMPS (AGENCIA ESPAÑOLA DE MEDICAMENTOS Y PRODUCTOS SANITARIOS)

PLACE & DATE OF FIRST ISSUE	MILANO – SEPTEMBER 2004
PLACE & DATE OF CURRENT ISSUE	SESTO SAN GIOVANNI (MI) – MARCH 2019
SIGNATURE **Legal Representative** **Dr.ssa Fiorenza Scozzesi**	

Rev:	05/2018

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EC DECLARATION OF CONFORMITY

MANUFACTURER	DIA.PRO DIAGNOSTIC BIOPROBES S.R.L. VIA G. CARDUCCI N° 27 – 20099 SESTO SAN GIOVANNI (MILANO) – ITALY
PRODUCT	**HP Ag** CODE: **HPAG.CE** (96 tests)
CLASSIFICATION	GENERAL IVD
CONFORMITY ASSESSMENT ROUTE	SELF CERTIFICATION

WE HEREBY DECLARE THAT THE ABOVE MENTIONED PRODUCT MEETS THE PROVISIONS OF THE COUNCIL DIRECTIVE 98/79/EC FOR IN VITRO DIAGNOSTIC DEVICES.

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Rev:	05/2018

HP Ag

Enzyme Immunoassay for the qualitative/quantitative determination of Helicobacter pylori Antigen in human stools

- for “in vitro” diagnostic use only -

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REF HPAG.CE.96
96 Tests

HP Ag

A. INTENDED USE
Enzyme Immunoassay (ELISA) for the one-step qualitative/quantitative determination of Helicobacter pylori Antigen (HP Ag) in human stools. The kit may be used for the follow-up of HP-infected patients and their pharmacological treatment. For "in vitro" diagnostic use only.

B. INTRODUCTION
Helicobacter pylori (Hp) is a Gram negative bacterium, firstly isolated in gastric mucosa by Marshall and Warren in 1983.
This bacterium is widely diffused in men, without limitations of sex and age; it has been found that infections can be transmitted directly by contact with contaminated biological fluids (saliva, stool, body secretions) and also from contaminated food and beverages.
H.pylori, and in particular some pathogenic strains (CagA +), is the etiological agent responsible of most of active infections and lesions of the gastric mucosa in man.
H.pylori infection moreover acts as cofactor in the development of tumoral pathologies of the gastric apparatus and it is suspected to be associated to some inflammatory pathologies of the genital female apparatus, evolving toward neoplastic transformation.
At the present time, the identification of Helicobacter pylori is mostly made with invasive histochemical techniques, with the determination of its urease activity on a isotopic substrate (breath test and mass analysis), with time-consuming bacteriological culture systems and with expensive molecular biology techniques (PCR).
ELISA for HP Ag have been only recently introduced as a specific, fast, non invasive (analysis of stools) and cheaper method of detection.

C. PRINCIPLE OF THE TEST
Stools from patients are used as a source of sample for the determination of HP antigen.
Microplates are coated with a cocktail of affinity purified mouse monoclonal antibodies directed to the most specific Helicobacter pylori antigens.
In the 1st incubation, the solid phase is treated with the sample, previously extracted from stools, and simultaneously with a mixture of monoclonal antibodies to Hp, conjugated with peroxidase (HRP).
After washing out all the other components of the sample, in the 2nd incubation the bound enzyme specifically present on the solid phase generates an optical signal that is proportional to the amount of H.pylori antigens present in the sample.

D. COMPONENTS
Code HPAG.CE.96 contains reagents to perform 96 tests.

1. Microplate MICROPLATE
8x12 microwell strips coated with anti HP Ag specific affinity purified mouse monoclonal antibodies and sealed into a bag with desiccant. Allow the microplate to reach room temperature before opening; reseal unused strips in the bag with desiccant and store at 4°C.

2. Calibration Set: CAL …
n° 1 set of 4 vials - Lyophilized calibrators. To be dissolved with EIA grade water. When dissolved, Calibrators have the following concentrations: **0 - 0.1 – 0.5 - 1.0 ug/ml HP Ag**
They contain fetal bovine serum, inactivated HP Ag, 10 mM phosphate buffer pH 7.4+/-0.1, 0.02% gentamicine sulphate and 0.045% ProClin 300 as preservatives.

Important Note: *Calibrators when dissolved are not stable. Proceed as described in the proper section for storage.*

3. Wash buffer concentrate WASHBUF 20X
1x60ml/bottle - 20x concentrated solution. Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2 and 0.05% Tween 20 and 0.045% ProClin 300

4. Enzyme Conjugate CONJ
1x16ml/vial - Ready to use component. It contains Horseradish Peroxidase (HRP) labeled mouse monoclonal antibodies to HP Ag , 10 mM Tris buffer pH 6.8+/-0.1, 2% BSA, 0.045% ProClin 300 and 0.02% gentamicine sulphate as preservatives.
The Enzyme Conjugate is color coded red.

5. Chromogen/Substrate SUBS TMB
1x25ml/vial - It contains a 50 mM citrate-phosphate buffered solution at pH 3.5-3.8, 0.03% tetra-methyl-benzidine (TMB) and 0.02% hydrogen peroxide (H_2O_2).
Note: To be stored protected from light as sensitive to strong illumination.

6. Specimen Diluent: DILSPE
2x60ml/vial - Buffered solution for the extraction of HP Ag from the specimen and preparation of the sample. It contains 10 mM Tris-HCl buffer pH 7.4+/-0.1, 2% BSA, 0.045% ProClin 300 and 0.02% gentamicine sulphate as preservatives.
The component is color coded blue.

7. Sulphuric Acid H_2SO_4 O.3 M
1x15ml/vial - It contains 0.3 M H2SO4 solution.
Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

8. Plate sealing foils: n° 2

9. Package insert: n° 1

Upon request:

HP Ag Extraction kit n° 1
The kit contains all what is necessary to prepare n° 50 samples extracted from stools collected by patients.

E. MATERIALS REQUIRED BUT NOT PROVIDED
1. Calibrated variable volume Micropipettes ranging 1000 ul and 200 ul; disposable plastic tips.
2. EIA grade water (double distilled or deionised, charcoal treated to remove oxidizing chemicals used as disinfectants).
3. Timer with 60 minute range or higher.
4. Absorbent paper tissues.
5. Calibrated ELISA microplate thermostatic incubator (dry or wet), set at +37°C.
6. Calibrated ELISA microwell reader with 450nm (reading) and with 620-630nm (blanking) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.
9. Disposable plastic micro-spoon stools collection container (available upon request from Dia.Pro s.r.l.)

F. WARNINGS AND PRECAUTIONS
1. The kit has to be used by skilled and properly trained technical personnel only, under the supervision of a medical doctor responsible of the laboratory.
2. All the personnel involved in performing the assay have to wear protective laboratory clothes, talc-free gloves and glasses. The use of any sharp (needles) or cutting (blades) devices should be avoided. All the personnel involved should be trained in biosafety procedures, as recommended by the Center for

Disease Control, Atlanta, U.S. and reported in the National Institute of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
3. The laboratory environment should be controlled so as to avoid contaminants such as dust or air-born microbial agents, when opening kit vials and microplates and when performing the test. Protect the Chromogen/Substrate from strong light and avoid vibration of the bench surface where the test is undertaken.
4. Upon receipt, store the kit at 2..8°C into a temperature controlled refrigerator or cold room.
5. Do not interchange components between different lots of the kits. It is recommended that components between two kits of the same lot should not be interchanged.
6. Check that the liquid components of the kit are clear and do not contain visible heavy particles or aggregates. If not, advise the laboratory supervisor to initiate the necessary procedures for kit replacement.
7. Avoid cross-contamination between samples by using disposable tips and changing them after each sample. Do not reuse disposable tips.
8. Avoid cross-contamination between kit components by using disposable tips and changing them between the use of each one. Do not reuse disposable tips.
9. Do not use the kit after the expiration date stated on the external container and internal (vials) labels.
10. Treat all specimens as potentially infective, according to national regulations and laws concerning biological sample handling and wasting.
11. The use of disposable plastic-ware is recommended in the preparation of the liquid components or in transferring components into automated workstations, in order to avoid cross contamination.
12. Wastes produced during the use of the kit have to be discarded in compliance with national directives and laws concerning laboratory waste of chemical and biological substances. In particular, liquid waste generated from the washing procedure, from residuals of controls and from samples has to be treated as potentially infective material and inactivated before waste. Suggested procedures of inactivation are treatment with a 10% final concentration of household bleach for 16-18 hrs or heat inactivation by autoclave at 121°C for 20 min..
13. Accidental spills from samples and operations have to be adsorbed with paper tissues soaked with household bleach and then with water. Tissues should then be discarded in proper containers designated for laboratory/hospital waste.
14. The Sulphuric Acid is an irritant. In case of spills, wash the surface with plenty of water
15. Other waste materials generated from the use of the kit (example: tips used for samples and controls, tools for the extraction of the sample from specimens, used microplates, etc.) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

G. SPECIMEN: COLLECTION, PREPARATION AND WARNINGS

1) It is recommended to collect fresh stools in the morning with the plastic collector provided on request together with the kit. Alternatively a conical bottomed disposable tube, provided by the laboratory to the patient, may be used.
2) The patient submitted to the test should not be under antibiotic or anti-bacterial treatments as this pharmaceutical therapy is known to affect H.pylori up to a certain extent, depending on the antibiotic used, giving origin to false interpretation.
3) The patient has to be asked to collect the specimen avoiding any possible contact with urine or water using the plastic spoon present in the stool collector and taking just the amount of specimen necessary to fill up the cavity of the spoon.
4) The patient is asked to deliver the specimen the same day to the laboratory. From the time of collection, the specimen can be stored in the laboratory up to 24 hr at 2..8°C or kept frozen at -20°C for longer time.
5) Specimens, and then samples derived from them, have to be clearly identified with codes or names in order to avoid misinterpretation of results. Bar code labeling and electronic reading is recommended when the number of samples on testing is pretty high.

***Important Note**: Degradation of HP antigen heavily occurs in stools after 24 hrs generating false negative results, even if the specimen is stored at 2..8°C.*

The next following operations are described and represented in figures in the Instructions for Use of the Stool Extraction Kit provided together with the kit.
Operate according to the following instructions:

1) Open the stool collection device and introduce the extraction brush deeply into the specimen. Rotate the brush 2-4 times in order to collect the right amount of biological material (about 0.2 gr).
2) Transfer the brush carefully into the test tube supplied in the kit and then add 1 ml Specimen Diluent. Keeping the brush inside the tube, mix vigorously on vortex for 1 min +/-10% in order to dissolve H.pylori into solution.
3) Discard the brush and insert the filtering piston, supplied with the kit, into the tube. Push gently the piston down into the tube in order to collect not more than 150-200 ul of the liquid phase of the suspension, volume enough to carry out the test.

***Important Notes**:*
***a)** Be careful not to apply a too strong manual pressure on the piston. The piston could break the tube and spills could be generated. If this should happen, use a paper towel soaked with an hospital disinfectant to clean up the contaminated surfaces.*

***b)** Avoid any addition of preservatives to samples, especially sodium azide as this chemical would affect the enzymatic activity of the conjugate, generating false negative results.*

H. PREPARATION OF COMPONENTS AND WARNINGS

A study conducted on an opened kit has not pointed out any relevant loss of activity up to 6 re-uses of the device and up to 3 months.

Microplates:
Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant has not turned dark green, indicating a defect in storing.
In this case, call Dia.Pro's customer service.
Unused strips have to be placed back inside the aluminum pouch, with the desiccant supplied, firmly zipped and stored at +2°..8°C.
***Important Note**: After first opening, remaining strips are stable until the humidity indicator inside the desiccant bag turns from yellow to green.*

Calibration Set:
Add the volume of ELISA grade water reported in the label to the lyophilized powder of each Calibrator. Let fully dissolve the content and then gently mix on vortex.
***Important Note**: When dissolved, Calibrators are not stable. Store Calibrators frozen in aliquots at –20°C, carefully labeled with the content of HP Ag present in each of them.*

Wash buffer concentrate:
The concentrated solution has to be diluted 20x with ELISA grade water and mixed gently end-over-end before use. During

preparation avoid foaming as the presence of bubbles could impact on the efficiency of the washing cycles.
Important Note: *Once diluted, the wash solution is stable for 1 week at +2..8° C.*

Enzyme Conjugate:
Ready to use. Mix well on vortex before use.

Chromogen/Substrate:
Ready to use. Mix well on vortex before use.
Avoid contamination of the liquid with oxidizing chemicals, air-driven dust or microbes. Do not expose to strong light, oxidizing agents and metallic surfaces.
If this component has to be transferred use only plastic, and if possible, sterile disposable container.

Specimen Diluent:
Ready to use. Mix well on vortex before use.

Sulphuric Acid:
Ready to use. Mix well on vortex before use.
Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

Legenda:

Warning **H statements:**
H315 – Causes skin irritation.
H319 – Causes serious eye irritation.

Precautionary **P statements:**
P280 – Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352 – IF ON SKIN: Wash with plenty of soap and water.
P332 + P313 – If skin irritation occurs: Get medical advice/attention.
P305 + P351 + P338 – IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337 + P313 – If eye irritation persists: Get medical advice/attention.
P362 + P363 – Take off contaminated clothing and wash it before reuse.

I. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

1. Micropipettes have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (70% ethanol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample or the components of the kit. They should also be regularly maintained in order to show a precision of 1% and a trueness of $\pm$2%.
2. The ELISA incubator has to be set at +37°C (tolerance of $\pm$0.5°C) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water baths are suitable for the incubations, provided that the instrument is validated for the incubation of ELISA tests.
3. The ELISA washer is extremely important to the overall performances of the assay. The washer must be carefully validated in advance, checked for the delivery of the right dispensation volume and regularly submitted to maintenance according to the manufacturer's instructions for use. In particular the washer, at the end of the daily workload, has to be extensively cleaned out of salts with deionized water. Before use, the washer has to be extensively primed with the diluted Washing Solution.
   The instrument weekly has to be submitted to decontamination according to its manual (NaOH 0.1 M decontamination suggested).
   5 washing cycles (aspiration + dispensation of 350ul/well of washing solution + 20 sec soaking = 1 cycle) are sufficient to ensure the assay with the declared performances. If soaking is not possible add one more cycle of washing.
   An incorrect washing cycle or salt-blocked needles are the major cause of false positive reactions. The washer has to be correctly optimized using the kit controls/calibrator and reference panels, before using the kit for routine laboratory tests.
   Important Note: *Due to the nature of the sample used and the possible presence of particles in the sample, be careful to control that the needles of the washer do not get blocked by the presence of stool bodies.*
4. Incubation times have a tolerance of $\pm$5%.
5. The ELISA microplate reader has to be equipped with a reading filter of 450nm and with a second filter of 620-630nm, mandatory for blanking purposes. Its standard performances should be (a) bandwidth $\leq$ 10 nm; (b) absorbance range from 0 to $\geq$ 2.0; (c) linearity to $\geq$ 2.0; repeatability $\geq$ 1%. Blanking is carried out on the well identified in the section "Internal Quality Control". The optical system of the reader has to be calibrated regularly to ensure that the correct optical density is measured. It should be regularly maintained according to the manufacturer 's instructions.
6. When using an ELISA automated workstation, all critical steps (dispensation, incubation, washing, reading, shaking, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the sections "Internal Quality Control". The assay protocol has to be installed in the operating system of the unit and validated as for the washer and the reader. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set. Particular attention must be paid to avoid carry over by the needles used for dispensing samples and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells due to strongly reactive samples, leading to false positive results. The use of ELISA automated work stations is recommended when the number of samples to be tested exceed 20-30 units per run.

Important Note: *Due to the nature of the sample used and the possible presence of particles in the sample, be careful to control that the needles of the workstation do not get blocked by the presence of stool bodies. We strongly suggest to use disposable sample tips in order to avoid any block or damage of fix probes.*

7. Dia.Pro's customer service offers support to the user in the setting and checking of instruments used in combination with the kit, in order to assure full compliance with the requirements described. Support is also provided for the installation of new instruments to be used with the kit.
8. Upon request, Dia.Pro srl offers a sample preparation device able to produce a particle free sample showing excellent performances in the assay. Please inquire.

L. PRE ASSAY CONTROLS AND OPERATIONS

1. Prepare the sample from stools as described in section G and represented in the Instructions for Use of the HP Ag Extraction Kit.
2. Check the expiration date of the kit printed on the external label of the kit box. Do not use if expired.
3. Check that the liquid components are not contaminated by naked-eye visible particles or aggregates. Check that the Chromogen/Substrate is colorless or pale blue by aspirating a small volume of it with a sterile transparent plastic pipette. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box. Check that the aluminum pouch, containing the microplate, is not punctured or damaged.

4. Dilute all the content of the 20x concentrated Wash Solution as described above.
5. Dissolve the Calibrator Set as described above.
6. Allow all the other components to reach room temperature (about 1 hr) and then mix as described.
7. Set the ELISA incubator at +37°C and prepare the ELISA washer by priming with the diluted washing solution, according to the manufacturers instructions. Set the right number of washing cycles reported in the specific section.
8. Check that the ELISA reader has been turned on at least 20 minutes before reading.
9. If using an automated workstation, turn it on, check settings and be sure to use the right assay protocol.
10. Check that the micropipettes are set to the required volume.
11. Check that all the other equipment is available and ready to use.
12. In case of problems, do not proceed further with the test and advise the supervisor.

M. ASSAY PROCEDURE

The assay has to be carried out according to what reported below, taking care to maintain the same incubation time for all the samples in testing.
Two procedures are available: a quantitative method able to provide a quantification of HP Ag in the specimen and a qualitative method.

A. Quantitative Assay

1. Place the required number of strips in the plastic holder and carefully identify the wells for calibrators and samples. Leave A1+B1 wells empty for blanking purposes.

2. Pipette 100 µl Calibrators in duplicate into the calibration wells (see the example of dispensation reported below).

3. With the Pasteur pipette supplied aspirate the liquid filtered up into the inner chamber of the piston and dispense 2 drops (about 100 µl) of sample into the sample well. Check for the presence of samples in wells by naked eye (there is a marked color difference between empty and full wells) or by reading at 450/620nm. (samples show OD values higher than 0.100).

4. Dispense then 100 µl Enzymatic Conjugate in all wells, except for A1+B1, used for blanking operations.

Important note: *Be careful not to touch the inner surface of the well with the pipette tip when the conjugate is dispensed. Contamination might occur.*

5. Following addition of the conjugate, check that the color of the samples has turned from brown to pale reddish and incubate the microplate for **120 min at +37°C**.

Important notes: *Strips have to be sealed with the adhesive sealing foil only when the test is performed manually. Do not cover strips when using ELISA workstations.*

6. When the first incubation is over, wash the microwells as previously described (section I.3)

7. Pipette 200 µl Chromogen/Substrate into all the wells, A1+B1 included. Incubate the microplate protected from light at **room temperature (18-24°C) for 20 min.**

Important note: *Do not expose to strong direct light as a high background might be generated.*

8. Pipette 100 µl Sulphuric Acid into all the wells to stop the enzymatic reaction, using the same pipetting sequence as in step 6.

9. Measure the color intensity of the solution in each well, as described in section I.5 using a 450nm filter (reading) and a 620-630nm filter (background subtraction, mandatory)), blanking the instrument on A1 or B1 or both.

An example of dispensation scheme is reported below:

Microplate

	1	2	3	4	5	6
A	BLK	CAL4				
B	BLK	CAL4				
C	CAL1	S1				
D	CAL1	S2				
E	CAL2	S3				
F	CAL2	S4				
G	CAL3	S5				
H	CAL3	S6				

Legenda: BLK = Blank CAL = Calibrator S = Sample

B. Qualitative Assay

1. Place the required number of strips in the plastic holder and carefully identify the wells for calibrators and samples. Leave A1well empty for blanking purposes.

2. Pipette 100 µl Calibrator 1 in duplicate, 100 µl Calibrator 2 in duplicate, 100 µl Calibrator 4 in single and then 100 µl samples. Check for the presence of samples in wells as reported before.

3. Dispense 100 µl Enzymatic Conjugate in all wells, except for A1, used for blanking operations.

Important note: *Be careful not to touch the inner surface of the well with the pipette tip when the conjugate is dispensed. Contamination might occur.*

4. Following addition of the conjugate, check that the color of the samples has turned from brown to pale reddish and then incubate the microplate for **120 min at +37°C**.

Important notes: *Strips have to be sealed with the adhesive sealing foil only when the test is performed manually. Do not cover strips when using ELISA workstations.*

5. When the first incubation is over, wash the microwells as previously described (section I.3)

6. Pipette 200 µl Chromogen/Substrate into all the wells, A1 included. Incubate the microplate protected from light at **room temperature (18-24°C) for 20 min.**

Important note: *Do not expose to strong direct light as a high background might be generated.*

7. Pipette 100 µl Sulphuric Acid into all the wells to stop the enzymatic reaction, using the same pipetting sequence as in step 6.

8. Measure the color intensity of the solution in each well, as described in section I.5 using a 450nm filter (reading) and a 620-630nm filter (background subtraction, mandatory), blanking the instrument on A1.

An example of dispensation scheme is reported below:

Microplate

	1	2	3	4	5	6
A	BLK	S3				
B	CAL1	S4				
C	CAL1	S5				
D	CAL2	S6				
E	CAL2	S7				
F	CAL4	S8				
G	S1	S9				
H	S2	S10				

Legenda: BLK = Blank CAL = Calibrator S = Sample

Important notes:

1. *Ensure that no fingerprints or dust are present on the external bottom of the microwell before reading They could generate false positive results on reading*
2. *Reading should ideally be performed immediately after the addition of the acid solution but definitely no longer than 20 minutes afterwards. Some self-oxidation of the chromogen can occur leading to a higher background.*

N. ASSAY SCHEME

Operations	Procedure
Calibrators&samples	100 ul
Enzyme Conjugate	100 ul
1st incubation	**120 min**
Temperature	+37°C
Washing steps	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
Chromogen/Substrate	200ul
2nd incubation	**20 min**
Temperature	room
Sulphuric Acid	100 ul
Reading OD	450nm / 620-630nm

O. INTERNAL QUALITY CONTROL

A check is performed on the controls/calibrator any time the kit is used in order to verify whether the expected OD450nm/620-630nm or S/Co values have been matched in the analysis.
Ensure that:

Parameter	Requirements
Blank well	< 0.100 OD450nm value
CAL 0 ug/ml	< 0.200 mean OD450nm value after blanking
CAL 0.1 ug/ml	OD450nm > OD450nm CAL 0 ug/ml + 0.100
CAL 1 ug/ml	> 1.000 OD450nm value

If the results of the test match the requirements stated above, proceed to the next section. If they do not, do not proceed any further and perform the following checks:

Problem	Check
Blank well > 0.100 OD450nm	1. that the Chromogen/Substrate solution has not become contaminated during the assay
CAL 0 ug/ml > 0.200 OD450nm after blanking	1. that the washing procedure and the washer settings are as validated in the pre qualification study; 2. that the proper washing solution has been used and the washer has been primed with it before use; 3. that no mistake has been done in the assay procedure (dispensation of positive calibrators instead of the negative one); 4. that no contamination of the calibrator or of the wells where the calibrator was dispensed has occurred due to spills of positive samples or of the enzyme conjugate; 5. that micropipettes have not become contaminated with positive samples or with the enzyme conjugate 6. that the washer needles are not blocked or partially obstructed.
CAL 0.1 ug/ml OD450nm < Cal 0 ug/ml + 0.100	1. that the procedure has been correctly performed; 2. that no mistake has occurred during its distribution (ex.: dispensation of negative calibrator instead) 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the calibrator has occurred.
CAL 1 ug/ml < 1.000 OD450nm	1. that the procedure has been correctly performed; 2. that no mistake has occurred during the distribution of the calibrator (dispensation of negative calibrator instead). 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the calibrator has occurred.

If any of the above problems have occurred, report the problem to the supervisor for further actions.
Important note:
The analysis must be done proceeding as the reading step described in the section M, points A8 and B8.

P. CALCULATION OF RESULTS

Quantitative Assay:
Calculate the mean OD450nm//620-630nm value of the calibrators. Then draw a calibration curve possibly using a 4 parameters fitting curve system. Then calculate on the curve the concentration of HP antigen in the sample.

Qualitative Assay:
The test results are calculated by means of a cut-off value determined from the O450nm/ 620-630nm value of the CAL 0 ug/ml (CAL 0) and the OD450nm/620-630nm of the CAL 0.1 ug/ml (CAL 0.1) with the following formula:

Cut-Off = (CAL 0 + CAL 0.1) / 2

Important note*: When the calculation of results is performed by the operating system of an ELISA automated work station, ensure that the proper formulation is used to calculate the cut-off value and generate the correct interpretation of results.*

Q. INTERPRETATION OF RESULTS

In the quantitative assay, samples showing a concentration of H.pylori antigen higher than 0.05 ug/ml are considered positive.
For the qualitative assay, test results are interpreted as a ratio of the sample OD450nm/620-630nm (S) and the Cut-Off value (Co), mathematically S/Co, according to the following table:

S/Co	Interpretation
< 1.0	Negative
1.0 – 1.1	Equivocal
> 1.1	Positive

A negative result indicates that the patient is not infected by H.pylori.
Any patient showing an equivocal result should be retested on a second sample.
A positive result is indicative of HP infection and therefore the patient should be treated accordingly.

Important notes:

1. *Interpretation of results should be done under the supervision of the laboratory supervisor to reduce the risk of judgment errors and misinterpretations.*
2. *Any positive result should be confirmed first by repeating the test and then, if still positive, by an alternative method before a diagnosis of HP infection is confirmed.*
3. *When test results are transmitted from the laboratory to another department, attention must be paid to avoid erroneous data transfer.*
4. *Diagnosis of HP infection has to be taken and released to the patient by a suitably qualified medical doctor. This should be done taking also into account other diagnostic evidences of infection.*

An example of qualitative method is reported below. (data obtained proceeding as the the reading step described in the section M, point B8):

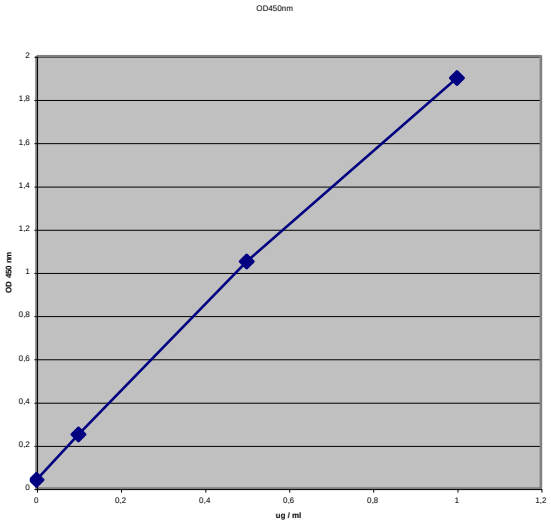
Note: The following data must not be used instead or real figures obtained by the user.

Cal 0 ug/ml: 0.040 –0.060 OD450nm
Mean Value: 0.050 OD450nm
Lower than 0.200 – Accepted

Cal 0.1 ug/ml: 0.210-0.230 OD450nm
Mean Value: 0.220 OD450nm
Higher than Cal 0 ug/ml + 0.100 – Accepted
Cut-Off = (CAL 0 + CAL 0.1) / 2 = 0.135
Calibrator 1 ug/ml: 2.000 OD450nm
OD450nm higher than 1.000 – Accepted

Sample 1: 0.028 OD450nm
Sample 2: 1.690 OD450nm
Sample 1 S/Co < 1.0 = negative
Sample 2 S/Co > 1.1 = positive

An Example of Calibration curve is reported below:



R. PERFORMANCE CHARACTERISTICS

Evaluation of Performances was conducted by testing negative and positive samples in an external clinical site and in the own laboratories as well.

1. Limit of detection

The limit of detection of the assay was calculated by examining serial dilution of HP antigen in Sample Diluent.
Results show that the analytical sensitivity of the assay is better than **0.05 ug/ml** when the limit of dilution is considered mean OD450nm/620-630nm CAL 0 ug/ml + 5 SD.

2. Diagnostic performances:

The diagnostic performances of the kit were studied in some external trials against (a) the “breath test”, considered by the medical literature the Gold Standard for HP Ag determination and (b) against a commercial CE-marked ELISA, as well.
In the studies were the breath test was used as reference stools were collected from patients the same day of the breath test.
In the study carried out with a commercial ELISA as reference the same samples were extracted with the specific devise supplied in combination with kits.
In the studies conducted in reference to the **breath test** the following mean values were obtained from two centers, one in Italy and one in Spain:

- Sensitivity: 98%
- Specificity: 96%

No confirmation by ELISA was possible on discrepant samples.

In the studies conducted in reference to a CE-marked commercial **ELISA**, similarly based on monoclonal antibodies to H.pylori, the following mean values were obtained:

- Sensitivity: 100%
- Specificity: 93%

The discrepant samples (7% “false positive”) turned to be positive in the breath test, revealing a better overall performance of our kit respect to the commercial reference one, when compared on the gold standard method.

Due to the highest specificity shown by the monoclonal antibodies used in the kit no cross-reaction was observed with Campilobacter species.

3. Precision:

The variability shown in the tables did not result in sample misclassification. CV values ranging 4-8%, depending on OD450nm/620-630nm values were observed.

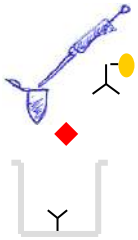
S. LIMITATIONS

False negative results were obtained from samples extracted from specimens stored for more than 1 day at 2..8°C. This finding is explain by the fact that the devise detects "live" H.pylori as confirmed by the results of excellent comparison to the breath test.

False positive results were mostly obtained from samples of liquid stools, difficult to handle and extract.

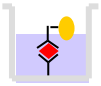
ASSAY GRAPHICAL SCHEME

Add 100 µl Calibrators, samples and conjugate to the plate and then incubate for 120 min at +37°C



Wash as described in the proper section

Add 200 µl Chromogen/Substrate and incubate 20 min at r.t.



Add 100 µl Sulphuric Acid

Read the plate at 450nm (reading) and at 620-630nm (blanking)

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All the IVD Products manufactured by the company are under the control of a certified Quality Management System in compliance with ISO 13485 rule. Each lot is submitted to a quality control and released into the market only if conforming with the EC technical specifications and acceptance criteria.

Manufacturer:
Dia.Pro Diagnostic Bioprobes Srl
Via G. Carducci n° 27 – Sesto San Giovanni (MI) – Italy

CE

MINISTERIO DE SANIDAD, CONSUMO Y BIENESTAR SOCIAL

agencia española de medicamentos y productos sanitarios

CERTIFICADO DE EXAMEN CE DE DISEÑO
de acuerdo con el Anexo IV, punto 4, de la Directiva 98/79/CE

EC DESIGN-EXAMINATION CERTIFICATE
in accordance with Annex IV, Section 4, Directive 98/79/EC

PRÓRROGA/*EXTENSION* — Fecha inicial/ *Initial date*: **14/02/2008**
Fecha de última prórroga/ *Last extension date*: **27/11/2013**

Certificado nº/*Certificate no*	Fecha de validez/*Date of validity*	ON nº/*NB no*
2008 02 0539 ED	**Desde/*From* 19/11/2018 Hasta/*To* 18/11/2023**	**0318**

A favor de /*In favour of:*

Fabricante/*Manufacturer*:
Nombre/*Name:* DIA. Pro Diagnostic Bioprobes S.r.l.
Dirección/*Address:* Via G. Carducci, 27 -20099- Sesto San Giovanni – Milano (Italy).
Representante autorizado ante la UE/*Authorized EU representative*:
Nombre/*Name:* Idem Dirección/*Address:* Idem

Para el producto/*For the product:*

Categoría/*Category:* Productos Sanitarios para Diagnóstico "In Vitro" / *In Vitro Diagnostic Medical Devices*
Grupo genérico/*Generic group*: Diagnóstico de enfermedades infecciosas / *Diagnostic of infectious diseases*
Tipo/*Type*: Especificados en Anexos de este Certificado/*Specified in Annexes to this Certificate.*

Elaborado en/*In the facilities:*

Dia. Pro Diagnostic Bioprobes S.r.l.
Via G. Carducci, 27 -20099- Sesto San Giovanni – Milano (Italy).

Este certificado debe ir acompañado por el certificado CE de Sistema de Garantía de Calidad Total Nº 2003 12 0388 CT/ *This certificate must be accompanied by the EC Full Quality Assurance System Certificate Nº 2003 12 0388 CT.*

Este certificado es consecuencia de la evaluación de la documentación técnica del diseño contenida en el expediente Nº 2003 05 0240, y garantiza que el diseño de los productos descritos cumple los requisitos de la Directiva/ *This certificate is issued on the assessment of the design documentation contained in dossier Nº 2003 05 0240, and guarantees that the design of the described products fulfil the requirements of the Directive.*

Madrid, 19 de noviembre de 2018
DIRECTORA DE LA AGENCIA ESPAÑOLA DE MEDICAMENTOS Y PRODUCTOS SANITARIOS

agencia española de medicamentos y productos sanitarios

Fdo. Mª Jesús Lamas Díaz

Firmado digitalmente por: Agencia Española de Medicamentos y Productos Sanitarios
Fecha de la firma: 19/11/2018
Localizador: 6XZPBYWEFC

Puede comprobar la autenticidad del documento en la aplicación Localizador de la Web de la AEMPS

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ORGANISMO NOTIFICADO 0318

CERTIFICADO DE EXAMEN CE DE DISEÑO
de acuerdo con el Anexo IV, punto 4, de la Directiva 98/79/CE

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Representante autorizado ante la UE/*Authorized EU representative:*
Nombre/*Name:* Idem Dirección/*Address:* Idem

Tipo de producto / *Device type*: **Reactivos y productos reactivos, calibradores y materiales de control para el diagnóstico de enfermedades infecciosas /** *Reagents, and reagent products, calibrators and control materials for diagnostic of human infectious diseases.*

Clasificación/*Classification:* **Lista A, Anexo II /** *List A, Annex II*

Reactivos y productos reactivos para la determinación, confirmación y cuantificación en muestras humanas de marcadores de infección por HIV 1 y 2, mediante técnicas de Inmunoabsorción enzimática (ELISA)/ *Reagents and reactive products for the determination, confirmation and quantification in human specimens of markers of HIV 1 and 2 infection, by Enzyme-linked immunosorbent assay (ELISA)* **[NANDO: IVD 0201]**

HIV Ab & Ag ELISA cualitativo / *ELISA qualitative*

- IVCOMB.CE (192 tests)
- IVCOMB.CE.96 (96 tests)
- IVCOMB.CE.480 (480 tests)
- IVCOMB.CE.960 (960 tests)
- IVCOMB.CE.DB (192 tests - For Dia.Blood application)

Este certificado ampara todas las marcas de estos productos incluidas por el fabricante en su declaración de conformidad. / *This certificate covers all trademarks of these products included by the manufacturer in his declaration of conformity*.

Madrid, 19 de noviembre de 2018
DIRECTORA DE LA AGENCIA ESPAÑOLA DE MEDICAMENTOS Y PRODUCTOS SANITARIOS

agencia española de medicamentos y productos sanitarios

Fdo. Mª Jesús Lamas Díaz

Firmado digitalmente por: Agencia Española de Medicamentos y Productos Sanitarios
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Dia.Pro
Diagnostic
BioProbes

EC DECLARATION OF CONFORMITY

MANUFACTURER	DIA.PRO DIAGNOSTIC BIOPROBES S.R.L. VIA G. CARDUCCI N° 27 – 20099 SESTO SAN GIOVANNI (MILANO) – ITALY
PRODUCT	**HIV Ab&Ag** CODES: **IVCOMB.CE (192 tests)** IVCOMB.CE.96 (96 tests) IVCOMB.CE.480 (480 tests) IVCOMB.CE.960 (960 tests) IVCOMB.CE.DB (192 tests)
CLASSIFICATION	ANNEX II – LIST A
CONFORMITY ASSESSMENT ROUTE	ANNEX IV

WE HEREBY DECLARE THAT THE ABOVE MENTIONED PRODUCT MEETS THE PROVISIONS OF THE COUNCIL DIRECTIVE 98/79/EC FOR IN VITRO DIAGNOSTIC DEVICES.

NOTIFIED BODY	AEMPS – n° 0318
(EC) CERTIFICATE(S)	• FULL QUALITY ASSURANCE SYSTEM N° 2003 12 0388 CT (in accordance with Annex IV – except Section IV) of the Directive 98/79/EC), RELEASED BY EC NOTIFIED BODY N° 0318 • DESIGN CERTIFICATE N° 2008 02 0539 ED RELEASED BY EC NOTIFIED BODY N° 0318 • UNE EN ISO 13485 N° 2013 11 0039 EN, RELEASED BY EC NOTIFIED BODY N° 0318

PLACE & DATE OF FIRST ISSUE	MILANO – FEBRUARY 2008
PLACE & DATE OF CURRENT EMISSION	SESTO SAN GIOVANNI (MI) – MARCH 2018
SIGNATURE **Legal Representative** **Dr.ssa Fiorenza Scozzesi**	DIA.PRO DIAGNOSTIC BIOPROBES srl

Rev:	0318

HIV Ab&Ag

Fourth generation Enzyme Immunoassay for the determination of antibodies to Human Immunodeficiency Virus or HIV type 1&2&O and P24 HIV-1 Antigen in human serum and plasma

- for “in vitro” diagnostic use only -

DIA.PRO
Diagnostic Bioprobes Srl
Via G. Carducci n° 27
20099 Sesto San Giovanni
(Milano) - Italy
Phone +39 02 27007161
Fax +39 02 26007726
e-mail: info@diapro.it

REF IVCOMB.CE
96/192/480/960 Tests

HIV Ab&Ag

A. INTENDED USE

The kit is a solid phase enzyme immunoassay for the in-vitro diagnostic screening of antibodies to all subtypes of HIV-1 and HIV-2 and HIV-1 antigen (p24) in human serum or plasma.
This kit is intended exclusively for ***In vitro*** diagnostic use in an authorized clinical laboratory and the test has to be carried out by specifically trained health-care professional personnel.
.

B. INTRODUCTION

Epidemiological evidence indicates that an infectious agent transmitted through intimate contact, intravenous drug use or use of infected blood or blood products leads to Acquired Immunodeficiency Syndrome (AIDS).
This disease affects T-cell mediated immunity, resulting in severe lymphopenia and a reduced subpopulation of helper T-lymphocytes. Destruction of this T-lymphocyte population by the virus causes an immune deficiency, resulting in a reduced or deficient response to subsequent infections.
Consequently, infections become more severe and may cause death. At present, there is no successful treatment for AIDS.

The etiological agent has been identified as a retrovirus, human immunodeficiency virus type 1 (HIV-1).
A closely related, but distinct type of immunodeficiency virus, designated HIV-2, has also been isolated. This virus causes a disease that is indistinguishable from AIDS.
Serological cross-reactivity between HIV-1 and HIV-2 has been shown to be highly variable from sample to sample.
This variability requires the inclusion of antigens to both HIV-1 and HIV-2 for the screening of antibodies to HIV-1 and HIV-2.
The presence of anti-HIV-1 and/or anti-HIV-2 and/or HIV p24 antigen in the blood indicates potential infection with HIV-1 and/or HIV-2 and consequently this blood should not be used for transfusion or for manufacture of injectable products.

C. PRINCIPLE OF THE TEST

Synthetic peptides representing immunodominant epitopes of HIV-1 and HIV-2 together with a monoclonal antibody to p24 HIV-1 antigen are coated onto wells of a microplate.
The peptides and the antibody have been carefully selected to ensure the screening of antibody and p24 antigen to all HIV-1 subtypes, including subtype O and HIV-2. Serum or plasma samples are added to these wells and, if antibodies specific to HIV-1 and/or HIV-2 (IgG, IgM or IgA) are present in the sample, they will form stable complexes with the HIV peptide antigens in the well. In case HIV-1 p24 is present in the sample, the antigen will be captured by the specific monoclonal antibody.
Antigen-antibody complexes are then identified through the successive addition of: (1) biotinylated peptides, a biotinylated monoclonal antibody to HIV-1 p24, and; (2) horseradish peroxidase HRP Streptavidin conjugate.
The hydrolytic activity of horseradish peroxidase allows for the quantification of these antibody-antigen complexes.
Peroxidase substrate solution is then added.
During incubation, a blue color will develop in proportion to the amount of anti-HIV-1/2 antibodies or HIV-1 p24 antigen bound to the well, thus establishing their presence or absence in the sample.
Wells containing samples negative for anti-HIV antibody and/or p24 antigen remain colorless.
A stop solution is added to each well and the resulting yellow color is read on a microplate reader at 450 nm.

D. COMPONENTS

The standard format of the product code IVCOMB.CE contains reagents for 192 tests.

1. Microplate MICROPLATE
n° 2 microplates. 12 strips of 8 breakable wells coated with HIV specific gp36, gp41 and gp120 peptides and with a Monoclonal Antibody specific to the HIV-1 p24 Ag. Plates are sealed into a bag with desiccant.

2. Negative Control CONTROL -
1x4.0ml/vial. Ready to use control. It contains animal serum negative for HIV antibodies and for p24 antigen, and 0.1% Kathon GC as preservatives. The negative control is yellow-brown color coded.

3. Positive Control HIV-1 Ab CONTROL 1+
1x4.0ml/vial. Ready to use control. It contains inactivated HIV 1 antibody positive serum, filtered HIV Ab&Ag negative animal serum and 0.1% Kathon GC as preservative. The Positive Control is light green color coded.
Important Note: *The positive control has been inactivated using B-propionolactone BPL/UV. This does not fully ensure the absence of viable pathogens, and therefore, the control should be handled as potentially biohazardous, in accordance with good laboratory practices.*

4. Positive Control HIV-2 Ab CONTROL 2+
1x4.0ml/vial. Ready to use control. It contains inactivated HIV2 antibody positive serum, filtered HIV Ab&Ag negative animal serum and 0.1% Kathon GC as preservatives. The Positive Control is dark green color coded.
Important Note: *The positive control has been inactivated using B-propionolactone BPL/UV. This does not fully ensure the absence of viable pathogens, and therefore, the control should be handled as potentially biohazardous, in accordance with good laboratory practices.*

5. HIV-1 P24 Ag Calibrator CAL Ag
2 vials. Lyophilized. It contains not infectious recombinant p24 antigen in a 10 mM phosphate buffer pH 7.0+/-0.2 with 0.3 mg/ml Gentamicine Sulphate and 0.1% Kathon GC as stabilizers. This component is calibrated against the NIBSC 1st International HIV-1 p24 Ag reference sample 90/636 (diluted 1:256) as well as the EFS HIV Ag performance panel (3015-3022).
Important Notes:
1) The Calibrator contains p24 recombinant Ag with a concentration of about 100 pg/ml, corresponding to about 4 IU/ml.
2) The volume necessary to dissolve the content of the vial may vary from lot to lot. Please use the right volume reported on the label .

6. Wash buffer concentrate WASHBUF 20X
2x60ml/bottle. 20x concentrated solution. It contains 0.1% Kathon GC. Once diluted, the wash solution contains 10 mM phosphate buffer saline pH 7.0+/-0.2 and 0.05% Tween 20.

7. Conjugate # 1 CONJ 1
8 vials. The vial contains lyophilized biotinylated HIV1&2&0 gp36, gp41 and gp120 peptides and a biotinylated monoclonal antibody specific for HIV 1 p24 antigen. Vials are to be resuspended with 6 ml of the Conjugate # 1 diluent.

8. Conjugate 1 Diluent CONJ 1 DIL
1x60ml/bottle. Used to dissolve the lyophilized powder of Conjugate # 1, it contains Tris saline Buffer supplemented with 0.05% Kathon GC, Tween 20 and BSA.

9. Conjugate # 2 CONJ 2
1x25ml/bottle The solution contains HRP conjugated with streptavidin in Tris saline Buffer supplemented with 0.05% Kathon GC, Tween 20 and BSA. This component is color coded in pink/red.

10. Chromogen/Substrate SUBS TMB
1x45ml/bottle Ready-to-use component. It contains 50 mM citrate buffer pH 3.5-3.8, 4% dimethylsulphoxide, 0.03% tetra-methyl-benzidine or TMB and 0.02% hydrogen peroxide or H2O2.
Note: *To be stored protected from light as sensitive to strong illumination.*

11. Sulphuric Acid H_2SO_4 O.3 M
1x25ml/bottle It contains 0.3 M H_2SO_4 solution.
Attention: Irritant (H315, H319; P280, P302+P352, 332+P313, P305+ P351+P338, P337+P313, P362+P363)

12. Sample Diluent: DILSPE
1x14ml/vial Contains Tris saline buffer supplemented with 0.05% Kathon GC, anti HAMA blocker, and Tween 20; used for specimen dilution. This component is color coded in light blue.

13. Plate sealing foils n° 4

14. Package insert n° 1

Important note: Upon request, Dia.Pro can supply reagents for 96, 480, 960 tests, as reported below **:**

1.Microplate	n°1	n°5	n°10
2.Negative Control	1x2.0ml/vial	1x10ml/vial	1x20ml/vial
3.Positive Control 1	1x2.0ml/vial	1x10ml/vial	1x20ml/vial
4.Positive Control 2	1x2.0ml/vial	1x10ml/vial	1x20ml/vial
5.HIV p24 Calibrator	n°1 vial	n° 5 vials	n° 10 vials
6.Wash buff conc	1x60ml/bottle	5x60ml/bottles	4x150ml/bottles
7.Conjugate # 1	n°4 vials	n°20 vials	n°40 vials
8.Conjugate 1 Diluent	1x30ml/vial	3x50ml/bottles	2x150ml/bottles
9.Conjugate # 2	1x15ml/vial	2x38ml/bottle	2x75ml/bottle
10.Chrom/Substrate	1x25ml/vial	3x42ml/bottle	2x125ml/bottles
11.Sulphuric Acid	1x15ml/vial	2x40ml/bottles	2x80ml/bottles
12.SampleDiluent	1x7ml/vial	1x35ml/bottle	1x70ml/bottle
Plate seal foils	n° 2	n° 10	n° 20
Pack. insert	n° 1	n°1	n° 1
Number of tests	**96**	**480**	**960**
Code	**IVCOMB.CE.96**	**IVCOMB.CE.480**	**IVCOMB.CE.960**

E. MATERIALS REQUIRED BUT NOT PROVIDED
1. Calibrated Micropipettes (200ul and 10ul) and disposable plastic tips.
2. EIA grade water (bidistilled or deionised, charcoal treated to remove oxidizing chemicals used as disinfectants).
3. Timer with 60 minute range or higher.
4. Absorbent paper tissues.
5. Calibrated ELISA microplate thermostatic incubator capable to provide a temperature of +37°C.
6. Calibrated ELISA microwell reader with 450nm (reading) and with 620-630nm (blanking) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.

F. WARNINGS AND PRECAUTIONS
1. The kit has to be used by skilled and properly trained technical personnel only, under the supervision of a medical doctor responsible of the laboratory.
2. When the kit is used for the screening of blood units and blood components, it has to be used in a laboratory certified and qualified by the national authority in that field (Ministry of Health or similar entity) to carry out this type of analysis.
3. All the personnel involved in performing the assay have to wear protective laboratory clothes, talc-free gloves and glasses. The use of any sharp (needles) or cutting (blades) devices should be avoided. All the personnel involved should be trained in biosafety procedures, as recommended by the Center for Disease Control, Atlanta, U.S. and reported in the National Institute of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
4. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.
5. The laboratory environment should be controlled so as to avoid contaminants such as dust or air-born microbial agents, when opening kit vials and microplates and when performing the test. Protect the Chromogen/Substrate from strong light and avoid vibration of the bench surface where the test is undertaken.
6. Upon receipt, store the kit at 2..8°C into a temperature controlled refrigerator or cold room.
7. Do not interchange components between different lots of the kits. It is recommended that components between two kits of the same lot should not be interchanged.
8. Check that the reagents are clear and do not contain visible heavy particles or aggregates. If not, advise the laboratory supervisor to initiate the necessary procedures for kit replacement.
9. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each sample.
10. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one.
11. Do not use the kit after the expiration date stated on the external container and internal (vials) labels.
12. Treat all specimens as potentially infective. All human serum specimens should be handled at Biosafety Level 2, as recommended by the Center for Disease Control, Atlanta, U.S. in compliance with what reported in the Institutes of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
13. The use of disposable plastic-ware is recommended in the preparation of the liquid components or in transferring components into automated workstations, in order to avoid cross contamination.
14. Waste produced during the use of the kit has to be discarded in compliance with national directives and laws concerning laboratory waste of chemical and biological substances. In particular, liquid waste generated from the washing procedure, from residuals of controls and from samples has to be treated as potentially infective material and inactivated before waste. Suggested procedures of inactivation are treatment with a 10% final concentration of household bleach for 16-18 hrs or heat inactivation by autoclave at 121°C for 20 min..
15. Accidental spills from samples and operations have to be adsorbed with paper tissues soaked with household bleach and then with water. Tissues should then be discarded in proper containers designated for laboratory/hospital waste.
16. The Sulphuric Acid is irritant. In case of spills, wash the surface with plenty of water.
17. Other waste materials generated from the use of the kit (example: tips used for samples and controls, used microplates) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

G. SPECIMEN: PREPARATION AND RECOMMENDATIONS
1.Blood is drawn aseptically by venepuncture and plasma or serum is prepared using standard techniques of preparation of samples for clinical laboratory analysis. No influence has been observed in the preparation of the sample with citrate, EDTA and heparin.
2. Avoid any addition of preservatives to samples; especially sodium azide as this chemical would affect the enzymatic activity of the conjugate, generating false negative results.
3. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results. When the kit is used for the screening of blood units, bar code labeling and electronic reading is strongly recommended.
4. Haemolysed (red) and visibly hyperlipemic ("milky") samples have to be discarded as they could generate false results.

Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they could give rise to false results.
5. Sera and plasma can be stored at +2°..8°C for up to seven days after collection. For longer storage periods, samples can be stored frozen at –20°C for several months. Any frozen samples should not be frozen/thawed more than once as this may generate particles that could affect the test result.
6. If particles are present filter using 0.2-0.8u filters to clean up the sample for testing.
7. Do not use heat inactivated samples as they could give origin to false reactivity.

H. PREPARATION OF COMPONENTS AND WARNINGS
A study conducted on an opened kit has not shown any relevant loss of activity up to 2 months.

Microplates:
Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the pouch is not broken or that some defect is present indicating a problem of storage. In this case call Dia.Pro's customer service.
Unused strips have to be placed back into the aluminum pouch, in presence of desiccant supplied, firmly zipped and stored at +2°..8°C. When opened the first time, residual strips are stable up to two months.

Negative Control:
Ready to use. Mix well on vortex before use.

Positive Controls Ab:
Positive controls are ready to use. Handle Positive Controls Ab as potentially infective, even if HIV, if present in the control, has been chemically inactivated.

Calibrator Ag
The Lyophilized Calibrator Ag contains a non-infectious recombinant p24 antigen. The volume of EIA grade water to be used for its dissolution and to reach the appropriate p24 concentration (about 100 pg/ml) is written on the vial label. To help dissolve the lyophilized pellet, vortex a few times, at regular intervals. Complete dissolution should be achieved within 2-5 minutes.
***Note**: When dissolved the Calibrator is not stable. Store in aliquots at –20°C.*

Wash buffer concentrate:
The 20x concentrated solution has to be diluted with EIA grade water up to 1200 ml and mixed gently end-over-end before use. As some salt crystals may be present into the vial, take care to dissolve all the content when preparing the solution.
In the preparation avoid foaming as the presence of bubbles could give origin to a bad washing efficiency.
Important Note: Once diluted, the wash solution is stable for 1 week at +2..8° C.

Conjugate # 1:
The Conjugate # 1 mix solution must be prepared immediately before starting the test. Add 6 ml of Conjugate 1 diluent directly to one Conjugate # 1 vial to dissolve the lyophilized powder. This preparation (a total of 6 ml in one vial) is sufficient for 32 tests, or 4 complete vertical strips of the microplate. To help dissolve the lyophilized powder, vortex a few times, at regular intervals.
Important Note: Any unused portion of this reconstituted Conjugate # 1 Solution may be stored at +2..8°C for no more than 12 hours. After this time it has to be discarded and the empty, used container has to be washed with EIA grade water and kept dry for any following re-use.

Conjugate # 2:
Ready to use reagent. Mix well end-over-end before use.

Chromogen/Substrate:
Ready to use. Mix well end-over-end before use.
Be careful not to contaminate the liquid with oxidizing chemicals, air-driven dust or microbes.
Do not expose to strong illumination, oxidizing agents and metallic surfaces.
If this component has to be transferred use only plastic, possible sterile disposable container.

Sulphuric Acid:
Ready to use. Mix well end-over-end before use.
Attention: Irritant (H315, H319; P280, P302+P352, 332+P313, P305+ P351+P338, P337+P313, P362+P364).

Legenda:

Warning **H statements:**
H315 – Causes skin irritation.
H319 – Causes serious eye irritation.

Precautionary **P statements:**
P280 – Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352 – IF ON SKIN: Wash with plenty of soap and water.
P332 + P313 – If skin irritation occurs: Get medical advice/attention.
P305 + P351 + P338 – IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337 + P313 – If eye irritation persists: Get medical advice/attention.
P362 + P363 - Take off contaminated clothing and wash it before reuse.

Sample Diluent:
Ready to use. Mix well end-over-end before use.

I. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT
1. **Micropipettes** have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (household alcohol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample. They should also be regularly maintained in order to show a precision of 1% and a trueness of +/-2%. Decontamination of spills or residues of kit components should also be carried out regularly.
2. The **ELISA incubator** has to be set at +37°C (tolerance of +/-0.5°C) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water baths are suitable for the incubations, provided that the instrument is validated for the incubation of ELISA tests.
3. The **ELISA washer** is extremely important to the overall performances of the assay. The washer must be carefully validated and correctly optimized using the kit controls and reference panels, before using the kit for routine laboratory tests. Usually 4-5 washing cycles (aspiration + dispensation of 350ul/well of washing solution = 1 cycle) are sufficient to ensure that the assay performs as expected. A soaking time of 20-30 seconds between cycles is suggested. In order to set correctly their number, it is recommended to run an assay with the kit controls and well characterized negative and positive reference samples, and check to match the values reported below in the sections "Validation of Test" and "Assay Performances". Regular calibration of the volumes delivered by, and maintenance (decontamination and cleaning of needles) of the washer has to be carried out according to the instructions of the manufacturer.
4. Incubation times have a tolerance of $\pm$5%.
5. The **ELISA microplate reader** has to be equipped with a reading filter of 450nm and with a second filter (620-630nm, strongly recommended) for blanking purposes. Its standard performances should be (a) bandwidth $\leq$ 10 nm; (b) absorbance range from 0 to $\geq$ 2.0; (c) linearity to $\geq$ 2.0; (d) repeatability $\geq$ 1%. Blanking is carried out on the well identified in the section "Assay Procedure". The optical system of the reader has to be calibrated regularly to ensure

that the correct optical density is measured. It should be regularly maintained according to the manufacturer 's instructions.

6. When using an **ELISA automated work station**, all critical steps (dispensation, incubation, washing, reading, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the sections "Internal Quality Control". The assay protocol has to be installed in the operating system of the unit and validated as for the washer and the reader. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set. Particular attention must be paid to avoid carry over by the needles used for dispensing and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells. The use of ELISA automated work stations is recommended for blood screening when the number of samples to be tested exceed 20-30 units per run.
7. When using automatic devices, in case the vial holder of the instrument does not fit with the vials supplied in the kit, transfer the solution into appropriate containers and label them with the same label peeled out from the original vial. This operation is important in order to avoid mismatching contents of vials, when transferring them. When the test is over, return the secondary labeled containers to 2..8°C, firmly capped.
8. Dia.Pro's customer service offers support to the user in the setting and checking of instruments used in combination with the kit, in order to assure compliance with the requirements described. Support is also provided for the installation of new instruments to be used with the kit.

L. PRE ASSAY CONTROLS AND OPERATIONS

1. Check the expiration date of the kit printed on the external label of the kit box. Do not use if expired.
2. Check that the liquid components are not contaminated by naked-eye visible particles or aggregates. Check that the Chromogen/Substrate is colorless or pale blue by aspirating a small volume of it with a sterile transparent plastic pipette. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box. Check that the aluminum pouch, containing the microplate, is not punctured or damaged.
3. Dilute all the content of the 20x concentrated Wash Solution as described above.
4. Dissolve the Calibrator Ag.
5. Dissolve the Conjugate # 1 vial containing lyophilzed powder with the Conjugate 1 Diluent (1 lyophilized Conjugate # 1 + 6ml Conjugate # 1 Diluent) to obtain the Conjugate # 1 Mix as described in the proper section.
6. Allow all the other components to reach room temperature (about 1 hr) and then mix as described.
7. Set the ELISA incubator at +37°C and prepare the ELISA washer by priming with the diluted washing solution, according to the manufacturers instructions. Set the right number of washing cycles as found in the validation of the instrument for its use with the kit.
8. Check that the ELISA reader has been turned on at least 20 minutes before reading.
9. If using an automated workstation, turn it on, check settings and be sure to use the right assay protocol.
10. Check that the micropipettes are set to the required volume.
11. Check that all the other equipment is available and ready to use.
12. In case of problems, do not proceed further with the test and advise the supervisor.

M. ASSAY PROCEDURE

The assay has to be carried out according to what reported below, taking care to maintain the same incubation time for all the samples in testing.

1. Automated assay:

In case the test is carried out automatically with an ELISA system, we suggest to make the instrument dispense 50 ul Sample Diluent first and then 150 ul controls and samples.
Before the next sample is aspirated, needles have to be duly washed to avoid any cross-contamination among samples or tips have to be changed.
For the next operations follow the operative instructions reported below for the Manual Assay.
It is strongly recommended to check that the time lap between the dispensation of the first and the last sample will be calculated by the instrument and taken into consideration by delaying the first washing operation accordingly.
The correct number of lyophilized Conjugate # 1 must be dissolved each with 6 ml Conjugate # 1 Diluent. Once the lyophilized powders are dissolved and mixed well, they are to be mixed together into a plastic container and the assay may begin.

2. Manual assay:

1. Dissolve the right number of lyophilized Conjugate # 1 with Conjugate # 1 Diluent before starting to dispense the samples and controls of the test.
2. Place the required number of strips in the microwell holder. Leave the 1st well empty for the operation of blanking.
3. Dispense 50 ul Sample Diluent in all the wells, except A1 used for blanking.
4. Dispense 150 ul of Negative Control in triplicate, 150 ul HIV1 Positive Control, 150 ul HIV2 Positive Control and 150 ul of Calibrator Ag in duplicate in proper wells.
5. Dispense 150 ul of Sample in each properly identified well. Mix gently the plate on the work surface, avoiding overflowing and contaminating adjacent wells, in order to fully disperse the sample into the diluent.
6. Incubate the microplate for **60 min at +37°C**.

Important note: *Strips have to be sealed with the adhesive sealing foil, supplied, only when the test is carried out manually. Do not cover strips when using ELISA automatic instruments.*

7. Wash the microplate with an automatic washer by delivering and aspirating 350ul/well of diluted washing solution as reported previously (section I.3).
8. Pipette 150 ul Conjugate # 1 mix, prepared as described before, into each well, except the 1st blanking well, and cover with the sealer.

Important note: *Be careful not to touch the plastic inner surface of the well with the tip filled with the Conjugate. Contamination might occur.*

9. Incubate the microplate for **30 min at +37°C**.
10. Pipette 100 ul of Conjugate # 2 in all the wells, except A1, and gently agitate the microplate to mix the two conjugates.

Important Note*: This solution must be added to the bottom of each well to ensure proper performance. Inadequate mixing of the two solutions (Conjugate 1 and Conjugate 2) may reduce the binding of streptavidin HRP (Conjugate 2) to the biotinylated reagents and consequently affect the performance of the assay. Be sure to provide an adequate mixing when the Conjugate # 2 is added, both in the manual and in the automated procedures.*

11. Incubate the microplate sealed for **30 min at +37°C**.
12. Wash as in section 7.
13. Dispense 200 ul of Chromogen/Substrate mixture into each well, the blank well included. Then incubate the microplate at **room temperature (18-25°C) for 30 minutes**. Start the timing immediately after addition of this component to the first well.

Important note: *Do not expose to strong direct illumination. High background might be generated.*

14. Pipette 100 ul Sulphuric Acid into all the wells using the same pipetting sequence as in step 13 to stop the enzymatic reaction. Addition of acid will turn the positive controls and positive samples from blue to yellow/brown.
15. Measure the color intensity of the solution in each well, as described in section I.5, at 450nm filter (reading) and at 620-630nm (background subtraction, strongly recommended), blanking the instrument on A1.

Important notes:

1. *If the second filter is not available ensure that no finger prints are present on the bottom of the microwell before reading at 450nm. Finger prints could generate false positive results on reading.*
2. *Reading has to be carried out just after the addition of the Stop Solution and anyway not any longer than 30 minutes after its addition. Some self oxidation of the chromogen can occur leading to high background.*
3. *The Calibrator (CAL) does not affect the cut-off calculation and therefore the test results calculation. The Calibrator may be used only when a laboratory internal quality control is required by the management.*

N. ASSAY SCHEME

Method	Operations
Sample Diluent	50 ul
Controls and calibrator	150 ul
Samples	150 ul
1st incubation	**60 min**
Temperature	+37°C
Wash step	4-5 cycles
Conjugate # 1	150 ul
2nd incubation	**30 min**
Temperature	+37°C
Conjugate # 2	100 ul
3rd incubation	**30 min**
Temperature	+37°C
Wash step	4-5 cycles
TMB/H2O2	200 ul
4th incubation	**30 min**
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm

An example of dispensation scheme is reported below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	CAL Ag										
B	NC	CAL Ag										
C	NC	S1										
D	NC	S2										
E	POS 1 Ab	S3										
F	POS 1 Ab	S4										
G	POS 2 Ab	S5										
H	POS 2 Ab	S6										

Legenda: BLK = Blank NC = Negative Control POS 1 Ab = HIV -1 Ab Positive Control, POS 2 Ab = HIV -2 Ab Positive, CAL Ag = HIV p24 Ag Calibrator, S = Sample

O. INTERNAL QUALITY CONTROL

A check is carried out on the controls and the calibrator any time the kit is used in order to verify whether their OD450nm values are as expected and reported in the table below.

Check	Requirements
Blank well	≤ 0.100 OD450nm value
Negative Control (NC)	≤ 0.200 mean OD450nm value after blanking Absorbance of individual negative control values must be less than or equal to 0.200. If one value is outside this range, discard this value and recalculate mean. If two values are outside this range the run should be repeated.
HIV-1 Ab Positive Control	Mean OD450nm ≥ 0.700.
HIV-2 Ab Positive Control	Mean OD450nm ≥ 0.700.
HIV Ag Calibrator	S/Co > 1

If the results of the test match the requirements stated above, proceed to the next section.
If they do not, do not proceed any further and operate as follows:

Problem	Check
Blank well > 0.100 OD450nm	1. that the Chromogen/Sustrate solution has not got contaminated during the assay
Negative Control (NC) > 0.200 OD450nm after blanking	1. that the washing procedure and the washer settings are as validated in the pre qualification study; 2. that the proper washing solution has been used and the washer has been primed with it before use; 3. that no mistake has been done in the assay procedure (dispensation of positive control instead of negative control; 4. that no contamination of the negative control or of their wells has occurred due to positive samples, to spills or to the enzyme conjugate; 5. that micropipettes haven't got contaminated with positive samples or with the enzyme conjugate 6. that the washer needles are not blocked or partially obstructed.
Positive Controls < 0.700 OD450nm	1. that the procedure has been correctly executed; 2. that no mistake has been done in the distribution of controls (dispensation of negative control instead of positive control. In this case, the negative control will have an OD450nm value > 0.200, too. 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the positive control has occurred.
HIV Ag Calibrator S/Co < 1	1. that the procedure has been correctly executed; 2. that no mistake has been done in the distribution of controls (dispensation of negative control instead of Calibrator Ag. In this case, the negative control will have an OD450nm value > 0.200, too. 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the positive control has occurred. 5.that the lyophilize powder was dissolved correctly with the correct volume of water written on the vial label.

Should these problems happen, after checking, report any residual problem to the supervisor for further actions.

P. CALCULATION OF THE CUT-OFF

The tests results are calculated by means of a cut-off value determined with the following formula on the mean OD450nm value of the Negative Control (NC):

NC + 0.125 = Cut-Off (Co)

The value found for the test is used for the interpretation of results as described in the next paragraph.

***Important note**: When the calculation of results is done by the operative system of an ELISA automated work station be sure that the proper formulation is used to calculate the cut-off value and generate the right interpretations of results.*

Q. INTERPRETATION OF RESULTS

Test results are interpreted as ratio of the sample OD450nm and the Cut-Off value (or S/Co) according to the following table:

S/Co	Interpretation
< 1	Negative
> 1	Positive

A negative result indicates that the patient has not been infected by HIV.
If the initial absorbance value is equal to or greater than the cut-off value, retest the sample in duplicate. If both retest values are less than the cut-off, the interpretation is not reactive for HIV antibody and/or antigen (negative).
If one or both retest values are equal to or greater than the cut-off the interpretation of the test results is repeatedly reactive. The sample should be considered reactive or positive for HIV antibody and/or antigen according to the criteria of this HIV ELISA test.
A positive result is indicative of HIV infection and therefore the patient should be treated accordingly.

Important notes:

1. *Interpretation of results should be done under the supervision of the responsible of the laboratory to reduce the risk of judgment errors and misinterpretations.*
2. *Rrepeatedly reactive specimens should be submitted to a Confirmation Assay before diagnosis of HIV infection is released.*
3. *When test results are transmitted from the laboratory to an informatics centre, attention has to be done to avoid erroneous data transfer.*
4. *Diagnosis of HIV infection has to be done and released to the patient only by a qualified medical doctor.*

An example of calculation is reported below:

The following data must not be used instead or real figures obtained by the user.

Negative Control: 0.110 – 0.120 - 0.115
OD450nm Mean Value: 0.115 OD450nm
Lower than 0.200 – Accepted

HIV 1 Ab Positive Control: 2.000 OD450nm mean value
Higher than 0.700 – Accepted

HIV 2 Ab Positive Control: 2.100 OD450nm mean value
Higher than 0.700 – Accepted

Calibrator Ag: 0.322 OD450nm mean value
S/Co > 1 - Accepted

Cut-Off = 0.115 +0.125 = 0.240
Sample 1: 0.070 OD450nm
Sample 2: 1.690 OD450nm
Sample 1 S/Co < 1 = negative
Sample 2 S/Co > 1 = positive

R. PERFORMANCES

Evaluation of Performances has been conducted in accordance to what reported in the Common Technical Specifications or CTS (art. 5, Chapter 3 of IVD Directive 98/79/EC).
The performance evaluation was carried out both in an external centre of excellence for HIV diagnosis, that examined the device on a population of antibody positive and negative samples against a CE-marked kit used in the laboratory as reference, and in DiaPro's laboratories as well to complete the study.

R.1 ANALYTICAL SENSITIVITY

The limit of detection (or analytical sensitivity) of the assay has been calculated by means of preparations specific for HIV-1 and HIV-2 antibody and HIV-1 p24 Ag detection, supplied by NIBSC Blanche Lane South Mimms Potters Bar Hertfordshire EN6 3QG, UK.
Samples were diluted in HIV Ab&Ag negative plasma to generate limiting dilution curves and examined in duplicate.
The tables below reports the mean OD450nm values and the S/Co index:

NIBSC anti-HIV 2 monitor sample code 99/674 – 005

Sample	Lot #	0506	Lot #	0706	Lot #	0906
Dilution	OD450nm	S/Co	OD450nm	S/Co	OD450nm	S/Co
1x	over	>14.5	over	>15.0	over	>15.1
2x	3.838	14.1	3.765	14.5	3.774	14.4
4x	2.371	8.7	2.268	8.7	2.319	8.9
8x	1.253	4.6	1.097	4.2	1.140	4.4
16x	0.700	2.6	0.712	2.7	0.735	2.8
32x	0.462	1.7	0.439	1.7	0.483	1.8
64x	0.281	1.0	0.260	1.0	0.294	1.1
128x	0.189	0.7	0.171	0.7	0.174	0.7
diluent	0.140	0.5	0.122	0.5	0.131	0.5

The device shows a limiting dilution value at 64x.

NIBSC British working standard for anti HIV 1 code 99/750 –007

Sample	Lot #	0506	Lot #	0706	Lot #	0906
Dilution	OD450nm	S/Co	OD450nm	S/Co	OD450nm	S/Co
1x	2.206	8.1	2.086	8.0	2.142	8.2
2x	0.999	3.7	0.925	3.6	1.027	3.9
4x	0.475	1.7	0.486	1.9	0.486	1.9
8x	0.295	1.1	0.301	1.2	0.289	1.1
16x	0.212	0.8	0.206	0.8	0.220	0.8
diluent	0.140	0.5	0.122	0.5	0.131	0.5

The devise shows a limiting dilution value at 8x.

NIBSC British working standard for anti HIV 1 code 99/710 – 007

Sample	Lot #	0506	Lot #	0706	Lot #	0906
Dilution	OD450nm	S/Co	OD450nm	S/Co	OD450nm	S/Co
1x	0.588	2.2	0.611	2.4	0.607	2.3
2x	0.301	1.1	0.309	1.2	0.312	1.2
4x	0.198	0.7	0.210	0.8	0.192	0.7
diluent	0.140	0.5	0.122	0.5	0.131	0.5

The devise shows a limiting dilution value at about 2x.

NIBSC HIV-1 p24 Antigen Monitor Sample code 02/146-002

Sample	Lot #	0506	Lot #	0706	Lot #	0906
Dilution	OD450nm	S/Co	OD450nm	S/Co	OD450nm	S/Co
1x	3.664	13.4	3.650	14.0	3.552	13.5
2x	2.151	7.9	2.133	8.2	2.086	8.0
4x	1.209	4.4	1.178	4.5	1.214	4.6
8x	0.734	2.7	0.729	2.8	0.780	3.0
16x	0.388	1.4	0.342	1.3	0.351	1.3
32x	0.259	***0.9***	0.236	***0.9***	0.229	***0.9***
diluent	0.140	0.5	0.122	0.5	0.131	0.5

The devise shows a limiting dilution value at about 16x.

NIBSC 1st International reference Reagent for HIV 1 Ag code 90/636 – (Version 4, 12 May 2009)

Sample	Lot #	1	Lot #	2	Lot #	3
IU/ml	OD450nm	S/Co	OD450nm	S/Co	OD450nm	S/Co
16	2.053	**14.8**	2.048	**14.8**	2.053	**14.5**
8	1.118	**8.0**	1.121	**8.1**	1.124	**8.0**
4	0.571	**4.1**	0.574	**4.1**	0.576	**4.1**
2	0.290	**2.1**	0.291	**2.1**	0.290	**2.0**
1	0.160	**1.2**	0.162	**1.2**	0.160	**1.1**
0.5	0.104	**0.7**	0.105	**0.8**	0.103	**0.7**
diluent	0.014	**//**	0.014	**//**	0.014	**//**

Note: Lot 1 = C2E2T2/6 – Lot 2 = C2E2T2/5 – Lot 3 = C2E2T2/4

The devise shows a sensitivity ≤ 2 IU/ml as required by CTS:2009.

R.2 DIAGNOSTIC SPECIFICITY AND SENSITIVITY

R.2.1 Diagnostic Specificity:

In addition to the first study, where a total of more than 5000 unselected blood donors, more than 200 hospitalized patients, (under examination for non HIV pathologies) and more than 100 potentially interfering specimens (other infectious diseases, E.coli antibody positive, patients affected by non viral hepatic diseases, dialysis patients, pregnant women, hemolized, lipemic, etc.) were tested, the diagnostic specificity was recently assessed by testing a total of 3268 negative samples on four different lots. A value of diagnostic specificity of 100% was observed.
No false reactivity due to the method of specimen preparation has been observed. Both plasma, derived with different standard techniques of preparation (citrate, EDTA and heparin), and sera have been used to determine the value of specificity.
Frozen specimens have been tested, as well, to check for interferences due to collection and storage.
No interference was observed.

R.2.2 Diagnostic Sensitivity

The diagnostic sensitivity of the test was determined on a population of HIV positive specimens.
Results are reported in the tables below.

Etablissement Francais du Sang
Mixed titer Performance
Panel HIV Ab (1-6) Lot # 11

ID	Composition	Lot # 0506	Lot # 0706	Lot # 0906	S/Co
		S/Co	S/Co	S/Co	mean
1	HIV2(1/200)	**>14.5**	**>15.0**	**>15.1**	**>14.9**
2	HIV2(1/800)	**10.0**	**10.2**	**10.2**	**10.1**
3	negative	0.4	0.4	0.4	0.4
4	HIV1(1/700)	**8.9**	**9.0**	**9.1**	**9.0**
5	HIV1(1/160)	**>14.5**	**>15.0**	**>15.1**	**>14.9**
6	HIV1(1/200)	**1.9**	**1.9**	**1.8**	**1.9**

BBI Anti-HIV 1 Low Titer
Performance Panel - PRB 108

Member ID #	IVCOMB.CE S/Co	IVAB.CE S/Co
1	**3.5**	**3.1**
2	0.5	0.2
3	**2.3**	**2.5**
4	**11.4**	**10.6**
5	**3.8**	**2.9**
6	**6.9**	**5.3**
7	**4.0**	**3.7**
8	**7.5**	**6.3**
9	**6.3**	**3.2**
10	**2.4**	**1.5**
11	**8.8**	**7.2**
12	**4.1**	**2.9**
13	**3.7**	**2.3**
14	**7.2**	**6.1**
15	**10.9**	**6.3**

BBI Anti-HIV 1 Low Titer
Performance Panel - PRB 107
(modified version)

Member ID #	IVCOMB.CE S/Co	Ref. Kit S/Co
2	**5.6**	**3.7**
3	**2.1**	**4.5**
5	0.5	0.1
6	**4.4**	**7.3**
7	**1.4**	**1.0**
8	**6.3**	**7.1**
10	**1.8**	**3.5**
12	**5.9**	**5.0**
13	**7.1**	**3.3**
15	**3.2**	**2.7**

BBI anti HIV-1 Low Titer
Performance Panel - PRB 106

Member ID #	IVCOMB.CE S/Co	Ref. Kit S/Co
1	**4.5**	**7.5**
2	**2.6**	**1.8**
3	**5.2**	**2.0**
4	**6.1**	**8.9**
5	**11.4**	**17.6**
6	0.5	0.1
7	**3.9**	**7.9**
8	**13.2**	**15.7**
9	**>14.5**	**16.6**
10	**6.1**	**12.7**
11	**4.8**	**6.8**
12	**3.0**	**2.4**
13	**5.9**	**10.5**
14	**7.7**	**6.0**
15	**>14.5**	**5.5**

BBI Anti-HIV 1 Mixed Titer
Performance Panel - PRB 204

Member ID #	IVCOMB.CE S/Co	Ref. Kit S/Co
1	**1.2**	**1.0**
2	**>14.5**	**65.2**
3	0.3	0.2
4	**>14.5**	**65.1**
5	**>14.5**	**66.1**
6	**>14.5**	**67.5**
7	**>14.5**	**66.0**
8	**>14.5**	**64.6**
9	**1.3**	**2.4**
10	**>14.5**	**65.1**
11	**>14.5**	**65.4**
12	**>14.5**	**63.8**
13	**4.7**	**67.0**
14	**>14.5**	**63.8**
15	**>14.5**	**63.6**
16	**>14.5**	**66.6**
17	**>14.5**	**68.8**
18	**>14.5**	**71.2**
19	**>14.5**	**71.5**
20	**>14.5**	**68.5**
21	**>14.5**	**71.7**
22	**>14.5**	**68.5**
23	0.3	0.2
24	**2.3**	**2.3**
25	0.8	0.9

BBI anti HIV 1/2 Combo Performance Panel - PRZ 206

Member ID #	IVCOMB.CE S/Co	Ref. Kit S/Co
1	**10.2**	**>15.7**
2	0.4	0.2
3	**>14.5**	**>15.7**
4	**>14.5**	**>15.7**
5	**6.6**	**9.2**
6	**>14.5**	**9.1**
7	0.4	0.2
8	**>14.5**	**>15.7**
9	**11.4**	**15.5**
10	**>14.5**	**>15.7**
11	**>14.5**	**>15.7**
12	**>14.5**	**12.8**
13	**>14.5**	**15.7**

BBI HIV-1 Incidence/Prevalence Performance Panel - PRB 601

Member ID #	IVCOMB.CE S/Co	Ref. Kit S/Co
1	**>14.5**	**18.5**
2	**>14.5**	**17.4**
3	**>14.5**	**17.4**
4	**>14.5**	**17.4**
5	**>14.5**	**15.8**
6	**>14.5**	**17.4**
7	**>14.5**	**18.5**
8	**>14.5**	**17.4**
9	**>14.5**	**17.4**
10	**>14.5**	**17.4**
11	**>14.5**	**18.5**
12	**>14.5**	**17.4**
13	**>14.5**	**17.4**
14	**>14.5**	**17.4**
15	**>14.5**	**17.4**

BBI Worldwide HIV Performance Panel - WWRB 303

Member ID #	IVCOMB.CE S/Co	Ref. Kit S/Co
1	**>14.5**	**>15.9**
2	**>14.5**	**>15.9**
3	**>14.5**	**>15.9**
4	**>14.5**	**>15.9**
5	**4.1**	**>15.9**
6	**>14.5**	**>15.9**
7	**>14.5**	**>15.9**
8	**>14.5**	**>15.9**
9	**1.1**	**2.4**
10	**>14.5**	**>15.9**
11	**11.3**	0.3
12	0.3	0.4
13	**>14.5**	**>15.9**
14	**>14.5**	**>15.9**
15	**>14.5**	**>15.9**

Etablissement Francais du Sang Performance Panel HIV Ag (3015-3022) lot 2004

Sample	IVCOMB.CE Lot # 0506 S/Co	Concentration HIV 1 p24 Ag [pg/ml]
3015	**8.7**	500
3016	**4.2**	250
3017	**1.8**	100
3018	**1.2**	50
3019	***0.9***	25
3020	0.6	10
3021	0.6	5
3022(diluent)	0.5	diluent

BBI HIV p24 Antigen Mixed Titer Performance Panel - PRA 203

Member ID #	IVCOMB.CE S/Co	Ref. Kit S/Co
1	**2.3**	**4.3**
2	**4.1**	**12.2**
3	**1.4**	6.0
4	**1.9**	**3.0**
5	Nd	Nd
6	**1.0**	**1.3**
7	**2.9**	**5.7**
8	**3.0**	**1.5**
9	0.4	0.5
10	**3.6**	**12.2**
11	**2.4**	**3.1**
12	**1.6**	**3.5**
13	**1.2**	**2.1**
14	0.4	0.9
15	**3.3**	**2.5**
16	**1.1**	**1.8**
17	**2.5**	**6.3**
18	**1.7**	**2.4**
19	0.4	0.5
20	**4.6**	**17.0**

Moreover, in the external Performance Evaluation a total of 651 positive samples, including HIV type 1, HIV type 2, HIV type 1 mixed subtypes (including 0), HIV 1 Antigen, more than 40 early seroconversion HIV samples and cell culture supernatants were evaluated and a value of 100% was found.

Finally, more than 30 **panels of seroconversion** containing samples of HIV 1/2/0 Antibodies and/or HIV-1 p24 Antigen positive**,** obtained from BBI, USA, were evaluated using IVCOMB.CE lot # 0506. In the table below results are reported.

Seroconversion Panel	**IVCOMB.CE** 4th Generation HIV Ab&Ag	**IVAB.CE** 3rd Generation HIV Ab
ID	**First specimen detected positive in the panel**	
PRB 910 (J)	2	3
PRB 916 (P)	4	5
PRB 917 (Q)	2	5
PRB 919 (S)	1	2
PRB 922 (V)	1	1
PRB 924 (X)	5	6
PRB 926 (Z)	3	5
PRB 927 (AB)	2	3
PRB 928 (AC)	2	3
PRB 929 (AD)	4	6
PRB 930 (AE)	2	3
PRB 931 (AF)	6	6
PRB 933 (AH)	2	2
PRB 935 (AJ)	6	7
PRB 937 (AL)	4	not detected
PRB 938 (AM)	1	3
PRB 939 (AN)	7	9
PRB 940 (AP)	2	4
PRB 941 (AQ)	4	5
PRB 942 (AR)	4	not detected
PRB 944 (AT)	3	5
PRB 946 (AV)	3	not detected
PRB 947 (AW)	2	4
PRB 948 (AX)	4	not detected
PRB 949 (AY)	4	5
PRB 950 (AZ)	3	4
PRB 952 (BB)	3	5
PRB 953 (BC)	3	4
PRB 955 (BE)	3	4
PRB 956 (BF)	5	not detected

The device shows a better sensitivity than the previous generation as it is able to detect the p24 antigen.

The results of the Performance Evaluation, correlate perfectly with what stated by EU CTS and show an overall value of diagnostic sensitivity of 100%

R.3 PRECISION

The precision of the device was assessed by determining its values in a within and between runs. In the table below results are reported for a negative sample and a low positive sample.

Average values N = 48	Negative Sample	Low Positive
OD450nm	0.136	0.916
Std.Deviation	0.011	0.022
CV %	7.6	4.0

S. LIMITATIONS

The user of this kit is advised to carefully read and understand this package insert. Strict adherence to the protocol is necessary in order to obtain reliable test results. In particular, accurate sample and reagent pipetting, along with careful washing and timing of incubation steps is essential for accurate and reproducible detection of HIV-1 and HIV-2 antibodies and HIV-1 p24 antigen.

After the EIA test is performed, repeatedly reactive specimens should be submitted for additional testing using Western Blot (WB), Immunofluorescence Assay (IFA), Radioimmunoprecipitation Assay (RIPA) tests and PCR for HIV nucleic acid.

The determination that a person's serum contains antibodies or p24 antigen to HIV has extensive medical, social, psychological and economic implications.

It is recommended that confidentiality, appropriate counselling and medical evaluation be considered an essential aspect of the testing sequence. AIDS and AIDS-related conditions are clinical diseases and their diagnosis can only be established clinically.

EIA testing alone cannot be used to diagnose AIDS.

A non-reactive test result at any point in the testing sequence does not preclude the possibility of exposure to or infection with HIV. The risk of an asymptomatic person, who is repeatably reactive, developing AIDS and/or AIDS-related conditions is not known.

Falsely reactive test results can be observed with a test kit of this nature. The proportion of reactive samples will depend on the sensitivity and specificity of the test kit and on the prevalence of HIV-1 and HIV-2 antibodies in the population to be screened.

Antibodies to HIV may occur due to voluntary participation in an HIV vaccine study.

Interpretation of this diagnostic test will depend on the type of vaccine given. Correlation with the medical history and additional testing may be necessary to accurately diagnose HIV in vaccine volunteers.

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All the IVD Products manufactured by the company are under the control of a certified Quality Management System approved by an EC Notified Body. Each lot is submitted to a quality control and released into the market only if conforming with the EC technical specifications and acceptance criteria.

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CE 0318

HSV1 IgG

Enzyme ImmunoAssay (ELISA) for the quantitative/qualitative determination of IgG antibodies to Herpes Simplex Virus type 1 in human serum and plasma

- for “in vitro” diagnostic use only -

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REF HSV1G.CE
96 Tests

HSV1 IgG

A. INTENDED USE

Enzyme ImmunoAssay (ELISA) for the quantitative/qualitative determination of IgG antibodies to Herpes Simplex Virus type 1 in human plasma and sera.
For "in vitro" diagnostic use only.

B. INTRODUCTION

Herpes Simplex Virus type 1 (HSV1) and type 2 (HSV2) are large complex DNA-containing viruses which have been shown to induce the synthesis of several proteins during infection, possessing an high number of crossreactive determinants and just a few of type-specific sequences.
The majority of primary and recurrent genital herpetic infections are caused by HSV2; while non genital infections, such as common cold sores, are caused primarily by HSV1.
The detection of virus specific IgG and IgM antibodies are important in the diagnosis of acute/primary virus infections or reactivations of a latent one, in the absence of evident clinical symptoms.
Asymptomatic infections may happen for HSV in apparently healthy individuals and during pregnancy. Severe herpetic infections may happen in immunocompromised and suppressed patients in which the disease may evolve toward critical pathologies.
The determination of HSV specific antibodies has then become important in the monitoring of "risk" patients and in the follow up of acute and severe infections.

C. PRINCIPLE OF THE TEST

Microplates are coated with native inactivated HSV1.
The solid phase is first treated with the diluted sample and IgG to HSV are captured, if present, by the antigens.
After washing out all the other components of the sample, in the 2nd incubation bound anti HSV1 IgG are detected by the addition of polyclonal specific anti hIgG antibodies, labelled with peroxidase (HRP).
The enzyme captured on the solid phase, acting on the substrate/chromogen mixture, generates an optical signal that is proportional to the amount of anti HSV1 IgG antibodies present in the sample. A Calibration Curve, calibrated against an internal Gold Standard, makes possible a quantitative determination of the IgG antibody in the patient.

D. COMPONENTS

Each kit contains sufficient reagents to perform 96 tests.

1. Microplate: MICROPLATE
n° 1. 12 strips x 8 microwells coated with native UV inactivated HSV1 in presence of bovine proteins.
Plates are sealed into a bag with desiccant. Allow the microplate to reach room temperature before opening; reseal unused strips in the bag with desiccant and store at 2..8°C.

2. Calibration Curve: CAL N° …
Ready to use and color coded standard curve derived from human plasma positive for HSV1 IgG ranging:
4ml CAL1 = 0 arbU/ml
4ml CAL2 = 5 arbU/ml
2ml CAL3 = 10 arbU/ml
2ml CAL4 = 20 arbU/ml
2mlCAL5 = 50 arbU/ml
4ml CAL6 = 100 arbU/ml.
Standards are calibrated in arbitrary units against an internal Gold Standard (or IGS).
It contains human serum proteins, 2% casein, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.1% Tween 20, 0.09% Na-azide and 0.045% ProClin 300 as preservatives. Standards are blue colored.

3. Control Serum: CONTROL …ml
1 vial. Lyophilized. It contains fetal bovine serum proteins, human IgG antibodies to HSV1 at about 20 arbU/ml ±20%, 0.2 mg/ml gentamicine sulphate and 0.045% ProClin 300 as preservatives.
Note: The volume necessary to dissolve the content of the vial may vary from lot to lot. Please use the right volume reported on the label.

4. Wash buffer concentrate: WASHBUF 20X
1x60ml/bottle20x concentrated solution. Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2, 0.05% Tween 20 and 0.045% ProClin 300.

5. Enzyme conjugate : CONJ
2x8ml/vial. Ready to use and red colour coded. It contains Horseradish peroxidase conjugated polyclonal antibodies to human IgG, 5% BSA, 10 mM Tris buffer pH 6.8+/-0.1, 0.045% ProClin 300, 0.02% gentamicine sulphate as preservatives and 0.01% red alimentary dye.

6. Chromogen/Substrate: SUBS TMB
1x16ml/vial. It contains 50 mM citrate-phosphate buffer pH 3.5-3.8, 4% dimethylsulphoxide, 0.03% tetra-methyl-benzidine (or TMB) and 0.02% hydrogen peroxide (or H_2O_2).
Note: To be stored protected from light as sensitive to strong illumination.

7. Sulphuric Acid: H_2SO_4 0.3 M
1x15ml/vial. It contains 0.3 M H_2SO_4 solution.
Attention: Irritant (H315, H319; P280, P302+P352, 332+P313, P305+ P351+P338, P337+P313, P362+P363)

8. Specimen Diluent: DILSPE
2x60ml/vial. It contains 2% casein, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.1% Tween 20, 0.09% Na-azide 0.1% and 0.045% ProClin 300 as preservatives. The reagent is blue colour coded.

9. Plate sealing foils n°2

10. Package insert n°1

E. MATERIALS REQUIRED BUT NOT PROVIDED

1. Calibrated Micropipettes (1000 ul, 100 ul and 10 ul) and disposable plastic tips.
2. EIA grade water (double distilled or deionised, charcoal treated to remove oxidizing chemicals used as disinfectants).
3. Timer with 60 minute range or higher.
4. Absorbent paper tissues.
5. Calibrated ELISA microplate thermostatic incubator (dry or wet), set at +37°C (+/-0.5°C tolerance)..
6. Calibrated ELISA microwell reader with 450nm (reading) and with 620-630nm (blanking) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.

F. WARNINGS AND PRECAUTIONS

1. The kit has to be used by skilled and properly trained technical personnel only, under the supervision of a medical doctor responsible of the laboratory.
2. All the personnel involved in performing the assay have to wear protective laboratory clothes, talc-free gloves and glasses. The use of any sharp (needles) or cutting (blades) devices should be avoided. All the personnel involved should be trained in biosafety procedures, as recommended by the Center for Disease Control, Atlanta, U.S. and reported in the National Institute of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
3. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.
4. The laboratory environment should be controlled so as to avoid contaminants such as dust or air-born microbial agents, when opening kit vials and microplates and when performing the test. Protect the Chromogen (TMB) from strong light and avoid vibration of the bench surface where the test is undertaken.
5. Upon receipt, store the kit at 2..8°C into a temperature controlled refrigerator or cold room.
6. Do not interchange components between different lots of the kits. It is recommended that components between two kits of the same lot should not be interchanged.
7. Check that the reagents are clear and do not contain visible heavy particles or aggregates. If not, advise the laboratory supervisor to initiate the necessary procedures for kit replacement.
8. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each sample. Do not reuse disposable tips.
9. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one. Do not reuse disposable tips.
10. Do not use the kit after the expiration date stated on the external container and internal (vials) labels. A study conducted on an opened kit did not pointed out any relevant loss of activity up to six 6 uses of the device and up to 3 months.
11. Treat all specimens as potentially infective. All human serum specimens should be handled at Biosafety Level 2, as recommended by the Center for Disease Control, Atlanta, U.S. in compliance with what reported in the Institutes of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
12. The use of disposable plastic-ware is recommended in the preparation of the liquid components or in transferring components into automated workstations, in order to avoid cross contamination.
13. Waste produced during the use of the kit has to be discarded in compliance with national directives and laws concerning laboratory waste of chemical and biological substances. In particular, liquid waste generated from the washing procedure, from residuals of controls and from samples has to be treated as potentially infective material and inactivated before waste. Suggested procedures of inactivation are treatment with a 10% final concentration of household bleach for 16-18 hrs or heat inactivation by autoclave at 121°C for 20 min..
14. Accidental spills from samples and operations have to be adsorbed with paper tissues soaked with household bleach and then with water. Tissues should then be discarded in proper containers designated for laboratory/hospital waste.
15. The Sulphuric Acid is an irritant. In case of spills, wash the surface with plenty of water
16. Other waste materials generated from the use of the kit (example: tips used for samples and controls, used microplates) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

G. SPECIMEN: PREPARATION AND WARNINGS

1. Blood is drawn aseptically by venepuncture and plasma or serum is prepared using standard techniques of preparation of samples for clinical laboratory analysis. No influence has been observed in the preparation of the sample with citrate, EDTA and heparin.
2. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results. Bar code labeling and electronic reading is strongly recommended.
3. Haemolysed ("red") and visibly hyperlipemic ("milky") samples have to be discarded as they could generate false results. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they could give rise to false results.
4. Sera and plasma can be stored at +2°…+8°C in primary collection tubes for up to five days after collection.

Do not freeze primary tubes of collection. For longer storage periods, sera and plasma samples, carefully removed from the primary collection tube, can be stored frozen at –20°C for several months. Any frozen samples should not be frozen/thawed more than once as this may generate particles that could affect the test result.

5. If particles are present, centrifuge at 2.000 rpm for 20 min or filter using 0.2-0.8u filters to clean up the sample for testing.

H. PREPARATION OF COMPONENTS AND WARNINGS

A study conducted on an opened kit has not pointed out any relevant loss of activity up to 6 re-uses of the device and up to 3 months.

Microplate:

Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant has not turned dark green, indicating a defect in storing.
In this case, call Dia.Pro's customer service.
Unused strips have to be placed back into the aluminum pouch, with the desiccant supplied, firmly zipped and stored at +2°..8°C.
After first opening, remaining strips are stable until the humidity indicator inside the desiccant bag turns from yellow to green.

Calibration Curve

Ready to use component. Mix carefully on vortex before use.

Control Serum

Add the volume of ELISA grade water, reported on the label, to the lyophilised powder; let fully dissolve and then gently mix on vortex.
Note: *The control after dissolution is not stable. Store frozen in aliquots at –20°C.*

Wash buffer concentrate:

The whole content of the concentrated solution has to be diluted 20x with bidistilled water and mixed gently end-over-end before use. During preparation avoid foaming as the presence of bubbles could impact on the efficiency of the washing cycles.
Note: *Once diluted, the wash solution is stable for 1 week at +2..8° C.*

Enzyme conjugate:

Ready to use. Mix well on vortex before use.
Be careful not to contaminate the liquid with oxidizing chemicals, air-driven dust or microbes.
If this component has to be transferred use only plastic, possibly sterile disposable containers.

Chromogen/Substrate:

Ready to use. Mix well on vortex before use.
Be careful not to contaminate the liquid with oxidizing chemicals, air-driven dust or microbes.
Do not expose to strong illumination, oxidizing agents and metallic surfaces.

If this component has to be transferred use only plastic, possible sterile disposable container

Sample Diluent
Ready to use component. Mix carefully on vortex before use.

Sulphuric Acid:
Ready to use. Mix well on vortex before use.
Attention: Irritant (H315, H319; P280, P302+P352, 332+P313, P305+ P351+P338, P337+P313, P362+P363).

Legenda:

Warning **H statements:**
H315 – Causes skin irritation.
H319 – Causes serious eye irritation.

Precautionary **P statements:**
P280 – Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352 – IF ON SKIN: Wash with plenty of soap and water.
P332 + P313 – If skin irritation occurs: Get medical advice/attention.
P305 + P351 + P338 – IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337 + P313 – If eye irritation persists: Get medical advice/attention.
P362 + P363 - Take off contaminated clothing and wash it before reuse.

I. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

1. Micropipettes have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (household alcohol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample. They should also be regularly maintained in order to show a precision of 1% and a trueness of +/-2%. Decontamination of spills or residues of kit components should also be carried out regularly.
2. The ELISA incubator has to be set at +37°C (tolerance of +/- 0.5°C) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water baths are suitable for the incubations, provided that the instrument is validated for the incubation of ELISA tests.
3. The **ELISA washer** is extremely important to the overall performances of the assay. The washer must be carefully validated in advance, checked for the delivery of the right dispensation volume and regularly submitted to maintenance according to the manufacturer's instructions for use. In particular the washer, at the end of the daily workload, has to be extensively cleaned out of salts with deionized water. Before use, the washer has to be extensively primed with the diluted Washing Solution.
   The instrument weekly has to be submitted to decontamination according to its manual (NaOH 0.1 M decontamination suggested).
   5 washing cycles (aspiration + dispensation of 350ul/well of washing solution + 20 sec soaking = 1 cycle) are sufficient to ensure the assay with the declared performances. If soaking is not possible add one more cycle of washing.
   An incorrect washing cycle or salt-blocked needles are the major cause of false positive reactions.
4. Incubation times have a tolerance of $\pm$5%.
5. The ELISA microplate reader has to be equipped with a reading filter of 450nm and with a second filter of 620-630nm, mandatory for blanking purposes. Its standard performances should be (a) bandwidth $\leq$ 10 nm; (b) absorbance range from 0 to $\geq$ 2.0; (c) linearity to $\geq$ 2.0; repeatability $\geq$ 1%. Blanking is carried out on the well identified in the section "Assay Procedure". The optical system of the reader has to be calibrated regularly to ensure that the correct optical density is measured. It should be regularly maintained according to the manufacturer 's instructions.
6. When using an ELISA automated work station, all critical steps (dispensation, incubation, washing, reading, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the section "Internal Quality Control".The assay protocol has to be installed in the operating system of the unit and validated as for the washer and the reader. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set. Particular attention must be paid to avoid carry over by the needles used for dispensing and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells. The use of ELISA automated work stations is recommended when the number of samples to be tested exceed 20-30 units per run.
7. Dia.Pro's customer service offers support to the user in the setting and checking of instruments used in combination with the kit, in order to assure compliance with the requirements described. Support is also provided for the installation of new instruments to be used with the kit.

L. PRE ASSAY CONTROLS AND OPERATIONS

1. Check the expiration date of the kit printed on the external label (primary container). Do not use if expired.
2. Check that the liquid components are not contaminated by visible particles or aggregates.
3. Check that the Chromogen (TMB) is colourless or pale blue by aspirating a small volume of it with a sterile plastic pipette.
4. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box (primary container). Check that the aluminium pouch, containing the microplate, is not punctured or damaged.
5. Dissolve the content of the lyophilised Control Serum as reported in the proper section.
6. Dilute all the content of the 20x concentrated Wash Solution as described above.
7. Allow all the other components to reach room temperature (about 1 hr) and then mix gently on vortex all liquid reagents.
8. Set the ELISA incubator at +37°C and prepare the ELISA washer by priming with the diluted washing solution, according to the manufacturers instructions. Set the right number of washing cycles as reported in the specific section.
9. Check that the ELISA reader is turned on or ensure it will be turned on at least 20 minutes before reading.
10. If using an automated work station, turn on, check settings and be sure to use the right assay protocol.
11. Check that the micropipettes are set to the required volume.
12. Check that all the other equipment is available and ready to use.
13. In case of problems, do not proceed further with the test and advise the supervisor.

M. ASSAY PROCEDURE

The assay has to be carried out according to what reported below, taking care to maintain the same incubation time for all the samples in testing.
The kit may be used for quantitative and qualitative determinations as well.

M1. QUANTITATIVE DETERMINATION:

Automated assay:

In case the test is carried out automatically with an ELISA system, we suggest to make the instrument aspirate 1000 µl Sample Diluent and then 10 µl sample (1:101 dilution factor). The whole content is then dispensed into a properly defined dilution tube. Before the next sample is aspirated, needles have

to be duly washed to avoid any cross-contamination among samples. When all the samples have been diluted make the instrument dispense 100 µl samples into the proper wells of the microplate.
This procedure may be carried out also in two steps of dilutions of 1:10 each (90 µl Sample Diluent + 10 µl sample) into a second dilution platform. Make then the instrument aspirate first 100 µl Sample Diluent, then 10 µl liquid from the first dilution in the platform and finally dispense the whole content in the proper well of the assay microplate.
Do not dilute Calibrators and the dissolved Control Serum as they are ready to use.
Dispense 100 µl calibrators/control in the appropriate calibration/control wells.
For the next operations follow the operative instructions reported below for the Manual Assay.
It is strongly recommended to check that the time lap between the dispensation of the first and the last sample will be calculated by the instrument and taken into consideration by delaying the first washing operation accordingly.

Manual assay:

1. Dilute samples 1:101 into a properly defined dilution tube (example: 1000 µl Sample Diluent + 10 µl sample). Do not dilute the Calibration Set as calibrators are ready to use. Mix carefully all the liquid components on vortex and then proceed as described below.
2. Place the required number of microwells in the microwell holder. Leave the A1 and B1 empty for the operation of blanking.
3. Dispense 100 µl of Calibrators and 100 µl Control Serum in duplicate. Then dispense 100 µl of diluted samples in each properly identified well.
4. Incubate the microplate for **60 min at +37°C**.

Important note: *Strips have to be sealed with the adhesive sealing foil, supplied, only when the test is carried out manually. Do not cover strips when using ELISA automatic instruments.*

5. Wash the microplate with an automatic washer as reported previously (section I.3).
6. Pipette 100 µl Enzyme Conjugate into each well, except A1+B1 blanking wells, and cover with the sealer. Check that this red coloured component has been dispensed in all the wells, except A1 and B1.

Important note: *Be careful not to touch the plastic inner surface of the well with the tip filled with the Enzyme Conjugate. Contamination might occur.*

7. Incubate the microplate for **60 min at +37°C**.
8. Wash microwells as in step 5.
9. Pipette 100 µl Chromogen/Substrate mixture into each well, the blank wells A1 and B1 included. Then incubate the microplate at **room temperature (18-24°C) for 20 minutes**.

Important note: *Do not expose to strong direct illumination. High background might be generated.*

10. Pipette 100 µl Sulphuric Acid to stop the enzymatic reaction into all the wells using the same pipetting sequence as in step 9. Addition of acid will turn the positive calibrators, the control serum and the positive samples from blue to yellow.
11. Measure the colour intensity of the solution in each well, as described in section I.5, at 450nm filter (reading) and at 620-630nm (background subtraction, mandatory), blanking the instrument on A1 or B1 or both.

M2. QUALITATIVE DETERMINATION
If only a qualitative determination is required, proceed as described below:

Automated assay:

Proceed as described in section M1.

Manual assay:

1. Dilute samples 1:101 into a properly defined dilution tube (example: 1000 µl Sample Diluent + 10 µl sample). Do not dilute the Calibration Set as calibrators are ready to use. Mix carefully all the liquid components on vortex and then proceed as described below.
2. Place the required number of Microwells in the microwell holder. Leave A1 well empty for the operation of blanking.
3. Dispense 100 µl of Calibrator 0 arbU/ml and Calibrator 5 arbU/ml in duplicate and Calibrator 100 arbU/ml in single. Then dispense 100 µl of diluted samples in each properly identified well.
4. Incubate the microplate for **60 min at +37°C**.

Important note: *Strips have to be sealed with the adhesive sealing foil, supplied, only when the test is carried out manually. Do not cover strips when using ELISA automatic instruments.*

5. Wash the microplate with an automatic washer as reported previously (section I.3).
6. Pipette 100 µl Enzyme Conjugate into each well, except the A1 well, and cover with the sealer. Check that this red coloured component has been dispensed in all the wells, except A1.

Important note: *Be careful not to touch the plastic inner surface of the well with the tip filled with the Enzyme Conjugate. Contamination might occur.*

7. Incubate the microplate for **60 min at +37°C**.
8. Wash microwells as in step 5.
9. Pipette 100 µl Chromogen/Substrate mixture into each well, the blank well included. Then incubate the microplate at **room temperature (18-24°C) for 20 minutes**.

Important note: *Do not expose to strong direct illumination. High background might be generated.*

10. Pipette 100 µl Sulphuric Acid into all the wells using the same pipetting sequence as in step 9. Addition of acid will turn the positive calibrators, the control serum and the positive samples from blue to yellow .
11. Measure the colour intensity of the solution in each well, as described in section I.5, at 450nm filter (reading) and at 620-630nm (background subtraction, mandatory), blanking the instrument on A1.

General Important notes:

1. *Ensure that no finger prints are present on the bottom of the microwell before reading. Finger prints could generate false positive results on reading.*

2. *Reading has to be carried out just after the addition of the Stop Solution and anyway not any longer than 20 minutes after its addition. Some self oxidation of the chromogen can occur leading to high background.*

N. ASSAY SCHEME

Method	Operations
Calibrators & Control (*) Samples diluted 1:101	100 µl 100 µl
1st incubation	60 min
Temperature	+37°C
Wash step	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
Enzyme conjugate	100 µl
2nd incubation	60 min
Temperature	+37°C
Wash step	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
TMB/H2O2	100 µl
3rd incubation	20 min
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm / 620-630nm

(*) Important Notes:

- *The Control Serum (CS) it does not affect the test's results calculation.*
- *The Control Serum (CS) used only if a laboratory internal quality control is required by the Management.*

An example of dispensation scheme for Quantitative Analysis is reported below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	CAL4	S 1									
B	BLK	CAL4	S 2									
C	CAL1	CAL5	S 3									
D	CAL1	CAL5	S 4									
E	CAL2	CAL6	S 5									
F	CAL2	CAL6	S 6									
G	CAL3	CS(*)	S 7									
H	CAL3	CS(*)	S 8									

Legenda: BLK = Blank CAL = Calibrator
CS(*) = Control Serum - Not mandatory S = Sample

An example of dispensation scheme in qualitative assays is reported below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S 3	S 11									
B	CAL1	S 4	S 12									
C	CAL1	S 5	S 13									
D	CAL2	S 6	S 14									
E	CAL2	S 7	S 15									
F	CAL6	S 8	S 16									
G	S 1	S 9	S 17									
H	S 2	S 10	S 18									

Legenda: BLK = Blank CAL = Calibrators
S = Sample

O. INTERNAL QUALITY CONTROL

A validation check is carried out on the calibrators any time the kit is used in order to verify whether the performances of the assay are as qualified.

Control that the following data are matched:

Check	Requirements
Blank well	< 0.050 OD450nm value
CAL 1 0 arbU/ml	< 0.150 mean OD450nm value after blanking coefficient of variation < 30%
CAL 2 5 arbU/ml	OD450nm > OD450nm CAL1 + 0.100
CAL 6 100 arbU/ml	OD450nm > 1.000

If the results of the test match the requirements stated above, proceed to the next section.

If they do not, do not proceed any further and operate as follows:

Problem	Check
Blank well > 0.050 OD450nm	1. that the Chromogen/Substrate solution has not got contaminated during the assay
CAL 1 **0 arbU/ml** > 0.150 OD450nm after blanking coefficient of variation > 30%	1. that the washing procedure and the washer settings are as validated in the pre qualification study; 2. that the proper washing solution has been used and the washer has been primed with it before use; 3. that no mistake has been done in the assay procedure (dispensation of a positive calibrator instead of the negative one; 4. that no contamination of the negative calibrator or of their wells has occurred due spills of positive samples or the enzyme conjugate; 5. that micropipettes haven't got contaminated with positive samples or with the enzyme conjugate 6. that the washer needles are not blocked or partially obstructed.
CAL 2 **5 arbU/ml** OD450nm < OD450nm CAL1 + 0.100	1. that the procedure has been correctly executed; 2. that no mistake has been done in its distribution (ex.: dispensation of a wrong calibrator instead); 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the calibrator has occurred.
CAL 6 **100 arbU/ml** < 1.000 OD450nm	1. that the procedure has been correctly executed; 2. that no mistake has been done in its distribution (dispensation of a wrong calibrator instead) ; 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the positive control has occurred.

Should one of these problems have happened, after checking, report to the supervisor for further actions.

**** Note:**

If Control Serum has used, verify the following data:

Check	Requirements
Control Serum	Mean OD450nm CAL 4 ± 20%

If the results of the test doesn't match the requirements stated above, operate as follows:

Problem	Check
Control Serum Different from expected value	1. that the procedure has been correctly executed; 2. that no mistake has been done in its distribution (dispensation of a wrong calibrator instead); 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the control serum has occurred.

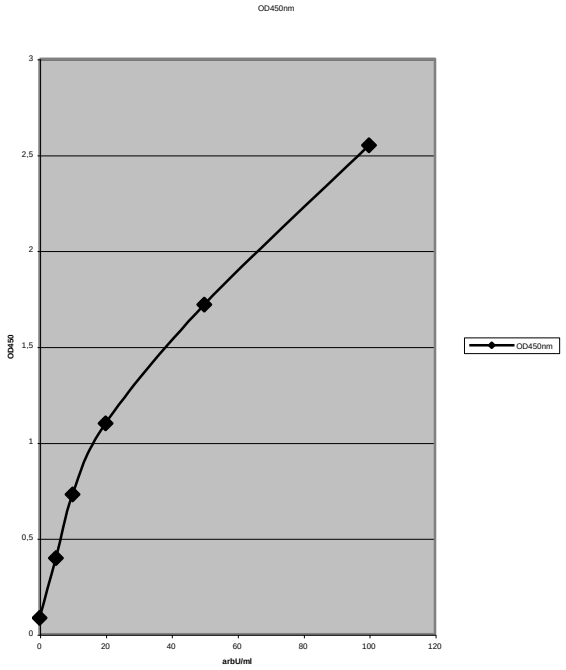
Anyway, if all other parameters (Blank, CAL1, CAL2, CAL6), match the established requirements, the test may be considered valid.

P. RESULTS

P.1 Quantitative method
If the test turns out to be valid, use for the quantitative method an approved curve fitting program to draw the calibration curve from the values obtained by reading at 450nm (4-parameters interpolation is suggested).
Then on the calibration curve calculate the concentration of anti Herpes Simplex Virus type 1 IgG antibody in samples.

An example of Calibration curve is reported in the next page.

Example of Calibration Curve :



Important Note:
Do not use the calibration curve above to make calculations.

P.2 Qualitative method
In the qualitative method, calculate the mean OD450nm values for the Calibrators 0 and 5 arbU/ml and then check that the assay is valid.

Example of calculation:

The following data must not be used instead or real figures obtained by the user.

Calibrator 0 arbU/ml: 0.020 – 0.024 OD450nm
Mean Value: 0.022 OD450nm
Lower than 0.150 – Accepted

Calibrator 5 arbU/ml: 0.350 – 0.370 OD450nm
Mean Value: 0.360 OD450nm
Higher than Cal 0 + 0.100 – Accepted

Calibrator 100 arbU/ml: 2.245 OD450nm
Higher than 1.000 – Accepted

Q. INTERPRETATION OF RESULTS
Samples with a concentration lower than 5 arbU/ml are considered negative for anti HSV1 IgG antibody.
Samples with a concentration higher than 5 arbU/ml are considered positive for anti HSV1 IgG antibody.

Particular attention in the interpretation of results has to be used in the follow-up of pregnancy for a primary infection of HSV due to the risk of neonatal malformations.

Important notes:
1. *Interpretation of results should be done under the supervision of the laboratory supervisor to reduce the risk of judgment errors and misinterpretations.*
2. *When test results are transmitted from the laboratory to another facility, attention must be paid to avoid erroneous data transfer.*
3. *In the follow-up of pregnancy for HSV infection a positive result (presence of IgG antibody > 5 arbU/ml) should be confirmed to ruled out the risk of a false positive result and a false definition of protection.*

R. PERFORMANCES

1. Limit of detection
The limit of detection of the assay has been calculated by means of an internal Gold Standard in absence of an international preparation to refer to.
The limit of detection has been calculated as mean OD450nm Calibrator 0 arbU/ml + 5 SD.
The table below reports the mean OD450nm values of this standard when diluted in negative plasma and then examined in the assay for three lots.

Mean OD450nm values (n = 2)

IgG arbU/ml	HSV1G.PU Lot # 0703	HSV1G.PU Lot # 1203	HSV1G.PU Lot # 0204/2
0	0.077	0.034	0.043
5	0.355	0.404	0.318
10	0.742	0.713	0.516
20	1.254	1.216	0.944
50	1.952	1.928	1.728
100	2.623	2.261	2.072

The assay shows a limit of detection far better than 5 arbU/ml.

In addition the preparation code Accurun n° 150, produced by Boston Biomedica Inc., BBI, USA, was tested in dilutions to determine the limit of its detection and provide a further value of analytical sensitivity

Mean OD450nm values (n = 2)

Dilution	HSV1G.CE Lot # 1004	HSV1G.PU Lot # 1203	HSV1G.PU Lot # 0204/2
1 X	1.248	1.218	1.300
2 X	0.860	0.848	0.876
4 X	0.545	0.526	0.583
8 X	0.315	0.300	0.329
16 X	0.164	0.152	0.148
32 X	0.082	0.064	0.072
0 arbU/ml	0.057	0.050	0.047
5 arbU/ml	0.288	0.355	0.318

2. Diagnostic sensitivity:
The diagnostic sensitivity has been tested in a performance evaluation study on panels of samples classified positive by a kit US FDA approved. Positive samples from different stage of HSV infection were tested. The value, obtained from the analysis of more than 300 specimens, has been > 98%.

3. Diagnostic specificity:
The diagnostic specificity has been determined on panels of negative samples from not infected individuals, classified negative with a kit US FDA approved.
Both plasma, derived with different standard techniques of preparation (citrate, EDTA and heparin), and sera have been used to determine the value of specificity.
Frozen specimens have been tested, as well, to check for interferences due to collection and storage.
No interference was observed.
Potentially interfering samples derived from patients with different pathologies (mostly ANA, AMA and RF positive) and from pregnant women were tested.
No crossreaction was observed.
An overall value > 98% of specificity was found when examined on more than 100 specimens.

3. Precision:
It has been calculated on the Calibrator 5 arbU/ml, considered the cut-off of the assay, examined in 16 replicates in three separate runs for three lots.
Results are reported as follows:

HSV1G.CE Lot # 1004

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.292	0.290	0.285	0.289
Std.Deviation	0.024	0.024	0.027	0.025
CV %	8.24	8.28	9.42	8.65

HSV1G.PU: lot 1203

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.365	0.382	0.378	0.375
Std.Deviation	0.022	0.029	0.018	0.023
CV %	6.02	7.59	4.76	6.12

HSV1G.PU: Lot 0204/2

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.322	0.298	0.304	0.308
Std.Deviation	0.018	0.019	0.016	0.018
CV %	5.59	6.38	5.26	5.74

The variability shown in the tables above did not result in sample misclassification.

S. LIMITATIONS OF THE PROCEDURE
Bacterial contamination or heat inactivation of the specimen may affect the absorbance values of the samples with consequent alteration of the level of the analyte.
Frozen samples containing fibrin particles or aggregates after thawing may generate some false results.
This test is suitable only for testing single samples and not pooled ones.
Diagnosis of an infectious disease should not be established on the basis of a single test result. The patient's clinical history, symptomatology, as well as other diagnostic data should be considered.

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All the IVD Products manufactured by the company are under the control of a certified Quality Management System in compliance with ISO 13485 rule. Each lot is submitted to a quality control and released into the market only if conforming with the EC technical specifications and acceptance criteria.

Manufacturer:
Dia.Pro Diagnostic Bioprobes Srl
Via G. Carducci n° 27 – Sesto San Giovanni (MI) – Italy

CE

Dia.Pro
Diagnostic
BioProbes

EC DECLARATION OF CONFORMITY

MANUFACTURER	DIA.PRO DIAGNOSTIC BIOPROBES S.R.L. VIA G. CARDUCCI N° 27 – 20099 SESTO SAN GIOVANNI (MILANO) – ITALY
PRODUCT	**HSV1 IgG** CODE: **HSV1G.CE** (96 tests)
CLASSIFICATION	GENERAL IVD
CONFORMITY ASSESSMENT ROUTE	SELF CERTIFICATION

WE HEREBY DECLARE THAT THE ABOVE MENTIONED PRODUCT MEETS THE PROVISIONS OF THE COUNCIL DIRECTIVE 98/79/EC FOR IN VITRO DIAGNOSTIC DEVICES.

ISO CERTIFICATE	UNE EN ISO 13485 N° 2013 11 0039 EN, RELEASED BY AEMPS (AGENCIA ESPAÑOLA DE MEDICAMENTOS Y PRODUCTOS SANITARIOS)

PLACE & DATE OF FIRST ISSUE	MILANO – OCTOBER 2004
PLACE & DATE OF CURRENT ISSUE	SESTO SAN GIOVANNI (MI) – MARCH 2019
SIGNATURE **Legal Representative** **Dr.ssa Fiorenza Scozzesi**	

Rev:	05/2018

HSV1 IgM

"Capture" Enzyme Immuno Assay (ELISA) for the determination of IgM antibodies to Herpes Simplex Virus type 1 in human plasma and sera

- for "in vitro" diagnostic use only -

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REF HSV1M.CE
96 tests

HSV1 IgM

A. INTENDED USE
Enzyme ImmunoAssay (ELISA) for the determination of IgM antibodies to Herpes Simplex Virus types 1 in human plasma and sera with the "capture" system. The device is intended for the follow-up of HSV1 infected patients and for the monitoring of risk of neonatal defects due to HSV infection during pregnancy.
For “in vitro” diagnostic use only.

B. INTRODUCTION
Herpes Simplex Virus type 1 (HSV1) and type 2 (HSV2) are large complex DNA-containing viruses which have been shown to induce the synthesis of several proteins during infection, possessing an high number of cross-reactive determinants and just a few of type-specific sequences.
The majority of primary and recurrent genital herpetic infections are caused by HSV2; while non genital infections, such as common cold sores, are caused primarily by HSV1.
The detection of virus specific IgG and IgM antibodies are important in the diagnosis of acute/primary virus infections or reactivations of a latent one, in the absence of evident clinical symptoms.
A-symptomatic infections may happen for HSV in apparently healthy individuals and during pregnancy. Severe herpetic infections may happen in immuno-compromised and suppressed patients in which the disease may evolve toward critical pathologies.
The determination of HSV specific antibodies has then become important in the monitoring of “risk” patients and in the follow up of acute and severe infections.

C. PRINCIPLE OF THE TEST
The assay is based on the principle of "IgM capture" where IgM class antibodies in the sample are first captured by the solid phase coated with anti hIgM antibody.
After washing out all the other components of the sample and in particular IgG antibodies, the specific IgM captured on the solid phase are detected by the addition of a preparation of inactivated HSV1, labeled with a HSV1 specific antibody conjugated with peroxidase (HRP).
After incubation, microwells are washed to remove unbound conjugate and then the chromogen/substrate is added.
In the presence of bound conjugate the colorless substrate is hydrolyzed to a colored end-product, whose optical density may be detected and is proportional to the amount of IgM antibodies to HSV1 present in the sample.
A system is described how to control whether the positivity shown by a sample is true or not (Confirmation Test), helpful for the clinician to make a correct interpretation of results.

D. COMPONENTS
The kit contains reagents for 96 tests.

1. Microplate: MICROPLATE
12 strips x 8 microwells coated with anti human IgM affinity purified goat antibody, in presence of bovine proteins.
Plates are sealed into a bag with desiccant. Allow the microplate to reach room temperature before opening; reseal unused strips in the bag with desiccant and store at 2..8°C.

2. Negative Control: CONTROL -
1x4.0 ml/vial. Ready to use control. It contains 1% human serum proteins, 2% casein, 10 mM tris buffer pH 6.0+/-0.1, 0.1% Tween 20, 0.09% sodium azide and 0.1% Kathon GC as preservatives.
The negative control is cpale yellow color coded..

3. Positive Control: CONTROL +
1x4.0 ml/vial. Ready to use control. It contains 1% human serum positive for HSV1 IgM, 2% casein, 10 mM tris buffer pH 6.0+/-0.1, 0.1% Tween 20, 0.09% sodium azide and 0.1% Kathon GC as preservatives.
The positive control is green colour coded.

4. Calibrator: CAL …ml
N° 1 lyophilized vial. To be dissolved with EIA grade water as reported in the label. It contains anti HSV1 IgM, fetal bovine serum, 0.2 mg/ml gentamicine sulphate and 0.1% Kathon GC as preservatives.
Note: The volume necessary to dissolve the content of the vial may vary from lot to lot. Please use the right volume reported on the label.

5. Lyophilized HSV1 Ag: AG HSV1
N° 6 lyophilized vials. The vials contain gamma-ray inactivated HSV1 in protein buffer. The solution contains 2% bovine proteins, 10 mM Tris HCl buffer pH 6.8+/-0.1, 0.2 mg/ml gentamicine sulphate and 0.1% Kathon GC. To be dissolved with 1.9 ml of Antigen Diluent as reported in the specific section.

6. Wash buffer concentrate: WASHBUF 20X
1x60ml/bottle. 20x concentrated solution. Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2, 0.05% Tween 20 and 0.1% Kathon GC.

7. Enzyme conjugate: CONJ 20X
1x0.8 ml/vial. 20x concentrated solution of a HSV1-specific antibody, labeled with HRP and diluted in a protein buffer containing 10 mM Tris buffer pH 6.8+/-0.1, 2% BSA, 0.1% Kathon GC and 0.2 mg/ml gentamicine sulphate as preservatives.

8. Antigen Diluent : AG DIL
n° 1 vial of 16 ml. Protein buffer solution for the preparation of the Immunocomplex. The solution contains 10 mM Tris buffer pH 6.8+/-0.1, 2% BSA, 0.1% Kathon GC and 0.2 mg/ml gentamicine sulphate as preservatives. The reagent is code coloured with 0.01% red alimentary dye

9. Specimen Diluent : DILSPE
2x60.0 ml/vial. Proteic buffered solution for the dilution of samples. It contains 2% casein, 10 mM tris buffer pH 6.0+/-0.1, 0.1% Tween 20, 0.09% sodium azide and 0.1% Kathon GC as preservatives.
The reagent is color coded with 0.01% blue alimentary dye.

10. Chromogen/Substrate : SUBS TMB
1x16ml/vial. It contains a 50 mM citrate-phosphate buffered solution at pH 3.5-3.8, 0.03% tetra-methyl-benzidine (TMB), 0.02% hydrogen peroxide (H_2O_2) and 4% dimethylsulphoxide.
Note: To be stored protected from light as sensitive to strong illumination.

11. Sulphuric Acid: H_2SO_4 0.3 M
1x15ml/vial. It contains 0.3 M H_2SO_4 solution.
Attention: Irritant (H315, H319; P280, P302+P352, 332+P313, P305+ P351+P338, P337+P313, P362+P363)

12. Plate sealing foils n° 2

13. Package insert n° 1

E. MATERIALS REQUIRED BUT NOT PROVIDED

1. Calibrated Micropipettes (1000 ul, 100 ul and 10 ul) and disposable plastic tips.
2. EIA grade water (double distilled or deionised, charcoal treated to remove oxidizing chemicals used as disinfectants).
3. Timer with 60 minute range or higher.
4. Absorbent paper tissues.
5. Calibrated ELISA microplate thermostatic incubator (dry or wet), set at +37°C (+/-0.5°C tolerance)..
6. Calibrated ELISA microwell reader with 450nm (reading) and with 620-630nm (blanking) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.

F. WARNINGS AND PRECAUTIONS

1. The kit has to be used by skilled and properly trained technical personnel only, under the supervision of a medical doctor responsible of the laboratory.
2. All the personnel involved in performing the assay have to wear protective laboratory clothes, talc-free gloves and glasses. The use of any sharp (needles) or cutting (blades) devices should be avoided. All the personnel involved should be trained in biosafety procedures, as recommended by the Center for Disease Control, Atlanta, U.S. and reported in the National Institute of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
3. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.
4. The laboratory environment should be controlled so as to avoid contaminants such as dust or air-born microbial agents, when opening kit vials and microplates and when performing the test. Protect the Chromogen (TMB) from strong light and avoid vibration of the bench surface where the test is undertaken.
5. Upon receipt, store the kit at 2..8°C into a temperature controlled refrigerator or cold room.
6. Do not interchange components between different lots of the kits. It is recommended that components between two kits of the same lot should not be interchanged.
7. Check that the reagents are clear and do not contain visible heavy particles or aggregates. If not, advise the laboratory supervisor to initiate the necessary procedures for kit replacement.
8. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each sample.
9. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one.
10. Do not use the kit after the expiration date stated on the external container and internal (vials) labels. A study conducted on an opened kit did not pointed out any relevant loss of activity up to six 6 uses of the device and up to 3 months.
11. Treat all specimens as potentially infective. All human serum specimens should be handled at Biosafety Level 2, as recommended by the Center for Disease Control, Atlanta, U.S. in compliance with what reported in the Institutes of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
12. The use of disposable plastic-ware is recommended in the preparation of the liquid components or in transferring components into automated workstations, in order to avoid cross contamination.
13. Waste produced during the use of the kit has to be discarded in compliance with national directives and laws concerning laboratory waste of chemical and biological substances. In particular, liquid waste generated from the washing procedure, from residuals of controls and from samples has to be treated as potentially infective material and inactivated before waste. Suggested procedures of inactivation are treatment with a 10% final concentration of household bleach for 16-18 hrs or heat inactivation by autoclave at 121°C for 20 min..
14. Accidental spills from samples and operations have to be adsorbed with paper tissues soaked with household bleach and then with water. Tissues should then be discarded in proper containers designated for laboratory/hospital waste.
15. The Sulphuric Acid is an irritant. In case of spills, wash the surface with plenty of water
16. Other waste materials generated from the use of the kit (example: tips used for samples and controls, used microplates) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

G. SPECIMEN: PREPARATION AND WARNINGS

1. Blood is drawn aseptically by venepuncture and plasma or serum is prepared using standard techniques of preparation of samples for clinical laboratory analysis. No influence has been observed in the preparation of the sample with citrate, EDTA and heparin.
2. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results. Bar code labeling and electronic reading is strongly recommended.
3. Haemolysed ("red") and visibly hyperlipemic ("milky") samples have to be discarded as they could generate false results. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they could give rise to false results.
4. Sera and plasma can be stored at +2°..8°C for up to five days after collection. For longer storage periods, samples can be stored frozen at –20°C for several months. Any frozen samples should not be frozen/thawed more than once as this may generate particles that could affect the test result.
5. If particles are present, centrifuge at 2.000 rpm for 20 min or filter using 0.2-0.8u filters to clean up the sample for testing.

H. PREPARATION OF COMPONENTS AND WARNINGS

A study conducted on an opened kit has not pointed out any relevant loss of activity up to 6 re-uses of the device and up to 3 months.

Microplate:

Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant has not turned dark green, indicating a defect in storing.
In this case, call Dia.Pro's customer service.
Unused strips have to be placed back into the aluminum pouch, with the desiccant supplied, firmly zipped and stored at +2°..8°C. After first opening, remaining strips are stable until the humidity indicator inside the desiccant bag turns from yellow to green.

Negative Control:

Ready to use. Mix well on vortex before use.

Positive Control:

Ready to use. Mix well on vortex before use.

Calibrator:

Add the volume of ELISA grade water reported on the label to the lyophilized powder. Let fully dissolve and then gently mix on vortex.
Important Note*: The solution is not stable. Store the Calibrator frozen in aliquots at –20°C.*

Wash buffer concentrate:

The whole content of the concentrated solution has to be diluted 20x with bidistilled water and mixed gently end-over-end before use. During preparation avoid foaming as the presence of bubbles could impact on the efficiency of the washing cycles.
Note: *Once diluted, the wash solution is stable for 1 week at +2..8° C.*

Ag/Ab Immunocomplex:
Proceed carefully as follows:
1. Dissolve the content of a lyophilized vial with 1.9 ml of Conjugate/Antigen Diluent. Let fully dissolved the lyophilized content and then gently mix on vortex.
2. Gently mix the concentrated Enzyme Conjugate on vortex. Then add 0.1 ml of it to the vial of the dissolved HSV1 Ag and mix gently on vortex.

Important Notes*:*
1. *Dissolve and prepare only the number of vials necessary to the test. The Immunocomplex obtained is not stable. Store any residual solution frozen in aliquots at –20°C.*
2. *The preparation of the Immucomplex has to be done **right before** the dispensation of samples and controls into the plate. Mix again on vortex gently just before its use.*

Specimen Diluent:
Ready to use. Mix well on vortex before use

Chromogen/Substrate:
Ready to use. Mix well on vortex before use.
Be careful not to contaminate the liquid with oxidizing chemicals, air-driven dust or microbes.
Do not expose to strong illumination, oxidizing agents and metallic surfaces.
If this component has to be transferred use only plastic, possible sterile disposable container

Sulphuric Acid:
Ready to use. Mix well on vortex before use.
Attention: Irritant (H315, H319; P280, P302+P352, 332+P313, P305+ P351+P338, P337+P313, P362+P363).

Legenda:

Warning **H statements:**
H315 – Causes skin irritation.
H319 – Causes serious eye irritation.

Precautionary **P statements:**
P280 – Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352 – IF ON SKIN: Wash with plenty of soap and water.
P332 + P313 – If skin irritation occurs: Get medical advice/attention.
P305 + P351 + P338 – IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337 + P313 – If eye irritation persists: Get medical advice/attention.
P362 + P363 - Take off contaminated clothing and wash it before reuse.

I. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

1. Micropipettes have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (household alcohol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample. They should also be regularly maintained in order to show a precision of 1% and a trueness of +/-2%. Decontamination of spills or residues of kit components should also be carried out regularly.
2. The ELISA incubator has to be set at +37°C (tolerance of +/- 0.5°C) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water baths are suitable for the incubations, provided that the instrument is validated for the incubation of ELISA tests.
3. The ELISA washer is extremely important to the overall performances of the assay. The washer must be carefully validated and correctly optimised using the kit controls and reference panels, before using the kit for routine laboratory tests. Usually 4-5 washing cycles (aspiration + dispensation of 350ul/well of washing solution = 1 cycle) are sufficient to ensure that the assay performs as expected. A soaking time of 20-30 seconds between cycles is suggested. In order to set correctly their number, it is recommended to run an assay with the kit controls and well characterized negative and positive reference samples, and check to match the values reported below in the section “Internal Quality Control”. Regular calibration of the volumes delivered by, and maintenance (decontamination and cleaning of needles) of the washer has to be carried out according to the instructions of the manufacturer.
4. Incubation times have a tolerance of $\pm$5%.
5. The ELISA microplate reader has to be equipped with a reading filter of 450nm and with a second filter (620-630nm, strongly recommended) for blanking purposes. Its standard performances should be (a) bandwidth $\leq$ 10 nm; (b) absorbance range from 0 to $\geq$ 2.0; (c) linearity to $\geq$ 2.0; repeatability $\geq$ 1%. Blanking is carried out on the well identified in the section “Assay Procedure”. The optical system of the reader has to be calibrated regularly to ensure that the correct optical density is measured. It should be regularly maintained according to the manufacturer ‘s instructions.
6. When using an ELISA automated work station, all critical steps (dispensation, incubation, washing, reading, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the section “Internal Quality Control”. The assay protocol has to be installed in the operating system of the unit and validated as for the washer and the reader. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set. Particular attention must be paid to avoid carry over by the needles used for dispensing and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells. The use of ELISA automated work stations is recommended when the number of samples to be tested exceed 20-30 units per run.
7. Dia.Pro’s customer service offers support to the user in the setting and checking of instruments used in combination with the kit, in order to assure compliance with the requirements described. Support is also provided for the installation of new instruments to be used with the kit.

L. PRE ASSAY CONTROLS AND OPERATIONS

1. Check the expiration date of the kit printed on the external label (primary container). Do not use the device if expired.
2. Check that the liquid components are not contaminated by visible particles or aggregates. Check that the Chromogen/Substrate is colorless or pale blue by aspirating a small volume of it with a sterile plastic pipette. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box (primary container). Check that the aluminum pouch, containing the microplate, is not punctured or damaged.
3. Dilute all the content of the 20x concentrated Wash Solution as described above.
4. Dissolve the Calibrator as described above and gently mix.
5. Allow all the other components to reach room temperature (about 1 hr) and then mix gently on vortex all liquid reagents.
6. Set the ELISA incubator at +37°C and prepare the ELISA washer by priming with the diluted washing solution, according to the manufacturers instructions. Set the right number of washing cycles as found in the validation of the instrument for its use with the kit.
7. Check that the ELISA reader is turned on or ensure it will be turned on at least 20 minutes before reading.
8. If using an automated work station, turn on, check settings and be sure to use the right assay protocol.
9. Check that the micropipettes are set to the required volume.

10. Check that all the other equipment is available and ready to use.
11. In case of problems, do not proceed further with the test and advise the supervisor.

M. ASSAY PROCEDURE

The assay has to be carried out according to what reported below, taking care to maintain the same incubation time for all the samples in testing.

M.1 Automated assay:

In case the test is carried out automatically with an ELISA system, we suggest to make the instrument aspirate 1000 µl Specimen Diluent and then 10 µl sample (1:101 dilution factor). The whole content is then dispensed into a properly defined dilution tube. Before the next sample is aspirated, needles have to be duly washed to avoid any cross-contamination among samples. When all the samples have been diluted make the instrument dispense 100 µl diluted samples into the proper wells of the microplate.

This procedure may be carried out also in two steps of dilutions of 1:10 each (90 µl Specimen Diluent + 10 µl sample) into a second dilution platform. Make then the instrument aspirate first 100 µl Specimen Diluent, then 10 µl liquid from the first dilution in the platform and finally dispense the whole content in the proper well of the assay microplate.

Do not dilute controls/calibrator as they are ready to use.

Dispense 100 µl calibrators/controls in the appropriate calibration/control wells.

For the next operations follow the operative instructions reported below for the Manual Assay.

It is strongly recommended to check that the time lap between the dispensation of the first and the last sample will be calculated by the instrument and taken into consideration by delaying the first washing operation accordingly.

M. 2 Manual assay:

1. Dilute samples 1:101 by dispensing first 10 µl sample and then 1 ml Specimen Diluent into a dilution tube; mix gently on vortex.
2. Place the required number of Microwells in the microwell holder. Leave the well in position A1 empty for the operation of blanking.
3. Dispense 100 µl of Negative Control and 100 µl of Calibrator in the proper wells in duplicate. Dispense 100 µl of Positive Control in single into the proper well. Do not dilute controls and the calibrator as they are ready to use !
4. Dispense 100 µl diluted samples in the proper sample wells and then check that all the samples wells are blue colored and that controls and calibrator have been dispensed.
5. Incubate the microplate for **60 min at +37°C** .

Important note: *Strips have to be sealed with the adhesive sealing foil, supplied, only when the test is carried out manually. Do not cover strips when using ELISA automatic instruments.*

6. Wash the microplate with an automatic washer as reported previously (section I.3).

7. Pipette 100 µl of the **Ag/Ab Immunocomplex** into each well, except the blanking well A1, and cover with the sealer. Check that all wells are red colored, except A1.

Important note: *Be careful not to touch the plastic inner surface of the well with the tip filled with the* **Ag/Ab Immunocomplex***. Contamination might occur.*

8. Incubate the microplate for **60 min at +37°C** .

9. Wash microwells as in step 6.

10. Pipette 100 µl Chromogen/Substrate mixture into each well, the blank well included. Then incubate the microplate at **room temperature (18-24°C) for 20 minutes**.

Important note: *Do not expose to strong direct illumination. High background might be generated.*

11. Pipette 100 µl Sulphuric Acid into all the wells using the same pipetting sequence as in step 10. Addition of acid will turn the positive control and positive samples from blue to yellow.
12. Measure the color intensity of the solution in each well, as described in section I.5, at 450nm filter (reading) and at 620-630nm (background subtraction, strongly recommeded), blanking the instrument on A1.

Important notes:

1. *If the second filter is not available ensure that no finger prints are present on the bottom of the microwell before reading at 450nm. Finger prints could generate false positive results on reading.*
2. *Reading has to be carried out just after the addition of the Stop Solution and anyway not any longer than 20 minutes after its addition. Some self oxidation of the chromogen can occur leading to high background.*

N. ASSAY SCHEME

Controls&calibrator(*) Samples diluted 1:101	100 ul 100 ul
1st incubation	**60 min**
Temperature	+37°C
Washing	4-5 cycles
Immunocomplex	100 ul
2nd incubation	**60 min**
Temperature	+37°C
Washing	4-5 cycles
TMB/H2O2 mix	100 ul
3rd incubation	**20 min**
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm

(*) Important Notes:

- *The Calibrator (CAL) does not affect the Cut Off calculation, therefore it does not affect the test's results calculation.*
- *The Calibrator (CAL) used only if a laboratory internal quality control is required by the Management.*

An example of dispensation scheme is reported below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S3										
B	NC	S4										
C	NC	S5										
D	CAL(*)	S6										
E	CAL(*)	S7										
F	PC	S8										
G	S1	S9										
H	S2	S10										

Legenda: BLK = Blank NC = Negative Control
CAL(*) = Calibrator-Not mandatory PC = Positive Control S = Sample

O. INTERNAL QUALITY CONTROL

A validation check is carried out on the controls any time the kit is used in order to verify whether the performances of the assay are as qualified.
Control that the following data are matched:

Parameter	Requirements
Blank well	< 0.05 OD450nm value
Negative Control mean value (NC)	< 0.200 OD450nm value after blanking coefficient of variation < 30%
Positive Control	> 1.000 OD450nm

If the results of the test match the requirements stated above, proceed to the next section.
If they do not, do not proceed any further and perform the following checks:

Problem	Check
Blank well > 0.05 OD450nm	1. that the Chromogen/Substrate solution has not become contaminated during the assay
Negative Control (NC) > 0.200 OD450nm after blanking coefficient of variation > 30%	1. that the washing procedure and the washer settings are as validated in the pre qualification study; 2. that the proper washing solution has been used and the washer has been primed with it before use; 3. that no mistake has been done in the assay procedure (dispensation of positive control instead of negative control; 4. that no contamination of the negative control or of the wells where the control was dispensed has occurred due to positive samples, to spills or to the enzyme conjugate; 5. that micropipettes have not become contaminated with positive samples or with the enzyme conjugate 6. that the washer needles are not blocked or partially obstructed.
Positive Control < 1.000 OD450nm	1. that the procedure has been correctly performed; 2. that no mistake has occurred during the distribution of the control (dispensation of negative control instead of positive control). 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the positive control has occurred.

If any of the above problems have occurred, report the problem to the supervisor for further actions.

**** Important Note:**
If the Calibrator has used, verify the following data:

Check	Requirements
Calibrator	S/Co > 1.2

If the results of the test doesn't match the requirements stated above, operate as follows:

Problem	Check
Calibrator S/Co < 1.2	1. that the procedure has been correctly performed; 2. that no mistake has occurred during its distribution (e.g.: dispensation of negative control instead); 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the calibrator has occurred.

Anyway, if all other parameters (Blank, Negative Control, Positive Control), match the established requirements, the test may be considered valid.

P. CALCULATION OF THE CUT-OFF

The test results are calculated by means of the mean OD450nm value of the Negative Control (NC) and a mathematical calculation, in order to define the following cut-off formulation:

Cut-Off = NC + 0.250

The value found for the test is used for the interpretation of results as described in the next paragraph.

***Important note**: When the calculation of results is performed by the operating system of an ELISA automated work station, ensure that the proper formulation is used to calculate the cut-off value and generate the correct interpretation of results.*

Q. INTERPRETATION OF RESULTS

Test results are interpreted as a ratio of the sample OD450nm and the Cut-Off value (or S/Co) according to the following table:

S/Co	Interpretation
< 1.0	Negative
1.0 - 1.2	Equivocal
> 1.2	Positive

A negative result indicates that the patient is not undergoing an acute infection of Herpes Simplex Virus type 1.
Any patient showing an equivocal result, should be re-tested by examining a second sample taken from the patient after 1-2 weeks from first testing.
A positive result is indicative of a Herpes Simplex Virus type 1 infection.

An example of calculation is reported below:

***Important Note:** The following data must not be used instead or real figures obtained by the user.*

Negative Control: 0.100 – 0.120 – 0.080 OD450nm
Mean Value: 0.100 OD450nm
Lower than 0.150 – Accepted
Positive Control: 1.850 OD450nm
Higher than 1.000 – Accepted

Cut-Off = 0.110+0.250 = 0.360

Calibrator: 1.000 - 0.900 OD450nm
Mean value: 0.950 OD450nm S/Co = 2.6
S/Co higher than 1.2 – Accepted

Sample 1: 0.075 OD450nm
Sample 2: 1.580 OD450nm
Sample 1 S/Co < 1 = negative
Sample 2 S/Co > 1.2 = positive

Important notes:

1. *Interpretation of results should be done under the supervision of the laboratory supervisor to reduce the risk of judgment errors and misinterpretations.*
2. *Particular attention in the interpretation of results has to be used in the follow-up of pregnancy for an infection of HSV due to the risk of severe neonatal malformations.*
3. *In pregnancy monitoring, it is strongly recommended that any positive result is confirmed first with the procedure described below and secondly with a different device for HSV IgM detection, before taking any preventive medical action.*
4. *Any positive sample should be submitted to the Confirmation Test reported in section T before giving a result of positivity. By carrying out this test, false reactions, leading to a misinterpretation of the analytical result, can be revealed and then ruled out.*
5. *When test results are transmitted from the laboratory to another facility, attention must be paid to avoid erroneous data transfer.*
6. *Diagnosis of infection has to be taken and released to the patient by a suitably qualified medical doctor.*

R. PERFORMANCE CHARACTERISTICS

1. Limit of detection

No international standard for HSV1&2 IgM Antibody detection has been defined so far by the European Community.
In its absence, an Internal Gold Standard (or IGS), calibrated on the preparation named "Accurun – Anti HSV2 IgM plasma" produced by Boston Biomedica Inc., USA, code 9106072, has been defined in order to provide the device with a constant and excellent sensitivity.
The limit of detection of the assay has been therefore calculated on the IGS. A limiting dilution curve was prepared in the Negative Control (NC).
Results of Quality Control are given in the following table:

OD450nm values

IGS	HSV1M.CE Lot # RD1	HSV1M.CE Lot # RD2	HSV1M.CE Lot # RD3
1X	0.450	0.460	0.455
2X	0.277	0.300	0.288
4X	0.216	0.198	0.185
NC	0.115	0.085	0.086

2. Diagnostic sensitivity:

The diagnostic sensitivity has been tested in a performance evaluation study on panels of 40 samples classified positive by a CE marked kit. The value obtained from the analysis was > 98%.

3. Diagnostic specificity:

The diagnostic specificity has been determined in the performance evaluation on panels of more than 300 specimens, negative with the reference kit, derived from normal individuals of European origin.
Both plasma, derived with different standard techniques of preparation (citrate, EDTA and heparin), and sera have been used to determine the specificity. No false reactivity due to the method of specimen preparation has been observed.
Frozen specimens have also been tested to check whether this interferes with the performance of the test. No interference was observed on clean and particle free samples.
A study conducted on more than 60 potentially cross-reactive samples has not revealed any interference in the system.
No cross reaction were observed.
The Performance Evaluation has provided a value > 98%.
False positive reactions may be anyway pointed out and then ruled out in the interpretation of results with the procedure reported in section T, able to verify whether or not a positive result is real.

4. Precision:

Results are reported as follows:

HSV1M.CE: lot # RD1

Negative (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.083	0.107	0.116	0.102
Std.Deviation	0.004	0.017	0.013	0.011
CV %	5.12	15.82	11.59	10.84

Low reactive (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.393	0.436	0.421	0.417
Std.Deviation	0.031	0.019	0.007	0.019
CV %	7.93	4.38	1.68	4.66

High reactive (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	1.469	1.530	1.541	1.513
Std.Deviation	0.034	0.055	0.037	0.042
CV %	2.31	3.60	2.39	2.77

HSV1M.CE: lot # RD2

Negative (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.101	0.099	0.097	0.099
Std.Deviation	0.009	0.011	0.013	0.011
CV %	8.91	11.11	13.40	11.14

Low reactive (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.412	0.395	0.420	0.409
Std.Deviation	0.015	0.009	0.012	0.012
CV %	3.64	2.27	2.86	2.92

High reactive (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	1.512	1.498	1.534	1.515
Std.Deviation	0.042	0.035	0.028	0.035
CV %	2.78	2.34	1.83	2.31

HSV1M.CE: lot # RD3

Negative (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.095	0.112	0.092	0.100
Std.Deviation	0.012	0.009	0.010	0.011
CV %	12.6	8.04	10.86	10.50

Low reactive (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.405	0.398	0.412	0.405
Std.Deviation	0.012	0.015	0.014	0.014
CV %	2.96	3.77	3.40	3.37

High reactive (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	1.489	1.475	1.518	1.494
Std.Deviation	0.025	0.032	0.028	0.028
CV %	1.68	2.17	1.84	1.90

S. LIMITATIONS

Frozen samples containing fibrin particles or aggregates may generate false positive results.

Bacterial contamination or heat inactivation of the specimen may affect the absorbance values of the samples with consequent alteration of the level of the analyte.

This test is suitable only for testing single samples and not pooled ones.

Diagnosis of an infectious disease should not be established on the basis of a single test result. The patient's clinical history, symptomatology, as well as other diagnostic data should be considered.

T. CONFIRMATION TEST

In order to provide the medical doctor with the best accuracy in the follow-up of pregnancy, where a false positive result could lead to an operation of abortion, a confirmation test is reported. The confirmation test has to be carried out on any positive sample before a diagnosis of primary infection of HSV is released to the doctor.

Proceed for confirmation as follows:

1. Prepare the Antigen/Conjugate Complex as described in the proper section. This reagent is called Solution A.
2. Then 25 ul concentrated Enzymatic Conjugate are diluted in 500 ul Antigen Diluent and mixed gently on vortex. Do not use any lyophilized antigen vial for this procedure ! This solution is called Solution B.
3. The well A1 of the strip is left empty for blanking.
4. The Negative Control is dispensed in the strip in positions B1+C1. This is used for the calculation of the cut-off and S/Co values.
5. The positive sample to be confirmed, diluted 1:101, is dispensed in the strip in position D1+E1.
6. The strip is incubated for 60 min at +37°C.
7. After washing, the blank well A1 is left empty.
8. 100 µl of Solution A are dispensed in wells B1+C1+D1.
9. Then 100 µl of Solution B are added to well E1.
10. The strip is incubated for 60 min at +37°C.
11. After washing, 100 µl Chromogen/Substrate are added to all the wells and the strip is incubated for 20 min at r.t.
12. 100 µl Sulphuric Acid are added to all the wells and then their color intensity is measured at 450nm (reading filter) and at 620-630nm (background subtraction,), blanking the instrument on A1.

Interpretation of results is carried out as follows:

1. If the sample in position D1 shows a S/Co value lower than 1.0 a problem of dispensation or contamination in the first test is likely to be occurred. The Assay Procedure in Section M has to be repeated to double check the analysis.
2. If the sample in position D1 shows a S/Co value higher than 1.2 and in position E1 shows a S/Co value still higher than 1.2 the sample is considered a **false positive**. The reactivity of the sample is in fact not dependent on the specific presence of HSV1 and a crossreaction with enzymatic conjugate has occurred.
3. If the sample in position D1 shows a S/Co value higher than 1.2 and in position E1 shows a S/Co value lower than 1.0 the sample is considered a **true positive**. The reactivity of the sample is in fact dependent on the specific presence of HSV and not due to any crossreaction.

The following table is reported for the interpretation of results

Well	S/Co		
D1	< 1.0	> 1.2	> 1.2
E1	< 1.0	> 1.2	< 1.0
Interpretation	Problem of contam.	False positive	True positive

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All the IVD Products manufactured by the company are under the control of a certified Quality Management System in compliance with ISO 13485 rule. Each lot is submitted to a quality control and released into the market only if conforming with the EC technical specifications and acceptance criteria.

Manufacturer:
Dia.Pro Diagnostic Bioprobes Srl
Via G. Carducci n° 27 – Sesto San Giovanni (MI) – Italy

CE

Dia.Pro
Diagnostic
BioProbes

EC DECLARATION OF CONFORMITY

MANUFACTURER	DIA.PRO DIAGNOSTIC BIOPROBES S.R.L. VIA G. CARDUCCI N° 27 – 20099 SESTO SAN GIOVANNI (MILANO) – ITALY
PRODUCT	**HSV1 IgM** CODE: **HSV1M.CE** (96 tests)
CLASSIFICATION	GENERAL IVD
CONFORMITY ASSESSMENT ROUTE	SELF CERTIFICATION

WE HEREBY DECLARE THAT THE ABOVE MENTIONED PRODUCT MEETS THE PROVISIONS OF THE COUNCIL DIRECTIVE 98/79/EC FOR IN VITRO DIAGNOSTIC DEVICES.

ISO CERTIFICATE	UNE EN ISO 13485 N° 2013 11 0039 EN, RELEASED BY AEMPS (AGENCIA ESPAÑOLA DE MEDICAMENTOS Y PRODUCTOS SANITARIOS)

PLACE & DATE OF FIRST ISSUE	MILANO – OCTOBER 2004
PLACE & DATE OF CURRENT ISSUE	SESTO SAN GIOVANNI (MI) – MARCH 2019
SIGNATURE **Legal Representative** **Dr.ssa Fiorenza Scozzesi**	

Rev:	05/2018

HSV2 IgG

Enzyme ImmunoAssay (ELISA) for the quantitative/qualitative determination of IgG antibodies to Herpes Simplex Virus type 2 in human serum and plasma

- for “in vitro” diagnostic use only -

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REF HSV2G.CE
96 Tests

HSV2 IgG

A. INTENDED USE

Enzyme ImmunoAssay (ELISA) for the quantitative/qualitative determination of IgG antibodies to Herpes Simplex Virus type 2 in human plasma and sera.
For "in vitro" diagnostic use only.

B. INTRODUCTION

Herpes Simplex Virus type 1 (HSV1) and type 2 (HSV2) are large complex DNA-containing viruses which have been shown to induce the synthesis of several proteins during infection, possessing an high number of crossreactive determinants and just a few of type-specific sequences.
The majority of primary and recurrent genital herpetic infections are caused by HSV2; while non genital infections, such as common cold sores, are caused primarily by HSV1.
The detection of virus specific IgG and IgM antibodies are important in the diagnosis of acute/primary virus infections or reactivations of a latent one, in the absence of evident clinical symptoms.
Asymptomatic infections may happen for HSV in apparently healthy individuals and during pregnancy. Severe herpetic infections may happen in immunocompromised and suppressed patients in which the disease may evolve toward critical pathologies.
The determination of HSV specific antibodies has then become important in the monitoring of "risk" patients and in the follow up of acute and severe infections.

C. PRINCIPLE OF THE TEST

Microplates are coated with synthetic HSV2 specific glycoprotein G or gG.
The solid phase is first treated with the diluted sample and IgG to HSV2 are captured, if present, by the antigens.
After washing out all the other components of the sample, in the 2nd incubation bound anti HSV2 IgG are detected by the addition of polyclonal specific anti hIgG antibodies, labelled with peroxidase (HRP).
The enzyme captured on the solid phase, acting on the substrate/chromogen mixture, generates an optical signal that is proportional to the amount of anti HSV2 IgG antibodies present in the sample. A Calibration Curve, calibrated against an internal Gold Standard, makes possible a quantitative determination of the IgG antibody in the patient.

D. COMPONENTS

Each kit contains sufficient reagents to perform 96 tests.

1. Microplate: MICROPLATE
n° 1. 12 strips x 8 microwells coated with synthetic HSV2-specific gG in presence of bovine proteins.
Plates are sealed into a bag with desiccant. Allow the microplate to reach room temperature before opening; reseal unused strips in the bag with desiccant and store at 2..8°C.

2. Calibration Curve: CAL N° …
Ready to use and color coded standard curve derived from human plasma positive for HSV2 IgG ranging:
4ml CAL1 = 0 arbU/ml
4ml CAL2 = 5 arbU/ml
2ml CAL3 = 10 arbU/ml
2ml CAL4 = 20 arbU/ml
2ml CAL5 = 50 arbU/ml
4ml CAL6 = 100 arbU/ml.
Standards are calibrated in arbitrary units against an internal Gold Standard (or IGS).
It contains human serum proteins, 2% casein, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.1% Tween 20, 0.09% Na-azide and ProClin 300 0.045% as preservatives. Standards are blue colored.

3. Control Serum: CONTROL …ml
1 vial. Lyophilized. It contains fetal bovine serum proteins, human IgG antibodies to HSV2 at about 20 arbU/ml ± 20%, 0.2 mg/ml gentamicine sulphate and 0.045% ProClin 300 as preservatives.
Note: The volume necessary to dissolve the content of the vial may vary from lot to lot. Please use the right volume reported on the label.

4. Wash buffer concentrate: WASHBUF 20X
1x60ml/bottle20x concentrated solution. Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2, 0.05% Tween 20 and 0.045% ProClin 300.

5. Enzyme conjugate : CONJ
2x8ml/vial. Ready to use and red colour coded. It contains Horseradish peroxidase conjugated polyclonal antibodies to human IgG, 5% BSA, 10 mM Tris buffer pH 6.8+/-0.1, 0.045% ProClin 300, 0.02% gentamicine sulphate as preservatives and 0.01% red alimentary dye.

6. Chromogen/Substrate: SUBS TMB
1x16ml/vial. It contains 50 mM citrate-phosphate buffer pH 3.5-3.8, 4% dimethylsulphoxide, 0.03% tetra-methyl-benzidine (or TMB) and 0.02% hydrogen peroxide (or H_2O_2).
Note: To be stored protected from light as sensitive to strong illumination.

7. Sulphuric Acid: H_2SO_4 0.3 M
1x15ml/vial. It contains 0.3 M H_2SO_4 solution.
Attention: Irritant (H315, H319; P280, P302+P352, 332+P313, P305+ P351+P338, P337+P313, P362+P363)

8. Specimen Diluent: DILSPE
2x60ml/vial. It contains 2% casein, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.1% Tween 20, 0.09% Na-azide 0.1% and 0.045% ProClin 300 as preservatives. The reagent is blue colour coded.

9. Plate sealing foils n°2

10. Package insert n°1

E. MATERIALS REQUIRED BUT NOT PROVIDED

1. Calibrated Micropipettes (1000 ul, 100 ul and 10 ul) and disposable plastic tips.
2. EIA grade water (double distilled or deionised, charcoal treated to remove oxidizing chemicals used as disinfectants).
3. Timer with 60 minute range or higher.
4. Absorbent paper tissues.
5. Calibrated ELISA microplate thermostatic incubator (dry or wet), set at +37°C (+/-0.5°C tolerance)..
6. Calibrated ELISA microwell reader with 450nm (reading) and with 620-630nm (blanking) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.

F. WARNINGS AND PRECAUTIONS

1. The kit has to be used by skilled and properly trained technical personnel only, under the supervision of a medical doctor responsible of the laboratory.
2. All the personnel involved in performing the assay have to wear protective laboratory clothes, talc-free gloves and glasses.

The use of any sharp (needles) or cutting (blades) devices should be avoided. All the personnel involved should be trained in biosafety procedures, as recommended by the Center for Disease Control, Atlanta, U.S. and reported in the National Institute of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
3. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.
4. The laboratory environment should be controlled so as to avoid contaminants such as dust or air-born microbial agents, when opening kit vials and microplates and when performing the test. Protect the Chromogen (TMB) from strong light and avoid vibration of the bench surface where the test is undertaken.
5. Upon receipt, store the kit at 2..8°C into a temperature controlled refrigerator or cold room.
6. Do not interchange components between different lots of the kits. It is recommended that components between two kits of the same lot should not be interchanged.
7. Check that the reagents are clear and do not contain visible heavy particles or aggregates. If not, advise the laboratory supervisor to initiate the necessary procedures for kit replacement.
8. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each sample. Do not reuse disposable tips.
9. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one. Do not reuse disposable tips.
10. Do not use the kit after the expiration date stated on the external container and internal (vials) labels. A study conducted on an opened kit did not pointed out any relevant loss of activity up to six 6 uses of the device and up to 3 months.
11. Treat all specimens as potentially infective. All human serum specimens should be handled at Biosafety Level 2, as recommended by the Center for Disease Control, Atlanta, U.S. in compliance with what reported in the Institutes of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
12. The use of disposable plastic-ware is recommended in the preparation of the liquid components or in transferring components into automated workstations, in order to avoid cross contamination.
13. Waste produced during the use of the kit has to be discarded in compliance with national directives and laws concerning laboratory waste of chemical and biological substances. In particular, liquid waste generated from the washing procedure, from residuals of controls and from samples has to be treated as potentially infective material and inactivated before waste. Suggested procedures of inactivation are treatment with a 10% final concentration of household bleach for 16-18 hrs or heat inactivation by autoclave at 121°C for 20 min..
14. Accidental spills from samples and operations have to be adsorbed with paper tissues soaked with household bleach and then with water. Tissues should then be discarded in proper containers designated for laboratory/hospital waste.
15. The Sulphuric Acid is an irritant. In case of spills, wash the surface with plenty of water
16. Other waste materials generated from the use of the kit (example: tips used for samples and controls, used microplates) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

G. SPECIMEN: PREPARATION AND WARNINGS

1. Blood is drawn aseptically by venepuncture and plasma or serum is prepared using standard techniques of preparation of samples for clinical laboratory analysis. No influence has been observed in the preparation of the sample with citrate, EDTA and heparin.
2. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results. Bar code labeling and electronic reading is strongly recommended.
3. Haemolysed ("red") and visibly hyperlipemic ("milky") samples have to be discarded as they could generate false results. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they could give rise to false results.
4. Sera and plasma can be stored at +2°…+8°C in primary collection tubes for up to five days after collection.
Do not freeze primary tubes of collection. For longer storage periods, sera and plasma samples, carefully removed from the primary collection tube, can be stored frozen at –20°C for several months. Any frozen samples should not be frozen/thawed more than once as this may generate particles that could affect the test result.
5. If particles are present, centrifuge at 2.000 rpm for 20 min or filter using 0.2-0.8u filters to clean up the sample for testing.

H. PREPARATION OF COMPONENTS AND WARNINGS

A study conducted on an opened kit has not pointed out any relevant loss of activity up to 6 re-uses of the device and up to 3 months.

Microplate:
Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant has not turned dark green, indicating a defect in storage.
In this case, call Dia.Pro's customer service.
Unused strips have to be placed back into the aluminum pouch, with the desiccant supplied, firmly zipped and stored at +2°..8°C.
After first opening, remaining strips are stable until the humidity indicator inside the desiccant bag turns from yellow to green.

Calibration Curve
Ready to use component. Mix carefully on vortex before use.

Control Serum
Add the volume of ELISA grade water, reported on the label, to the lyophilised powder; let fully dissolve and then gently mix on vortex.
Note: *The control after dissolution is not stable. Store frozen in aliquots at –20°C.*

Wash buffer concentrate:
The whole content of the concentrated solution has to be diluted 20x with bidistilled water and mixed gently end-over-end before use. During preparation avoid foaming as the presence of bubbles could impact on the efficiency of the washing cycles.
Note: *Once diluted, the wash solution is stable for 1 week at +2..8° C.*

Enzyme conjugate:
Ready to use. Mix well on vortex before use.
Be careful not to contaminate the liquid with oxidizing chemicals, air-driven dust or microbes.
If this component has to be transferred use only plastic, possibly sterile disposable containers.

Chromogen/Substrate:
Ready to use. Mix well on vortex before use.
Be careful not to contaminate the liquid with oxidizing chemicals, air-driven dust or microbes.
Do not expose to strong illumination, oxidizing agents and metallic surfaces.
If this component has to be transferred use only plastic, possible sterile disposable container

Sample Diluent
Ready to use component. Mix carefully on vortex before use.

Sulphuric Acid:
Ready to use. Mix well on vortex before use.
Attention: Irritant (H315, H319; P280, P302+P352, 332+P313, P305+ P351+P338, P337+P313, P362+P363).

Legenda:

Warning **H statements:**
H315 – Causes skin irritation.
H319 – Causes serious eye irritation.

Precautionary **P statements:**
P280 – Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352 – IF ON SKIN: Wash with plenty of soap and water.
P332 + P313 – If skin irritation occurs: Get medical advice/attention.
P305 + P351 + P338 – IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337 + P313 – If eye irritation persists: Get medical advice/attention.
P362 + P363 - Take off contaminated clothing and wash it before reuse.

I. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

1. Micropipettes have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (household alcohol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample. They should also be regularly maintained in order to show a precision of 1% and a trueness of +/-2%. Decontamination of spills or residues of kit components should also be carried out regularly.
2. The ELISA incubator has to be set at +37°C (tolerance of +/- 0.5°C) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water baths are suitable for the incubations, provided that the instrument is validated for the incubation of ELISA tests.
3. The **ELISA washer** is extremely important to the overall performances of the assay. The washer must be carefully validated in advance, checked for the delivery of the right dispensation volume and regularly submitted to maintenance according to the manufacturer's instructions for use. In particular the washer, at the end of the daily workload, has to be extensively cleaned out of salts with deionized water. Before use, the washer has to be extensively primed with the diluted Washing Solution.
   The instrument weekly has to be submitted to decontamination according to its manual (NaOH 0.1 M decontamination suggested).
   5 washing cycles (aspiration + dispensation of 350ul/well of washing solution + 20 sec soaking = 1 cycle) are sufficient to ensure the assay with the declared performances. If soaking is not possible add one more cycle of washing.
   An incorrect washing cycle or salt-blocked needles are the major cause of false positive reactions.
4. Incubation times have a tolerance of $\pm$5%.
5. The ELISA microplate reader has to be equipped with a reading filter of 450nm and with a second filter (620-630nm, mandatory) for blanking purposes. Its standard performances should be (a) bandwidth $\leq$ 10 nm; (b) absorbance range from 0 to $\geq$ 2.0; (c) linearity to $\geq$ 2.0; repeatability $\geq$ 1%. Blanking is carried out on the well identified in the section "Assay Procedure". The optical system of the reader has to be calibrated regularly to ensure that the correct optical density is measured. It should be regularly maintained according to the manufacturer 's instructions.
6. When using an ELISA automated work station, all critical steps (dispensation, incubation, washing, reading, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the section "Internal Quality Control''. The assay protocol has to be installed in the operating system of the unit and validated as for the washer and the reader. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set. Particular attention must be paid to avoid carry over by the needles used for dispensing and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells. The use of ELISA automated work stations is recommended when the number of samples to be tested exceed 20-30 units per run.
7. Dia.Pro's customer service offers support to the user in the setting and checking of instruments used in combination with the kit, in order to assure compliance with the requirements described. Support is also provided for the installation of new instruments to be used with the kit.

L. PRE ASSAY CONTROLS AND OPERATIONS

1. Check the expiration date of the kit printed on the external label (primary container). Do not use if expired.
2. Check that the liquid components are not contaminated by visible particles or aggregates.
3. Check that the Chromogen (TMB) is colourless or pale blue by aspirating a small volume of it with a sterile plastic pipette.
4. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box (primary container). Check that the aluminium pouch, containing the microplate, is not punctured or damaged.
5. Dissolve the content of the lyophilised Control Serum as reported in the proper section.
6. Dilute all the content of the 20x concentrated Wash Solution as described above.
7. Allow all the other components to reach room temperature (about 1 hr) and then mix gently on vortex all liquid reagents.
8. Set the ELISA incubator at +37°C and prepare the ELISA washer by priming with the diluted washing solution, according to the manufacturers instructions. Set the right number of washing cycles as reported in the specific section.
9. Check that the ELISA reader is turned on or ensure it will be turned on at least 20 minutes before reading.
10. If using an automated work station, turn on, check settings and be sure to use the right assay protocol.
11. Check that the micropipettes are set to the required volume.
12. Check that all the other equipment is available and ready to use.
13. In case of problems, do not proceed further with the test and advise the supervisor.

M. ASSAY PROCEDURE

The assay has to be carried out according to what reported below, taking care to maintain the same incubation time for all the samples in testing.
The kit may be used for quantitative and qualitative determinations as well.

M1. QUANTITATIVE DETERMINATION:

Automated assay:

In case the test is carried out automatically with an ELISA system, we suggest to make the instrument aspirate 1000 µl Sample Diluent and then 10 µl sample (1:101 dilution factor). The whole content is then dispensed into a properly defined dilution tube. Before the next sample is aspirated, needles have to be duly washed to avoid any cross-contamination among samples. When all the samples have been diluted make the instrument dispense 100 µl samples into the proper wells of the microplate.
This procedure may be carried out also in two steps of dilutions of 1:10 each (90 µl Sample Diluent + 10 µl sample) into a second dilution platform. Make then the instrument aspirate first

100 µl Sample Diluent, then 10 µl liquid from the first dilution in the platform and finally dispense the whole content in the proper well of the assay microplate.
Do not dilute Calibrators and the dissolved Control Serum as they are ready to use.
Dispense 100 µl calibrators/control in the appropriate calibration/control wells.
For the next operations follow the operative instructions reported below for the Manual Assay.
It is strongly recommended to check that the time lap between the dispensation of the first and the last sample will be calculated by the instrument and taken into consideration by delaying the first washing operation accordingly.

Manual assay:

1. Dilute samples 1:101 into a properly defined dilution tube (example: 1000 µl Sample Diluent + 10 µl sample). Do not dilute the Calibration Set as calibrators are ready to use. Mix carefully all the liquid components on vortex and then proceed as described below.
2. Place the required number of microwells in the microwell holder. Leave the A1 and B1 empty for the operation of blanking.
3. Dispense 100 µl of Calibrators and 100 µl Control Serum in duplicate. Then dispense 100 µl of diluted samples in each properly identified well.
4. Incubate the microplate for **60 min at +37°C**.

Important note: *Strips have to be sealed with the adhesive sealing foil, supplied, only when the test is carried out manually. Do not cover strips when using ELISA automatic instruments.*

5. Wash the microplate with an automatic washer as reported previously (section I.3).
6. Pipette 100 µl Enzyme Conjugate into each well, except A1+B1 blanking wells, and cover with the sealer. Check that this red coloured component has been dispensed in all the wells, except A1 and B1.

Important note: *Be careful not to touch the plastic inner surface of the well with the tip filled with the Enzyme Conjugate. Contamination might occur.*

7. Incubate the microplate for **60 min at +37°C**.
8. Wash microwells as in step 5.
9. Pipette 100 µl Chromogen/Substrate mixture into each well, the blank wells A1 and B1 included. Then incubate the microplate at **room temperature (18-24°C) for 20 minutes**.

Important note: *Do not expose to strong direct illumination. High background might be generated.*

10. Pipette 100 µl Sulphuric Acid to stop the enzymatic reaction into all the wells using the same pipetting sequence as in step 9. Addition of acid will turn the positive calibrators, the control serum and the positive samples from blue to yellow.
11. Measure the colour intensity of the solution in each well, as described in section I.5, at 450nm filter (reading) and at 620-630nm (background subtraction, mandatory), blanking the instrument on A1 or B1 or both.

M2. QUALITATIVE DETERMINATION
If only a qualitative determination is required, proceed as described below:

Automated assay:

Proceed as described in section M1.

Manual assay:

1. Dilute samples 1:101 into a properly defined dilution tube (example: 1000 µl Sample Diluent + 10 µl sample). Do not dilute the Calibration Set as calibrators are ready to use. Mix carefully all the liquid components on vortex and then proceed as described below.
2. Place the required number of Microwells in the microwell holder. Leave A1 well empty for the operation of blanking.
3. Dispense 100 µl of Calibrator 0 arbU/ml and Calibrator 5 arbU/ml in duplicate and Calibrator 100 arbU/ml in single. Then dispense 100 µl of diluted samples in each properly identified well.
4. Incubate the microplate for **60 min at +37°C**.

Important note: *Strips have to be sealed with the adhesive sealing foil, supplied, only when the test is carried out manually. Do not cover strips when using ELISA automatic instruments.*

5. Wash the microplate with an automatic washer as reported previously (section I.3).
6. Pipette 100 µl Enzyme Conjugate into each well, except the A1 well, and cover with the sealer. Check that this red coloured component has been dispensed in all the wells, except A1.

Important note: *Be careful not to touch the plastic inner surface of the well with the tip filled with the Enzyme Conjugate. Contamination might occur.*

7. Incubate the microplate for **60 min at +37°C**.
8. Wash microwells as in step 5.
9. Pipette 100 µl Chromogen/Substrate mixture into each well, the blank well included. Then incubate the microplate at **room temperature (18-24°C) for 20 minutes**.

Important note: *Do not expose to strong direct illumination. High background might be generated.*

10. Pipette 100 µl Sulphuric Acid into all the wells using the same pipetting sequence as in step 9. Addition of acid will turn the positive calibrators, the control serum and the positive samples from blue to yellow.
11. Measure the colour intensity of the solution in each well, as described in section I.5, at 450nm filter (reading) and at 620-630nm (background subtraction, mandatory), blanking the instrument on A1.

General Important notes:

1. *Ensure that no finger prints are present on the bottom of the microwell before reading. Finger prints could generate false positive results on reading.*

2. *Reading has to be carried out just after the addition of the Stop Solution and anyway not any longer than 20 minutes after its addition. Some self oxidation of the chromogen can occur leading to high background.*

N. ASSAY SCHEME

Method	Operations
Calibrators & Control (*) Samples diluted 1:101	100 µl 100 µl
1st incubation	60 min
Temperature	+37°C
Wash step	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
Enzyme conjugate	100 µl
2nd incubation	60 min
Temperature	+37°C
Wash step	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
TMB/H2O2	100 µl
3rd incubation	20 min
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm / 620-630nm

(*) Important Notes:

- *The Control Serum (CS) it does not affect the test's results calculation.*
- *The Control Serum (CS) used only if a laboratory internal quality control is required by the Management.*

An example of dispensation scheme for Quantitative Analysis is reported below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	CAL4	S 1									
B	BLK	CAL4	S 2									
C	CAL1	CAL5	S 3									
D	CAL1	CAL5	S 4									
E	CAL2	CAL6	S 5									
F	CAL2	CAL6	S 6									
G	CAL3	CS(*)	S 7									
H	CAL3	CS(*)	S 8									

Legenda: BLK = Blank CAL = Calibrator
CS(*) = Control Serum - Not mandatory S = Sample

An example of dispensation scheme in qualitative assays is reported below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S 2	S 10									
B	CAL1	S 3	S 11									
C	CAL1	S 4	S 12									
D	CAL2	S 5	S 13									
E	CAL2	S 6	S 14									
F	CAL6	S 7	S 15									
G	S1	S 8	S 16									
H	S2	S 9	S 17									

Legenda: BLK = Blank CAL = Calibrators
S = Sample

O. INTERNAL QUALITY CONTROL

A validation check is carried out on the calibrators any time the kit is used in order to verify whether the performances of the assay are as qualified.

Control that the following data are matched:

Check	Requirements
Blank well	< 0.050 OD450nm value
CAL 1 0 arbU/ml	< 0.150 mean OD450nm value after blanking coefficient of variation < 30%
CAL 2 5 arbU/ml	OD450nm ≥ OD450nm CAL1 + 0.100
CAL 6 100 arbU/ml	OD450nm ≥ 1.000

If the results of the test match the requirements stated above, proceed to the next section.

If they do not, do not proceed any further and operate as follows:

Problem	Check
Blank well > 0.050 OD450nm	1. that the Chromogen/Sustrate solution has not got contaminated during the assay
CAL 1 **0 arbU/ml** > 0.150 OD450nm after blanking coefficient of variation > 30%	1. that the washing procedure and the washer settings are as validated in the pre qualification study; 2. that the proper washing solution has been used and the washer has been primed with it before use; 3. that no mistake has been done in the assay procedure (dispensation of a positive calibrator instead of the negative one; 4. that no contamination of the negative calibrator or of their wells has occurred due spills of positive samples or the enzyme conjugate; 5. that micropipettes haven't got contaminated with positive samples or with the enzyme conjugate 6. that the washer needles are not blocked or partially obstructed.
CAL 2 **5 arbU/ml** OD450nm ≤ OD450nm CAL1 + 0.100	1. that the procedure has been correctly executed; 2. that no mistake has been done in its distribution (ex.: dispensation of a wrong calibrator instead); 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the calibrator has occurred.
CAL 6 **100 arbU/ml** ≤ 1.000 OD450nm	1. that the procedure has been correctly executed; 2. that no mistake has been done in its distribution (dispensation of a wrong calibrator instead) ; 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the positive control has occurred.

Should one of these problems have happened, after checking, report to the supervisor for further actions.

**** Note:**

If Control Serum has used, verify the following data:

Check	Requirements
Control Serum	Mean OD450nm CAL 4 ± 20%

If the results of the test doesn't match the requirements stated above, operate as follows:

Problem	Check
Control Serum Different from expected value	1. that the procedure has been correctly executed; 2. that no mistake has been done in its distribution (dispensation of a wrong calibrator instead); 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the control serum has occurred.

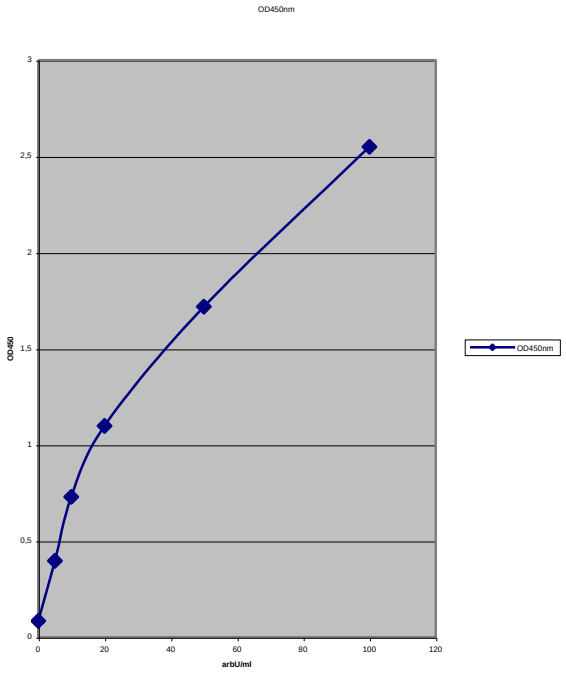
Anyway, if all other parameters (Blank, CAL1, CAL2, CAL6), match the established requirements, the test may be considered valid.

P. RESULTS

P.1 Quantitative method
If the test turns out to be valid, use for the quantitative method an approved curve fitting program to draw the calibration curve from the values obtained by reading at 450nm (4-parameters interpolation is suggested).
Then on the calibration curve calculate the concentration of anti Herpes Simplex Virus type 2 IgG antibody in samples.

An example of Calibration curve is reported in the next page.

Example of Calibration Curve :



Important Note:
Do not use the calibration curve above to make calculations.

P.2 Qualitative method
In the qualitative method, calculate the mean OD450nm values for the Calibrators 0 and 5 arbU/ml and then check that the assay is valid.

Example of calculation:

The following data must not be used instead or real figures obtained by the user.

Calibrator 0 arbU/ml: 0.020 – 0.024 OD450nm
Mean Value: 0.022 OD450nm
Lower than 0.150 – Accepted

Calibrator 5 arbU/ml: 0.350 – 0.370 OD450nm
Mean Value: 0.360 OD450nm
Higher than Cal 0 + 0.100 – Accepted

Calibrator 100 arbU/ml: 2.245 OD450nm
Higher than 1.000 – Accepted

Q. INTERPRETATION OF RESULTS
Samples with a concentration lower than 5 arbU/ml are considered negative for anti HSV2 IgG antibody.
Samples with a concentration higher than 5 arbU/ml are considered positive for anti HSV2 IgG antibody.

Particular attention in the interpretation of results has to be used in the follow-up of pregnancy for a primary infection of HSV due to the risk of neonatal malformations.

Important notes:
1. *Interpretation of results should be done under the supervision of the laboratory supervisor to reduce the risk of judgment errors and misinterpretations.*
2. *When test results are transmitted from the laboratory to another facility, attention must be paid to avoid erroneous data transfer.*
3. *In the follow-up of pregnancy for HSV infection a positive result (presence of IgG antibody > 5 arbU/ml) should be confirmed to ruled out the risk of a false positive result and a false definition of protection.*

R. PERFORMANCES

1. Limit of detection
The limit of detection of the assay has been calculated by means of an internal Gold Standard in absence of an international preparation to refer to.
The limit of detection has been calculated as mean OD450nm Calibrator 0 arbU/ml + 5 SD.
The table below reports the mean OD450nm values of this standard when diluted in negative plasma and then examined in the assay for three lots.

Mean OD450nm values (n = 2)

IgG arbU/ml	HSV2G.PU Lot # 1203	HSV2G.PU Lot # 1103	HSV2G Lot # 0304/2
0	0.022	0.030	0.014
5	0.353	0.384	0.269
10	0.596	0.606	0.557
20	1.169	1.471	0.895
50	2.030	2.276	1.776
100	3.102	3.353	2.893

The assay shows a limit of detection far better than 5 arbU/ml.

2. Diagnostic sensitivity:
The diagnostic sensitivity has been tested in a performance evaluation study on panels of samples classified positive by a kit US FDA approved. Positive samples from different stage of HSV infection were tested. The value, obtained from the analysis of more than 300 specimens, has been $\geq$ 98%.

3. Diagnostic specificity:
The diagnostic specificity has been determined on panels of negative samples from not infected individuals, classified negative with a kit US FDA approved.
Both plasma, derived with different standard techniques of preparation (citrate, EDTA and heparin), and sera have been used to determine the value of specificity.
Frozen specimens have been tested, as well, to check for interferences due to collection and storage.
No interference was observed.
Potentially interfering samples derived from patients with different pathologies (mostly ANA, AMA and RF positive) and from pregnant women were tested.
No crossreaction was observed.
An overall value > 98% of specificity was found when examined on more than 100 specimens.

3. Precision:
It has been calculated on the Calibrator 5 arbU/ml, considered the cut-off of the assay, examined in 16 replicates in three separate runs for three lots.

Results are reported as follows:

HSV2G.CE: lot 1004

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.286	0.303	0.256	0.282
Std.Deviation	0.022	0.037	0.020	0.026
CV %	7.7	12.4	7.74	9.28

HSV2G.PU: lot 1103

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.375	0.384	0.394	0.384
Std.Deviation	0.019	0.022	0.015	0.019
CV %	5.07	5.73	3.81	4.87

HSV2G.PU: lot 1203

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.352	0.345	0.332	0.343
Std.Deviation	0.017	0.020	0.024	0.020
CV %	4.83	5.78	7.23	5.95

The variability shown in the tables above did not result in sample misclassification.

S. LIMITATIONS OF THE PROCEDURE
Bacterial contamination or heat inactivation of the specimen may affect the absorbance values of the samples with consequent alteration of the level of the analyte.
Frozen samples containing fibrin particles or aggregates after thawing may generate some false results.
This test is suitable only for testing single samples and not pooled ones.
Diagnosis of an infectious disease should not be established on the basis of a single test result. The patient's clinical history, symptomatology, as well as other diagnostic data should be considered.

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All the IVD Products manufactured by the company are under the control of a certified Quality Management System in compliance with ISO 13485 rule. Each lot is submitted to a quality control and released into the market only if conforming with the EC technical specifications and acceptance criteria.

Manufacturer:
Dia.Pro Diagnostic Bioprobes S.r.l.
Via G. Carducci n° 27 – Sesto San Giovanni (MI) – Italy

CE

Dia.Pro
Diagnostic
BioProbes

EC DECLARATION OF CONFORMITY

MANUFACTURER	DIA.PRO DIAGNOSTIC BIOPROBES S.R.L. VIA G. CARDUCCI N° 27 – 20099 SESTO SAN GIOVANNI (MILANO) – ITALY
PRODUCT	**HSV2 IgG** CODE: **HSV2G.CE** (96 tests)
CLASSIFICATION	GENERAL IVD
CONFORMITY ASSESSMENT ROUTE	SELF CERTIFICATION

WE HEREBY DECLARE THAT THE ABOVE MENTIONED PRODUCT MEETS THE PROVISIONS OF THE COUNCIL DIRECTIVE 98/79/EC FOR IN VITRO DIAGNOSTIC DEVICES.

ISO CERTIFICATE	UNE EN ISO 13485 N° 2013 11 0039 EN, RELEASED BY AEMPS (AGENCIA ESPAÑOLA DE MEDICAMENTOS Y PRODUCTOS SANITARIOS)

PLACE & DATE OF FIRST ISSUE	MILANO – OCTOBER 2004
PLACE & DATE OF CURRENT ISSUE	SESTO SAN GIOVANNI (MI) – MARCH 2019
SIGNATURE **Legal Representative** **Dr.ssa Fiorenza Scozzesi**	

Rev:	05/2018

HSV2 IgM

"Capture" Enzyme Immuno Assay (ELISA) for the determination of IgM antibodies to Herpes Simplex Virus type 2 in human plasma and sera

- for "in vitro" diagnostic use only -

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REF HSV2M.CE
96 tests

HSV2 IgM

A. INTENDED USE

Enzyme ImmunoAssay (ELISA) for the determination of IgM antibodies to Herpes Simplex Virus types 2 in human plasma and sera with the "capture" system. The devise is intended for the follow-up of HSV2 infected patients and for the monitoring of risk of neonatal defects due to HSV infection during pregnancy.
For “in vitro” diagnostic use only.

B. INTRODUCTION

Herpes Simplex Virus type 1 (HSV1) and type 2 (HSV2) are large complex DNA-containing viruses which have been shown to induce the synthesis of several proteins during infection, possessing an high number of cross-reactive determinants and just a few of type-specific sequences.
The majority of primary and recurrent genital herpetic infections are caused by HSV2; while non genital infections, such as common cold sores, are caused primarily by HSV1.
The detection of virus specific IgG and IgM antibodies are important in the diagnosis of acute/primary virus infections or reactivations of a latent one, in the absence of evident clinical symptoms.
A-symptomatic infections may happen for HSV in apparently healthy individuals and during pregnancy. Severe herpetic infections may happen in immuno-compromised and suppressed patients in which the disease may evolve toward critical pathologies.
The determination of HSV specific antibodies has then become important in the monitoring of “risk” patients and in the follow up of acute and severe infections.

C. PRINCIPLE OF THE TEST

The assay is based on the principle of "IgM capture" where IgM class antibodies in the sample are first captured by the solid phase coated with anti hIgM antibody.
After washing out all the other components of the sample and in particular IgG antibodies, the specific IgM captured on the solid phase are detected by the addition of a preparation of inactivated HSV2, labeled with a HSV2 specific antibody conjugated with peroxidase (HRP).
After incubation, microwells are washed to remove unbound conjugate and then the chromogen/substrate is added.
In the presence of bound conjugate the colorless substrate is hydrolyzed to a colored end-product, whose optical density may be detected and is proportional to the amount of IgM antibodies to HSV2 present in the sample.
A system is described how to control whether the positivity shown by a sample is true or not (Confirmation Test), helpful for the clinician to make a correct interpretation of results.

D. COMPONENTS

The kit contains reagents for 96 tests.

1. Microplate: MICROPLATE
12 strips x 8 microwells coated with anti human IgM affinity purified goat antibody, in presence of bovine proteins.
Plates are sealed into a bag with desiccant. Allow the microplate to reach room temperature before opening; reseal unused strips in the bag with desiccant and store at 2..8°C.

2. Negative Control: CONTROL -
1x4.0 ml/vial. Ready to use control. It contains 1% human serum proteins, 2% casein, 10 mM tris buffer pH 6.0+/-0.1, 0.1% Tween 20, 0.09% sodium azide and 0.045% ProClin 300 as preservatives.
The negative control is pale yellow color coded.

3. Positive Control: CONTROL +
1x4.0 ml/vial. Ready to use control. It contains 1% human serum positive for HSV2 IgM, 2% casein, 10 mM tris buffer pH 6.0+/-0.1, 0.1% Tween 20, 0.09% sodium azide and 0.045% ProClin 300 as preservatives.
The positive control is green colour coded.

4. Calibrator: CAL …ml
N° 1 lyophilized vial. To be dissolved with EIA grade water as reported in the label. It contains anti HSV2 IgM, fetal bovine serum, 0.2 mg/ml gentamicine sulphate and 0.045% ProClin 300 as preservatives.
Note: The volume necessary to dissolve the content of the vial may vary from lot to lot. Please use the right volume reported on the label.

5. Lyophilized HSV2 Ag: AG HSV2
N° 6 lyophilized vials. The vials contain lyophilized gamma-ray inactivated HSV2 in protein buffer. The solution contains 2% bovine proteins, 10 mM Tris HCl buffer pH 6.8+/-0.1, 0.2 mg/ml gentamicine sulphate and 0.045% ProClin 300. To be dissolved with 1.9 ml of Antigen Diluent as reported in the specific section.

6. Wash buffer concentrate: WASHBUF 20X
1x60ml/bottle. 20x concentrated solution. Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2, 0.05% Tween 20 and 0.045% ProClin 300.

7. Enzyme conjugate: CONJ 20X
1x0.8 ml/vial. 20x concentrated solution of a HSV2-specific antibody, labeled with HRP and diluted in a protein buffer containing 10 mM Tris buffer pH 6.8+/-0.1, 2% BSA, 0.045% ProClin 300 and 0.2 mg/ml gentamicine sulphate as preservatives.

8. Antigen Diluent : AG DIL
n° 1 vial of 16 ml. Protein buffer solution for the preparation of the Immunocomplex. The solution contains 10 mM Tris buffer pH 6.8+/-0.1, 2% BSA, 0.045% ProClin 300 and 0.2 mg/ml gentamicine sulphate as preservatives. The reagent is code coloured with 0.01% red alimentary dye

9. Specimen Diluent : DILSPE
2x60.0 ml/vial. Proteic buffered solution for the dilution of samples. It contains 2% casein, 10 mM tris buffer pH 6.0+/-0.1, 0.1% Tween 20, 0.09% sodium azide and 0.045% ProClin 300 as preservatives.
The reagent is color coded with 0.01% blue alimentary dye.

10. Chromogen/Substrate : SUBS TMB
1x16ml/vial. It contains a 50 mM citrate-phosphate buffered solution at pH 3.5-3.8, 0.03% tetra-methyl-benzidine (TMB), 0.02% hydrogen peroxide (H_2O_2) and 4% dimethylsulphoxide.
Note: To be stored protected from light as sensitive to strong illumination.

11. Sulphuric Acid: H_2SO_4 0.3 M
1x15ml/vial. It contains 0.3 M H_2SO_4 solution.
Attention: Irritant (H315, H319; P280, P302+P352, 332+P313, P305+ P351+P338, P337+P313, P362+P363)

12. Plate sealing foils n° 2

13. Package insert n° 1

E. MATERIALS REQUIRED BUT NOT PROVIDED

1. Calibrated Micropipettes (1000 ul, 100 ul and 10 ul) and disposable plastic tips.
2. EIA grade water (double distilled or deionised, charcoal treated to remove oxidizing chemicals used as disinfectants).
3. Timer with 60 minute range or higher.
4. Absorbent paper tissues.
5. Calibrated ELISA microplate thermostatic incubator (dry or wet), set at +37°C (+/-0.5°C tolerance).
6. Calibrated ELISA microwell reader with 450nm (reading) and with 620-630nm (blanking) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.

F. WARNINGS AND PRECAUTIONS

1. The kit has to be used by skilled and properly trained technical personnel only, under the supervision of a medical doctor responsible of the laboratory.
2. All the personnel involved in performing the assay have to wear protective laboratory clothes, talc-free gloves and glasses. The use of any sharp (needles) or cutting (blades) devices should be avoided. All the personnel involved should be trained in biosafety procedures, as recommended by the Center for Disease Control, Atlanta, U.S. and reported in the National Institute of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
3. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.
4. The laboratory environment should be controlled so as to avoid contaminants such as dust or air-born microbial agents, when opening kit vials and microplates and when performing the test. Protect the Chromogen (TMB) from strong light and avoid vibration of the bench surface where the test is undertaken.
5. Upon receipt, store the kit at 2..8°C into a temperature controlled refrigerator or cold room.
6. Do not interchange components between different lots of the kits. It is recommended that components between two kits of the same lot should not be interchanged.
7. Check that the reagents are clear and do not contain visible heavy particles or aggregates. If not, advise the laboratory supervisor to initiate the necessary procedures for kit replacement.
8. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each sample. Do not reuse disposable tips.
9. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one. Do not reuse disposable tips.
10. Do not use the kit after the expiration date stated on the external container and internal (vials) labels. A study conducted on an opened kit did not pointed out any relevant loss of activity up to six 6 uses of the device and up to 3 months.
11. Treat all specimens as potentially infective. All human serum specimens should be handled at Biosafety Level 2, as recommended by the Center for Disease Control, Atlanta, U.S. in compliance with what reported in the Institutes of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
12. The use of disposable plastic-ware is recommended in the preparation of the liquid components or in transferring components into automated workstations, in order to avoid cross contamination.
13. Waste produced during the use of the kit has to be discarded in compliance with national directives and laws concerning laboratory waste of chemical and biological substances. In particular, liquid waste generated from the washing procedure, from residuals of controls and from samples has to be treated as potentially infective material and inactivated before waste. Suggested procedures of inactivation are treatment with a 10% final concentration of household bleach for 16-18 hrs or heat inactivation by autoclave at 121°C for 20 min..
14. Accidental spills from samples and operations have to be adsorbed with paper tissues soaked with household bleach and then with water. Tissues should then be discarded in proper containers designated for laboratory/hospital waste.
15. The Sulphuric Acid is an irritant. In case of spills, wash the surface with plenty of water
16. Other waste materials generated from the use of the kit (example: tips used for samples and controls, used microplates) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

G. SPECIMEN: PREPARATION AND WARNINGS

1. Blood is drawn aseptically by venepuncture and plasma or serum is prepared using standard techniques of preparation of samples for clinical laboratory analysis. No influence has been observed in the preparation of the sample with citrate, EDTA and heparin.
2. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results. Bar code labeling and electronic reading is strongly recommended.
3. Haemolysed ("red") and visibly hyperlipemic ("milky") samples have to be discarded as they could generate false results. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they could give rise to false results.
4. Sera and plasma can be stored at +2°…+8°C in primary collection tubes for up to five days after collection. Do not freeze primary tubes of collection. For longer storage periods, sera and plasma samples, carefully removed from the primary collection tube, can be stored frozen at –20°C for at least 12 months. Any frozen samples should not be frozen/thawed more than once as this may generate particles that could affect the test result.
5. If particles are present, centrifuge at 2.000 rpm for 20 min or filter using 0.2-0.8u filters to clean up the sample for testing.

H. PREPARATION OF COMPONENTS AND WARNINGS

A study conducted on an opened kit has not pointed out any relevant loss of activity up to 6 re-uses of the device and up to 3 months.

Microplate:
Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant has not turned dark green, indicating a defect in storing.
In this case, call Dia.Pro's customer service.
Unused strips have to be placed back into the aluminum pouch, with the desiccant supplied, firmly zipped and stored at +2°..8°C. After first opening, remaining strips are stable until the humidity indicator inside the desiccant bag turns from yellow to green.

Negative Control:
Ready to use. Mix well on vortex before use.

Positive Control:
Ready to use. Mix well on vortex before use.

Calibrator:
Add the volume of ELISA grade water reported on the label to the lyophilized powder. Let fully dissolve and then gently mix on vortex.
Important Note*: The solution is not stable. Store the Calibrator frozen in aliquots at –20°C.*

Wash buffer concentrate:
The whole content of the concentrated solution has to be diluted 20x with bidistilled water and mixed gently end-over-end before

use. During preparation avoid foaming as the presence of bubbles could impact on the efficiency of the washing cycles.
Note: *Once diluted, the wash solution is stable for 1 week at +2..8° C.*

Ag/Ab Immunocomplex:
Proceed carefully as follows:
1. Dissolve the content of a lyophilized vial with 1.9 ml of Conjugate/Antigen Diluent. Let fully dissolved the lyophilized content and then gently mix on vortex.
2. Gently mix the concentrated Enzyme Conjugate on vortex. Then add 0.1 ml of it to the vial of the dissolved HSV2 Ag and mix gently on vortex.

Important Notes*:*
1. *Dissolve and prepare only the number of vials necessary to the test. The Immunocomplex obtained is not stable. Store any residual solution frozen in aliquots at –20°C.*
2. *The preparation of the Immucomplex has to be done **right before** the dispensation of samples and controls into the plate. Mix again on vortex gently just before its use.*

Specimen Diluent:
Ready to use. Mix well on vortex before use

Chromogen/Substrate:
Ready to use. Mix well on vortex before use.
Be careful not to contaminate the liquid with oxidizing chemicals, air-driven dust or microbes.
Do not expose to strong illumination, oxidizing agents and metallic surfaces.
If this component has to be transferred use only plastic, possible sterile disposable container

Sulphuric Acid:
Ready to use. Mix well on vortex before use.
Attention: Irritant (H315, H319; P280, P302+P352, 332+P313, P305+ P351+P338, P337+P313, P362+P363).

Legenda:

Warning **H statements:**
H315 – Causes skin irritation.
H319 – Causes serious eye irritation.

Precautionary **P statements:**
P280 – Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352 – IF ON SKIN: Wash with plenty of soap and water.
P332 + P313 – If skin irritation occurs: Get medical advice/attention.
P305 + P351 + P338 – IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337 + P313 – If eye irritation persists: Get medical advice/attention.
P362 + P363 - Take off contaminated clothing and wash it before reuse.

I. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT
1. Micropipettes have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (household alcohol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample. They should also be regularly maintained in order to show a precision of 1% and a trueness of +/-2%. Decontamination of spills or residues of kit components should also be carried out regularly.
2. The ELISA incubator has to be set at +37°C (tolerance of +/- 0.5°C) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water baths are suitable for the incubations, provided that the instrument is validated for the incubation of ELISA tests.
3. The **ELISA washer** is extremely important to the overall performances of the assay. The washer must be carefully validated in advance, checked for the delivery of the right dispensation volume and regularly submitted to maintenance according to the manufacturer's instructions for use. In particular the washer, at the end of the daily workload, has to be extensively cleaned out of salts with deionized water. Before use, the washer has to be extensively primed with the diluted Washing Solution.
   The instrument weekly has to be submitted to decontamination according to its manual (NaOH 0.1 M decontamination suggested).
   5 washing cycles (aspiration + dispensation of 350ul/well of washing solution + 20 sec soaking = 1 cycle) are sufficient to ensure the assay with the declared performances. If soaking is not possible add one more cycle of washing.
   An incorrect washing cycle or salt-blocked needles are the major cause of false positive reactions.
4. Incubation times have a tolerance of $\pm$5%.
5. The ELISA microplate reader has to be equipped with a reading filter of 450nm and with a second filter de 620-630nm, mandatory for blanking purposes. Its standard performances should be (a) bandwidth $\leq$ 10 nm; (b) absorbance range from 0 to $\geq$ 2.0; (c) linearity to $\geq$ 2.0; repeatability $\geq$ 1%. Blanking is carried out on the well identified in the section "Assay Procedure". The optical system of the reader has to be calibrated regularly to ensure that the correct optical density is measured. It should be regularly maintained according to the manufacturer 's instructions.
6. When using an ELISA automated work station, all critical steps (dispensation, incubation, washing, reading, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the section "Internal Quality Control". The assay protocol has to be installed in the operating system of the unit and validated as for the washer and the reader. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set. Particular attention must be paid to avoid carry over by the needles used for dispensing and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells. The use of ELISA automated work stations is recommended when the number of samples to be tested exceed 20-30 units per run.
7. Dia.Pro's customer service offers support to the user in the setting and checking of instruments used in combination with the kit, in order to assure compliance with the requirements described. Support is also provided for the installation of new instruments to be used with the kit.

L. PRE ASSAY CONTROLS AND OPERATIONS
1. Check the expiration date of the kit printed on the external label (primary container). Do not use the device if expired.
2. Check that the liquid components are not contaminated by visible particles or aggregates. Check that the Chromogen/Substrate is colorless or pale blue by aspirating a small volume of it with a sterile plastic pipette. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box (primary container). Check that the aluminum pouch, containing the microplate, is not punctured or damaged.
3. Dilute all the content of the 20x concentrated Wash Solution as described above.

4. Dissolve the Calibrator as described above and gently mix.
5. Allow all the other components to reach room temperature (about 1 hr) and then mix gently on vortex all liquid reagents.
6. Set the ELISA incubator at +37°C and prepare the ELISA washer by priming with the diluted washing solution, according to the manufacturers instructions. Set the right number of washing cycles as reported in the specific section.
7. Check that the ELISA reader is turned on or ensure it will be turned on at least 20 minutes before reading.
8. If using an automated work station, turn on, check settings and be sure to use the right assay protocol.
9. Check that the micropipettes are set to the required volume.
10. Check that all the other equipment is available and ready to use.
11. In case of problems, do not proceed further with the test and advise the supervisor.

M. ASSAY PROCEDURE

The assay has to be carried out according to what reported below, taking care to maintain the same incubation time for all the samples in testing.

M.1 Automated assay:

In case the test is carried out automatically with an ELISA system, we suggest to make the instrument aspirate 1000 µl Specimen Diluent and then 10 µl sample (1:101 dilution factor). The whole content is then dispensed into a properly defined dilution tube. Before the next sample is aspirated, needles have to be duly washed to avoid any cross-contamination among samples. When all the samples have been diluted make the instrument dispense 100 µl diluted samples into the proper wells of the microplate.

This procedure may be carried out also in two steps of dilutions of 1:10 each (90 µl Specimen Diluent + 10 µl sample) into a second dilution platform. Make then the instrument aspirate first 100 µl Specimen Diluent, then 10 µl liquid from the first dilution in the platform and finally dispense the whole content in the proper well of the assay microplate.

Do not dilute controls/calibrator as they are ready to use.

Dispense 100 µl calibrators/control in the appropriate calibration/control wells.

For the next operations follow the operative instructions reported below for the Manual Assay.

It is strongly recommended to check that the time lap between the dispensation of the first and the last sample will be calculated by the instrument and taken into consideration by delaying the first washing operation accordingly.

M. 2 Manual assay:

1. Dilute samples 1:101 by dispensing first 10 µl sample and then 1 ml Specimen Diluent into a dilution tube; mix gently on vortex.
2. Place the required number of Microwells in the microwell holder. Leave the well in position A1 empty for the operation of blanking.
3. Dispense 100 µl of Negative Control and 100 µl of Calibrator in the proper wells in duplicate. Dispense 100 µl of Positive Control in single into the proper well. Do not dilute controls and the calibrator as they are ready to use !
4. Dispense 100 µl diluted samples in the proper sample wells and then check that all the samples wells are blue colored and that controls and calibrator have been dispensed.
5. Incubate the microplate for **60 min at +37°C** .

Important note: *Strips have to be sealed with the adhesive sealing foil, supplied, only when the test is carried out manually. Do not cover strips when using ELISA automatic instruments.*

6. Wash the microplate with an automatic as reported previously (section I.3).
7. Pipette 100 µl of the **Ag/Ab Immunocomplex** into each well, except the blanking well A1, and cover with the sealer. Check that all wells are red colored, except A1.

Important note: *Be careful not to touch the plastic inner surface of the well with the tip filled with the* **Ag/Ab Immunocomplex***. Contamination might occur.*

8. Incubate the microplate for **60 min at +37°C** .
9. Wash microwells as in step 6.
10. Pipette 100 µl Chromogen/Substrate mixture into each well, the blank well included. Then incubate the microplate at **room temperature (18-24°C) for 20 minutes**.

Important note: *Do not expose to strong direct illumination. High background might be generated.*

11. Pipette 100 µl Sulphuric Acid into all the wells using the same pipetting sequence as in step 10. Addition of acid will turn the positive control and positive samples from blue to yellow .
12. Measure the color intensity of the solution in each well, as described in section I.5, at 450nm filter (reading) and at 620-630nm (background subtraction, mandatory), blanking the instrument on A1.

Important notes:

1. *Ensure that no finger prints are present on the bottom of the microwell before reading. Finger prints could generate false positive results on reading.*
2. *Reading has to be carried out just after the addition of the Stop Solution and anyway not any longer than 20 minutes after its addition. Some self oxidation of the chromogen can occur leading to high background.*

N. ASSAY SCHEME

Controls&calibrator (*) Samples diluted 1:101	100 ul 100 ul
1st incubation	**60 min**
Temperature	+37°C
Washing	n° 5 cycles with 20" of soaking OR n° 6 cycles without soaking
Immunocomplex	100 ul
2nd incubation	**60 min**
Temperature	+37°C
Washing	n° 5 cycles with 20" of soaking OR n° 6 cycles without soaking
TMB/H2O2 mix	100 ul
3rd incubation	**20 min**
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm / 620-630nm

(*) Important Notes:

- *The Calibrator (CAL) does not affect the Cut Off calculation, therefore it does not affect the test's results calculation.*
- *The Calibrator (CAL) used only if a laboratory internal quality control is required by the Management.*

An example of dispensation scheme is reported below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S3										
B	NC	S4										
C	NC	S5										
D	CAL(*)	S6										
E	CAL(*)	S7										
F	PC	S8										
G	S1	S9										
H	S2	S10										

Legenda: BLK = Blank NC = Negative Control
CAL(*) = Calibrator–Not Mandatory PC = Positive Control S = Sample

O. INTERNAL QUALITY CONTROL

A validation check is carried out on the controls any time the kit is used in order to verify whether the performances of the assay are as qualified.
Control that the following data are matched:

Parameter	Requirements
Blank well	< 0.05 OD450nm value
Negative Control mean value (NC)	< 0.200 OD450nm value after blanking coefficient of variation < 30%
Positive Control	> 1.000 OD450nm

If the results of the test match the requirements stated above, proceed to the next section.
If they do not, do not proceed any further and perform the following checks:

Problem	Check
Blank well > 0.05 OD450nm	1. that the Chromogen/Substrate solution has not become contaminated during the assay
Negative Control (NC) > 0.200 OD450nm after blanking coefficient of variation > 30%	1. that the washing procedure and the washer settings are as validated in the pre qualification study; 2. that the proper washing solution has been used and the washer has been primed with it before use; 3. that no mistake has been done in the assay procedure (dispensation of positive control instead of negative control; 4. that no contamination of the negative control or of the wells where the control was dispensed has occurred due to positive samples, to spills or to the enzyme conjugate; 5. that micropipettes have not become contaminated with positive samples or with the enzyme conjugate 6. that the washer needles are not blocked or partially obstructed.
Positive Control < 1.000 OD450nm	1. that the procedure has been correctly performed; 2. that no mistake has occurred during the distribution of the control (dispensation of negative control instead of positive control). 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the positive control has occurred.

If any of the above problems have occurred, report the problem to the supervisor for further actions.

**** Important Notes:**
The analysis must be done proceeding as the reading step described in the section M, point 12.

If the Calibrator has used, verify the following data:

Check	Requirements
Calibrator	S/Co > 1.2

If the results of the test doesn't match the requirements stated above, operate as follows:

Problem	Check
Calibrator S/Co < 1.2	1. that the procedure has been correctly performed; 2. that no mistake has occurred during its distribution (e.g.: dispensation of negative control instead) 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the calibrator has occurred.

Anyway, if all other parameters (Blank, Negative Control, Positive Control), match the established requirements, the test may be considered valid.

P. CALCULATION OF THE CUT-OFF

The test results are calculated by means of the mean OD450nm/620-630nm value of the Negative Control (NC) and a mathematical calculation, in order to define the following cut-off formulation:

Cut-Off = NC + 0.250

The value found for the test is used for the interpretation of results as described in the next paragraph.

***Important note**: When the calculation of results is performed by the operating system of an ELISA automated work station, ensure that the proper formulation is used to calculate the cut-off value and generate the correct interpretation of results.*

Q. INTERPRETATION OF RESULTS

Test results are interpreted as a ratio of the sample OD450nm/620-630nm and the Cut-Off value (or S/Co) according to the following table:

S/Co	Interpretation
< 1.0	Negative
1.0 - 1.2	Equivocal
> 1.2	Positive

A negative result indicates that the patient is not undergoing an acute infection of Herpes Simplex Virus type 2.
Any patient showing an equivocal result, should be re-tested by examining a second sample taken from the patient after 1-2 weeks from first testing.
A positive result is indicative of a Herpes Simplex Virus type 2 infection.

An example of calculation is reported below (data obtained proceeding as the reading step described in the section M, point 12).

Important Note: *The following data must not be used instead of real figures obtained by the user.*

Negative Control: 0.090 – 0.110 – 0.070 OD450nm
Mean Value: 0.100 OD450nm
Lower than 0.200 – Accepted
Positive Control: 1.850 OD450nm
Higher than 1.000 – Accepted

Cut-Off = 0.100+0.250 = 0.350

Calibrator: 0.900 – 1.100 OD450nm
Mean value: 1.000 OD450nm S/Co = 2.8
S/Co higher than 1.2 – Accepted

Sample 1: 0.070 OD450nm
Sample 2: 1.690 OD450nm
Sample 1 S/Co < 1 = negative
Sample 2 S/Co > 1.2 = positive

Important notes:

1. *Interpretation of results should be done under the supervision of the laboratory supervisor to reduce the risk of judgment errors and misinterpretations.*
2. *Particular attention in the interpretation of results has to be used in the follow-up of pregnancy for an infection of HSV due to the risk of severe neonatal malformations.*
3. *In pregnancy monitoring, it is strongly recommended that any positive result is confirmed first with the procedure described below and secondly with a different device for HSV IgM detection, before taking any preventive medical action.*
4. *Any positive sample should be submitted to the Confirmation Test reported in section T before giving a result of positivity. By carrying out this test, false reactions, leading to a misinterpretation of the analytical result, can be revealed and then ruled out.*
5. *When test results are transmitted from the laboratory to another facility, attention must be paid to avoid erroneous data transfer.*
6. *Diagnosis of infection has to be taken and released to the patient by a suitably qualified medical doctor.*

R. PERFORMANCE CHARACTERISTICS

1. Limit of detection

No international standard for HSV1&2 IgM Antibody detection has been defined so far by the European Community.
In its absence, an Internal Gold Standard (or IGS), calibrated on the preparation named "Accurun – Anti HSV2 IgM plasma" produced by Boston Biomedica Inc., USA, code 9106072, has been defined in order to provide the device with a constant and excellent sensitivity..
The limit of detection of the assay has been therefore calculated on the IGS. A limiting dilution curve was prepared in Negative Control (NC).
Results of Quality Control are given in the following table:

OD450nm values

IGS	HSV2M.CE Lot # RD1	HSV2M.CE Lot # RD2	HSV2M.CE Lot # RD3
1X	0.560	0.572	0.590
2X	0.343	0.324	0.348
4X	0.239	0.218	0.225
NC	0.145	0.132	0.139

2. Diagnostic sensitivity:

The diagnostic sensitivity has been tested in a clinical trial on panels of 40 samples classified positive by a kit US FDA approved. The value obtained from the analysis was > 98%.

3. Diagnostic specificity:

The diagnostic specificity has been determined in a performance evaluation study on panels of more than 300 specimens, negative with the reference kit, derived from normal individuals of European origin.
Both plasma, derived with different standard techniques of preparation (citrate, EDTA and heparin), and sera have been used to determine the specificity. No false reactivity due to the method of specimen preparation has been observed.
Frozen specimens have also been tested to check whether this interferes with the performance of the test. No interference was observed on clean and particle free samples.
A study conducted on more than 60 potentially cross-reactive samples has not revealed any interference in the system.
No cross reaction were observed.
The Performance Evaluation has provided a value > 98%.
False positive reactions may be anyway pointed out and then ruled out in the interpretation of results with the procedure reported in section T, able to verify whether or not a positive result is real.

4. Precision:

Results are reported as follows:

HSV2M.CE: lot # RD1

Negative (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.092	0.113	0.097	0.101
Std.Deviation	0.011	0.019	0.010	0.013
CV %	12.25	16.83	10.24	13.11

Low reactive (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.451	0.471	0.435	0.452
Std.Deviation	0.018	0.000	0.033	0.017
CV %	3.92	0.00	7.48	3.8

High reactive (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	1.530	1.574	1.527	1.543
Std.Deviation	0.023	0.052	0.006	0.027
CV %	1.48	3.33	0.37	1.73

HSV2M.CE: lot # RD2

Negative (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.095	0.101	0.097	0.098
Std.Deviation	0.006	0.008	0.005	0.006
CV %	6.30	7.92	5.15	6.45

Low reactive (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.431	0.428	0.453	0.437
Std.Deviation	0.023	0.018	0.023	0.021
CV %	5.3	4.2	5.10	4.9

High reactive (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	1.558	1.552	1.541	1.550
Std.Deviation	0.031	0.025	0.039	0.032
CV %	1.98	1.61	2.53	2.04

HSV2M.CE: lot # RD3

Negative (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.104	0.108	0.099	0.104
Std.Deviation	0.015	0.010	0.011	0.012
CV %	14.4	9.2	11.11	11.57

Low reactive (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.425	0.436	0.440	0.434
Std.Deviation	0.008	0.006	0.009	0.008
CV %	1.8	1.4	2.0	1.7

High reactive (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	1.571	1.562	1.558	1.564
Std.Deviation	0.040	0.034	0.024	0.033
CV %	2.54	2.17	1.54	2.08

Important note:
The performance data have been obtained proceeding as the reading step described in the section M, point 12.

S. LIMITATIONS

Frozen samples containing fibrin particles or aggregates may generate false positive results.
Bacterial contamination or heat inactivation of the specimen may affect the absorbance values of the samples with consequent alteration of the level of the analyte.
This test is suitable only for testing single samples and not pooled ones.
Diagnosis of an infectious disease should not be established on the basis of a single test result. The patient's clinical history, symptomatology, as well as other diagnostic data should be considered.

T. CONFIRMATION TEST

In order to provide the medical doctor with the best accuracy in the follow-up of pregnancy, where a false positive result could lead to an operation of abortion, a confirmation test is reported. The confirmation test has to be carried out on any positive sample before a diagnosis of primary infection of HSV is released to the doctor.
Proceed for confirmation as follows:

1. Prepare the Antigen/Conjugate Complex as described in the proper section. This reagent is called Solution A.
2. Then 25 ul concentrated Enzymatic Conjugate are diluted in 500 ul Antigen Diluent and mixed gently on vortex. Do not use any lyophilized antigen vial for this procedure ! This solution is called Solution B.
3. The well A1 of the strip is left empty for blanking.
4. The Negative Control is dispensed in the strip in positions B1+C1. This is used for the calculation of the cut-off and S/Co values.
5. The positive sample to be confirmed, diluted 1:101, is dispensed in the strip in position D1+E1.
6. The strip is incubated for 60 min at +37°C.
7. After washing, the blank well A1 is left empty.
8. 100 µl of Solution A are dispensed in wells B1+C1+D1.
9. Then 100 µl of Solution B are added to well E1.
10. The strip is incubated for 60 min at +37°C.
11. After washing, 100 µl Chromogen/Substrate are added to all the wells and the strip is incubated for 20 min at r.t.
12. 100 µl Sulphuric Acid are added to all the wells and then their color intensity is measured at 450nm (reading filter) and at 620-630nm (background subtraction), blanking the instrument on A1.

Interpretation of results is carried out as follows:

1. If the sample in position D1 shows a S/Co value lower than 1.0 a problem of dispensation or contamination in the first test is likely to be occurred. The Assay Procedure in Section M has to be repeated to double check the analysis.
2. If the sample in position D1 shows a S/Co value higher than 1.2 and in position E1 shows a S/Co value still higher than 1.2 the sample is considered a **false positive**. The reactivity of the sample is in fact not dependent on the specific presence of HSV2 and a crossreaction with enzymatic conjugate has occurred.
3. If the sample in position D1 shows a S/Co value higher than 1.2 and in position E1 shows a S/Co value lower than 1.0 the sample is considered a **true positive**. The reactivity of the sample is in fact dependent on the specific presence of HSV and not due to any crossreaction.

The following table is reported for the interpretation of results

Well	S/Co		
D1	< 1.0	> 1.2	> 1.2
E1	< 1.0	> 1.2	< 1.0
Interpretation	Problem of contam.	False positive	True positive

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Manufacturer:
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CE

HSV2 IgM

Ensayo inmunoenzimático (ELISA) de "captura" para la determinación de anticuerpos IgM al Virus Herpes Simplex tipo 2 en plasma y suero humanos

- Uso exclusivo para diagnóstico "in vitro"-

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REF HSV2M.CE
96 pruebas

HSV2 IgM

A. OBJETIVO DEL ESTUCHE.
Ensayo inmunoenzimático (ELISA) para la determinación de anticuerpos IgM al Virus Herpes Simplex tipo 2, en plasma y suero humanos, mediante un sistema de "captura".
El estuche ha sido concebido para el seguimiento de pacientes infectados con HSV y para el monitoreo de la infección durante el embarazo, causa de riesgo de malformaciones en el neonato.
Uso exclusivo para diagnóstico "in vitro".

B. INTRODUCCIÓN.
Los Virus del Herpes Simplex tipos 1 (HSV1) y 2 (HSV2) son grandes y complejos virus ADN que inducen la síntesis de diversas proteinas durante la infección, poseen un alto número de determinantes de reactividad cruzada y pocas secuencias tipo específicas.La mayor parte de las infecciones herpéticas primarias y recurrentes son causadas por HSV2, mientras que aquellas infecciones no asociadas a los genitales son causadas fundamentalmente por HSV1.
La detección de anticuerpos IgG e IgM específicos al virus, es importante en el diagnóstico de las infecciones agudas/primarias, así como en las reactivaciones de una infección latente, en ausencia de síntomas clínicos evidentes.
En individuos aparentemente sanos y durante el embarazo, pueden aparecer infecciones asintomáticas debidas a HSV. En pacientes inmunocomprometidos se pueden presentar severas infecciones herpéticas, donde la enfermedad evoluciona hacia patologías clínicas.
La determinación de anticuerpos específicos al virus constituye un elemento importante para el seguimiento de pacientes en grupos de riesgo, así como para el monitoreo de las infecciones severas y agudas.

C. PRINCIPIOS DEL ENSAYO.
El ensayo se basa en el principio de "captura de IgM", donde los anticuerpos de esta clase presentes en la muestra, son capturados por la fase sólida recubierta con un anticuerpo anti-IgM humano.
Luego del lavado, que elimina el resto de los componentes de la muestra en particular los anticuerpos IgG, se adiciona una preparación purificada de HSV 2, inactivado y marcado con un anticuerpo específico conjugado con Peroxidasa (HRP), lo cual permite detectar los anticuerpos IgM inmovilizados en la fase sólida. Posteriormente a la incubación, los pocillos se lavan para eliminar cualquier traza de conjugado en exceso y se añade el substrato cromogénico. En presencia del conjugado, el substrato es hidrolizado generándose una señal coloreada proporcional a la cantidad de anticuerpos IgM al HSV 2, presentes en la muestra.
La Prueba de Confirmación controla la ocurrencia de falsos positivos, lo cual permite a los clínicos una correcta interpretación de los resultados.

D. COMPONENTES.
Cada estuche contiene reactivos suficientes para realizar 96 pruebas.

1. Microplaca: MICROPLATE
12 tiras de 8 pocillos recubiertos con anticuerpos de cabra anti-IgM humano, purificados por afinidad, en presencia de proteinas de bovino.
Las placas están en una bolsa sellada con desecante. Se deben poner las mismas a temperatura ambiente antes de abrirlas, sellar las tiras sobrantes en la bolsa con el desecante y almacenar entre 2 y 8ºC.

2. Control Negativo: CONTROL -
1x4.0 ml/vial. Listo para el uso. Contiene 1% de proteinas del suero humano, 2% de caseína, tampón Tris10 mM pH 6.0+/-0.1, 0.1% de Tween 20, además de azida sódica 0.09% y ProClin 300 0.045% como preservativos.
El control negativo está codificado con el color amarillo pálido.

3. Control Positivo: CONTROL +
1x4.0 ml/vial. Listo para el uso. Contiene 1% de suero humano positivo a IgM HSV2, 2% de caseína, tampón Tris10 mM pH 6.0+/-0.1, 0.1% de Tween 20, además de azida sódica 0.09% y ProClin 300 0.045% como preservativos.
El control positivo está codificado con el color verde.

4. Calibrador: CAL …ml
n° 1 vial. Liofilizado. Para disolver en agua calidad EIA como se indica en la etiqueta. Contiene anticuerpos IgM a HSV2, suero fetal bovino, además de sulfato de gentamicina 0.2 mg/ml y ProClin 300 0.045% como preservativos.
Nota: El volumen necesario para disolver el contenido del frasco varía en cada lote. Se recomienda usar el volumen indicado en la etiqueta.

5. Antígenos liofilizados HSV2 Ag: AG HSV2
N° 6 viales liofilizados. Contienen antígenos de HSV2 en un tampón proteico, inactivados por radiaciones gamma, 2% de proteinas de bovino, tampón Tris HCl 10 mM pH 6.8+/-0.1 además de 0.2 mg/ml de sulfato de gentamicina y 0.045% de ProClin 300. Debe disolverse con 1.9 ml de Diluente de Antígeno, según se indica más adelante.

6. Tampón de Lavado Concentrado: WASHBUF 20X
1x60ml/botella. Solución concentrada 20x. Una vez diluida, la solución de lavado contiene tampón fosfato 10 mM a pH 7.0 +/-0.2, Tween 20 al 0.05% y ProClin 300 al 0.045%.

7. Conjugado: CONJ 20X
1x0.8 ml/vial. Solución concentrada 20x. Contiene un anticuerpo específico anti-HSV2 conjugado con peroxidasa (HPR) diluído en un tampón proteico, tampón Tris 10mM a pH 6.8 +/- 0.1, 2% de BSA, además de 0.2 mg/ml de sulfato de gentamicina y ProClin 300 0.045% como preservativos.

8. Diluente de Antígeno: AG DIL
n° 1 vial de 16 ml. Solución tamponada proteica para la preparación del inmunocomplejo. Contiene tampón Tris 10mM a pH 6.8 +/- 0.1, 2% de BSA, además de 0.2 mg/ml de sulfato de gentamicina y ProClin 300 0.045% como preservativos. El reactivo está codificado con el color rojo (0.01% de colorante rojo).

9. Diluente de muestras : DILSPE
2x60ml/vial. Solución tamponada proteica para la dilución de las muestras. Contiene 2% de caseína, tampón Tris 10 mM a pH 6.0 +/- 0.1, 0.2% de Tween 20, además de azida sódica al 0.09% y 0.045 de ProClin 300 como preservativos.
El reactivo está codificado con el color azul (0.01% de colorante azul).

10. Cromógeno/Substrato: SUBS TMB
1x16ml/vial. Contiene una solución tamponada citrato-fosfato 50mM pH 3.5-3.8, tetra-metil-benzidina (TMB) 0.03% y peróxido de hidrógeno (H_2O_2) 0.02% así como dimetilsulfóxido al 4%.
Nota: Evitar la exposición a la luz, la sustancia es fotosensible.

11. Ácido Sulfúrico: H_2SO_4 0.3 M
1x15ml/vial. Contiene solución de H_2SO_4 0.3M
Atención: Irritante (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

12. Sellador adhesivo, n° 2

13. Manual de instrucciones, n° 1

E. MATERIALES NECESARIOS NO SUMINISTRADOS.

1. Micropipetas calibradas (1000 ul, 100 ul and 10 ul) y puntas plásticas desechables.
2. Agua de calidad EIA (bidestilada o desionizada, tratada con carbón para remover químicos oxidantes usados como desinfectantes).
3. *Timer* con un rango de 60 minutos como mínimo.
4. Papel absorbente.
5. Incubador termostático de microplacas ELISA, calibrado (en seco o húmedo) fijo a 37ºC (+/-0.5°C tolerancia).
6. Lector calibrado de microplacas de ELISA con filtros de 450nm (lectura) y de 620-630 nm.
7. Lavador calibrado de microplacas ELISA.
8. Vórtex o similar.

F. ADVERTENCIAS Y PRECAUCIONES.

1. El estuche debe ser usado por personal técnico adecuadamente entrenado, bajo la supervisión de un doctor responsable del laboratorio.
2. Todas las personas encargadas de la realización de las pruebas deben llevar las ropas protectoras adecuadas de laboratorio, guantes y gafas. Evitar el uso de objetos cortantes (cuchillas) o punzantes (agujas). El personal debe ser adiestrado en procedimientos de bioseguridad, según ha sido recomendado por el Centro de Control de Enfermedades de Atlanta, Estados Unidos, y publicado por el Instituto Nacional de Salud: “Biosafety in Microbiological and Biomedical Laboratories”, ed.1984.
3. Todo el personal involucrado en el manejo de muestras debe estar vacunado contra HBV y HAV, para lo cual existen vacunas disponibles, seguras y eficaces.
4. Se debe controlar el ambiente del laboratorio para evitar la contaminación de los componentes con polvo o agentes microbianos cuando se abran los estuches, así como durante la realización del ensayo. Evitar la exposición del substrato a la luz y las vibraciones de la mesa de trabajo durante el ensayo.
5. Conservar el estuche a temperaturas entre 2-8 ºC, en un refrigerador con temperatura regulada o en cámara fría.
6. No intercambiar reactivos de diferentes lotes ni tampoco de diferentes estuches.
7. Comprobar que los reactivos no contienen precipitados ni agregados en el momento del uso. De darse el caso, informar al supervisor para realizar el procedimiento pertinente y reemplazar el estuche.
8. Evitar contaminación cruzada entre muestras de suero/ plasma usando puntas desechables y cambiándolas luego de cada uso. No reutilizar puntas desechables.
9. Evitar contaminación cruzada entre los reactivos del estuche usando puntas desechables y cambiándolas luego de cada uso. No reutilizar puntas desechables.
10. No usar el producto después de la fecha de caducidad indicada en el estuche e internamente en los reactivos. Según estudios realizados, no se ha detectado pérdida relevante de actividad en estuches abiertos, en uso por un período de hasta 3 meses.
11. Tratar todas las muestras como potencialmente infecciosas. Las muestras de suero humano deben ser manipuladas al nivel 2 de bioseguridad, según ha sido recomendado por el Centro de Control de Enfermedades de Atlanta, Estados Unidos y publicado por el Instituto Nacional de Salud: “Biosafety in Microbiological and Biomedical Laboratories”, ed.1984.
12. Se recomienda el uso de material plástico desechable para la preparación de las soluciones de lavado y para la transferencia de los reactivos a los diferentes equipos automatizados a fin de evitar contaminaciones.
13. Los desechos producidos durante el uso del estuche deben ser eliminados según lo establecido por las directivas nacionales y las leyes relacionadas con el tratamiento de los residuos químicos y biológicos de laboratorio. En particular, los desechos líquidos provenientes del proceso de lavado deben ser tratados como potencialmente infecciosos y deben ser inactivados. Se recomienda la inactivación con lejía al 10% de 16 a 18 horas o el uso de la autoclave a 121°C por 20 minutos.
14. En caso de derrame accidental de algún producto, se debe utilizar papel absorbente embebido en lejía y posteriormente en agua. El papel debe eliminarse en contenedores designados para este fin en hospitales y laboratorios.
15. El ácido sulfúrico es irritante. En caso de derrame, se debe lavar la superficie con abundante agua.
16. Otros materiales de desecho generados durante la utilización del estuche (por ejemplo: puntas usadas en la manipulación de las muestras y controles, microplacas usadas) deben ser manipuladas como fuentes potenciales de infección de acuerdo a las directivas nacionales y leyes para el tratamiento de residuos de laboratorio.

G. MUESTRA: PREPARACIÓN Y RECOMENDACIONES.

1. Extraer la sangre asépticamente por punción venosa y preparar el suero o plasma según las técnicas estándar de los laboratorios de análisis clínico. No se ha detectado que el tratamiento con citrato, EDTA o heparina afecte las muestras.
2. Las muestras deben estar identificadas claramente mediante código de barras o nombres, a fin de evitar errores en los resultados. Se recomienda el uso del código de barras.
3. Las muestras hemolizadas (color rojo) o hiperlipémicas (aspecto lechoso) deben ser descartadas para evitar falsos resultados, al igual que aquellas donde se observe la presencia de precipitados, restos de fibrina o filamentos microbianos.
4. El suero y el plasma pueden conservarse a una temperatura entre +2° y +8°C en tubos de recolección principales hasta cinco días después de la extracción. No congelar tubos de recolección principales. Para periodos de almacenamiento más prolongados, las muestras de plasma o suero, retiradas cuidadosamente del tubo de extracción principal, pueden almacenarse congeladas a –20°C durante almenos 12 meses. Evitar congelar/descongelar cada muestra más de una vez, ya que pueden generarse partículas que podrían afectar al resultado de la prueba.
5. Si hay presencia de agregados, la muestra se puede aclarar mediante centrifugación a 2000 rpm durante 20 minutos o por filtración con un filtro de 0,2-0,8 micras.

H. PREPARACIÓN DE LOS COMPONENTES Y PRECAUCIONES.

Estudios de estabilidad realizados en estuches en uso (hasta 6 veces) no han arrojado pérdida de actividad significativa en un período de 3 meses.

Microplacas:

Dejar la microplaca a temperatura ambiente (aprox. 1 hora) antes de abrir el envase. Compruebe que el desecante no esté de un color verde oscuro, lo que indicaría un defecto de concervación. De ser así, debe solicitar el servicio de Dia.Pro: atención al cliente.

Las tiras de pocillos no utilizadas, deben guardarse herméticamente cerradas en la bolsa de aluminio con el desecante a 2-8ºC. Una vez abierto el envase, las tiras sobrantes, se mantienen estables hasta que el indicador de humedad dentro de la bolsa del desecante cambie de amarillo a verde.

Control Negativo:
Listo para el uso. Mezclar bien con la ayuda de un vórtex, antes de usar.
Control Positivo:
Listo para el uso. Mezclar bien con la ayuda de un vórtex, antes de usar.

Calibrador:
Añadir al polvo liofilizado, el volumen de agua de calidad ELISA indicado en la etiqueta. Dejar disolver completamente y luego mezclar cuidadosamnete con el vórtex antes de usar.
Nota: *Para preservar la reactividad se recomienda mantenerla congelada en alícuotas a –20ºC. No recongelar.*
Solución de Lavado Concentrada:
Todo el contenido de la solución concentrada debe diluirse 20x con agua bidestilada y mezclarse suavemente antes de usarse. Durante la preparación evitar la formación de espuma y burbujas, lo que podría influir en la eficiencia de los ciclos de lavado.
Nota: *Una vez diluida, la solución es estable por una semana a temperaturas entre +2 y 8ºC.*

Inmunocomplejo Ag/Ab:
Proceder cuidadosamente según se indica:
1. Disolver el contenido de un vial liofilizado utilizando 1.9 ml de Diluente Antígeno. Dejar disolver completamente y luego mezclar cuidadosamente con el vórtex.
2. Mezclar el Conjugado concentrado con ayuda del vórtex. Añadir luego 0.1 ml del mismo al vial del Ag HSV2 disuelto y mezclar suavemente en el vórtex.

Notas Importantes*:*
1. *Disolver y preparar solamente los viales necesarios para la prueba. El inmunocomplejo obtenido no es estable. Almacenar la solución sobrante en alícuotas a –20°C.*
2. *La preparación del inmunocomplejo debe realizarse **justo antes** de dispensar las muestras y los controles en la placa. Mezclar nuevamente en vórtex justo antes de usar.*

Diluente de muestras :
Listo para el uso. Mezclar bien con un vórtex antes de usar.

Cromógeno/ Substrato:
Listo para el uso. Mezclar bien con un vórtex antes de usar. Evitar posible contaminación del líquido con oxidantes químicos, polvo o microbios. Evitar la exposición a la luz, agentes oxidantes y superficies metálicas. En caso de que deba transferirse el reactivo, usar contenedores de plástico, estériles y desechables, siempre que sea posible.

Ácido Sulfúrico:
Listo para el uso. Mezclar bien con un vórtex antes de usar.
Leyenda:

Indicación de peligro, **Frases H**
H315 – Provoca irritación cutánea.
H319 – Provoca irritación ocular grave.

Consejo de prudencia, **Frases P**
P280 – Llevar guantes/prendas/gafas/máscara de protección.
P302 + P352 – EN CASO DE CONTACTO CON LA PIEL: Lavar con agua y jabón abundantes.
P332 + P313 – En caso de irritación cutánea: Consultar a un médico.
P305 + P351 + P338 – EN CASO DE CONTACTO CON LOS OJOS: Aclarar cuidadosamente con agua durante varios minutos. Quitar las lentes de contacto, si lleva y resulta fácil. Seguir aclarando.
P337 + P313 – Si persiste la irritación ocular: Consultar a un médico.
P362 + P363 – Quitarse las prendas contaminadas y lavarlas antes de volver a usarlas.

I. INSTRUMENTOS Y EQUIPAMIENTO UTILIZADOS EN COMBINACIÓN CON EL ESTUCHE.
1. Las micropipetas deben ser calibradas para dispensar correctamente el volumen requerido en el ensayo y sometidas a una descontaminación periódica de las partes que pudieran entrar accidentalmente en contacto con la muestra o los reactivos (acohol 70%, lejía 10%, de calidad de los desinfectantes hospitalarios). Deben además, ser regularmente revisadas para mantener una precisión del 1% y una confiabilidad de +/- 2%. Deben descontaminarse periodicamente los residuos de los componentes del estuche.
2. La incubadora de ELISA debe ser ajustada a 37ºC (+/- 0.5ºC de tolerancia) y controlada periódicamente para mantener la temperatura correcta. Pueden emplearse incubadoras secas o baños de agua siempre que estén validados para la incubación de pruebas de ELISA.
3. El **lavador ELISA** es extremadamente importante para el rendimiento global del ensayo. El lavador debe ser validado de forma minuciosa previamente, revisado para comprobar que suministra el volumen de dispensación correcto y enviado regularmente a mantenimiento de acuerdo con las instrucciones de uso del fabricante. En particular, deben lavarse minuciosamente las sales con agua desionizada del lavador al final de la carga de trabajo diaria. Antes del uso, debe suministrarse extensivamente solución de lavado diluida al lavador. Debe enviarse el instrumento semanalmente a descontaminación según se indica en su manual (se recomienda descontaminación con NaOH 0.1 M). Para asegurar que el ensayo se realiza conforme a los rendimientos declarados, basta con 5 ciclos de lavado (aspiración + dispensado de 350 µl/pocillo de solución de lavado + 20 segundos de remojo = 1 ciclo). Si no es posible remojar, añadir un ciclo de lavado adicional. Un ciclo de lavado incorrecto o agujas obstruidas con sal son las principales causas de falsas reacciones positivas.
4. Los tiempos de incubación deben tener un margen de ±5%.
5. El lector de microplacas ELISA debe estar provisto de un filtro de lectura de 450nm y de un segundo filtro de 620-630nm, obligatorio para reducir interferencias en la lectura. El procedimiento estándar debe contemplar: a) Ancho de banda <= 10nm b) Rango de absorbancia de 0 a >=2.0, c) Linealidad >=2.0, reproducibilidad >=1%. El blanco se prueba en el pocillo indicado en la sección “Control de calidad interno”. El sistema óptico del lector debe ser calibrado periódicamente para garantizar la correcta medición de la densidad óptica, según las normas del fabricante.
6. En caso de usar un sistema automatizado de ELISA, los pasos críticos (dispensado, incubación, lavado, lectura, agitación y procesamiento de datos) deben ser cuidadosamente fijados, calibrados, controlados y periódicamente ajustados, para garantizar los valores indicados en la sección “Control de calidad interno”. El protocolo del ensayo debe ser instalado en el sistema operativo de la unidad y validado tanto para el lavador como para el lector. Por otro lado, la parte del sistema que maneja los líquidos (dispensado y lavado) debe ser validada y fijada correctamente. Debe prestarse particular atención a evitar el arrastre por las agujas de dispensación y de lavado, a fin de minimizar la posibilidad de ocurrencia de falsos positivos por contaminación de los pocillos adyacentes por muestras fuertemente reactivas. Se recomienda el uso de sistemas automatizados para el pesquisaje en unidades de sangre y cuando la cantidad de muestras supera las 20-30 unidades por ensayo.
7. El servicio de atención al cliente de Dia.Pro, ofrece apoyo al usuario para calibrar, ajustar e instalar los equipos a usar en combinación con el estuche, con el propósito de asegurar el cumplimiento de los requerimientos descritos.

L. OPERACIONES Y CONTROLES PREVIOS AL ENSAYO.

1. Compruebe la fecha de caducidad indicada en la parte externa del estuche (envase primario). No usar si ha caducado.
2. Compruebe que los componentes líquidos no están contaminados con partículas o agregados visibles. Asegúrese de que el cromógeno (TMB) es incoloro o azul pálido, aspirando un pequeño volumen del mismo con una pipeta estéril de plástico. Compruebe que no han ocurrido rupturas ni derrames de líquido dentro de la caja (envase primario) durante el transporte. Asegurarse de que la bolsa de aluminio que contiene la microplaca no esté rota o dañada.
3. Diluir totalmente la Solución de Lavado Concentrada 20X, como se ha descrito anteriormente.
4. Disolver el Calibrador como se ha descrito anteriormente y mezclar suavemente.
5. Dejar los componentes restantes alcanzar la temperatura ambiente (aprox. 1 hora), mezclar luego suavemente en el vórtex todos los reactivos líquidos.
6. Ajustar la incubadora de ELISA a 37ºC y cebar el lavador de ELISA utilizando la solución de lavado, según las instrucciones del fabricante. Fijar el número de ciclos de lavado lavado según se indica en la sección específica.
7. Comprobar que el lector de ELISA esté encendido al menos 20 minutos antes de realizar la lectura.
8. En caso de trabajar automáticamente, encender el equipo y comprobar que los protocolos estén correctamente programados.
9. Comprobar que las micropipetas estén fijadas en el volumen requerido.
10. Asegurarse de que el equipamiento a usar esté en perfecto estado, disponible y listo para el uso.
11. En caso de surgir algún problema, se debe detener el ensayo y avisar al supervisor.

M. PROCEDIMIENTO DEL ENSAYO.

El ensayo debe realizarse según las instrucciones que siguen a continuación, es importante mantener en todas las muestras el mismo tiempo de incubación.

M.1 Ensayo automatizado:

En el caso de que el ensayo se realice de manera automatizada con un sistema ELISA, se recomienda programar al equipo para aspirar 1000µl de Diluente de Muestras, y posteriormente 10µl de muestra (factor de dilución 1:101).

La mezcla debe ser dispensada cuidadosamente en un tubo de dilución. Antes de aspirar la muestra siguiente, las agujas deben lavarse debidamente para evitar cualquier contaminación cruzada entre las muestras. Cuando todas las muestras han sido diluídas, programar el equipo para dispensar 100 µl de las mismas en los pocillos correspondientes.

Este procedimiento puede realizarse en dos pasos de dilución de 1:10 cada uno (90 µl de Diluente de Muestras + 10 µl de muestra) en una segunda plataforma de dilución. Programar el equipo para aspirar primeramente 100 µl de Diluente de Muestras, luego 10 µl de la primera dilución en la plataforma y finalmente dispensar todo el contenido en los pocillos apropiados de la microplaca.

No diluir el Calibrador ni los controles, ya que están listos para el uso.

Dispensar 100ul de controles/calibrador en los pocillos correspondientes.

Para las operaciones siguientes, consulte las instrucciones que aparecen debajo para el Ensayo Manual.

Es muy importante comprobar que el tiempo entre el dispensado de la primera y la última muestra sea calculado por el instrumento y considerado para los lavados.

M.2 Ensayo Manual.

1. Diluir las muestras 1:101 dispensando primeramente 10 µl de muestra y luego 1 ml de Diluente de Muestra en un tubo de dilución, mezclar bien con vórtex.
2. Poner el número de tiras necesarias en el soporte plástico. Dejar el pocillo A1 vacío para el blanco.
3. Dispensar 100 µl del Control Negativo y 100µl del Calibrador por duplicado. Luego dispensar 100µl del Control Positivo (sencillo) en los respectivos pocillos. No diluir los controles ni el calibrador ya que están listos para el uso.
4. Dispensar 100 µl de las muestras diluídas en los pocillos correspondientes y chequear luego que estos pocillos son de color azul y que los controles y el calibrador han sido añadidos.
5. Incubar la microplaca **60 min a +37°C**.

***Nota importante**: Las tiras se deben sellar con el adhesivo suministrado solo cuando se hace el test manualmente. No sellar cuando se emplean equipos automatizados de ELISA.*

6. Lavar la microplaca según se indica en la seción I.3.
7. Dispensar 100uL del **Inmunocomplejo Ag/Ab** en todos los pocillos, excepto en el A1 y cubrir con el sellador. Compruebe que este reactivo de color rojo ha sido añadido en todos los pocillos excepto el A1.

***Nota importante**: Tener cuidado de no tocar la pared interna del pocillo con la punta de la pipeta al dispensar el **Inmunocomplejo Ag/Ab**. Podría producirse contaminación.*

8. Incubar la microplaca **60 min a +37°C**.
9. Lavar la microplaca, de igual forma que en el paso 6.
10. Dispensar 100µl del Cromógeno/Substrato en todos los pocillos, incluido el A1. Incubar la microplaca a **temperatura ambiente (18-24°C) durante 20 minutos**.

***Nota importante**: No exponer directamente a fuerte iluminación, de lo contrario se generan interferencias.*

11. Dispensar 100µl de Ácido Sulfúrico en todos los pocillos para detener la reacción enzimática, usar la misma secuencia que en el paso 10. La adición del ácido cambia el color de los controles positivos y las muestras positivas de azul a amarillo.
12. Medir la intensidad del color con el lector, según se describe en la sección I.5, utilizando un filtro de 450 nm (lectura) y otro de 620-630 nm (substracción del fondo, obligatorio), calibrando el instrumento con el pocillo A1 (blanco).

Notas generales importantes:

1. *Segurarse de que no hay impresiones digitales en el fondo de los pocillos antes de leer. Podrían generarse falsos positivos en la lectura.*
2. *La lectura debe hacerse inmediatamente después de añadir la solución de parada y, en cualquier caso, nunca transcurridos 20 minutos después de su adición. Se podría producir auto oxidación del cromógeno causando un elevado fondo.*

N. ESQUEMA DEL ENSAYO.

Controles&Calibrador (*) Muestras diluídas 1:101	100 ul 100 ul
1ra incubación	**60 min**
Temperatura	+37°C
Lavado	5 ciclos con 20''de remojo o 6 ciclos sin remojo
Inmunocomplejo	100 ul
2da incubación	**60 min**

Temperatura	+37°C
Lavado	5 ciclos con 20''de remojo o 6 ciclos sin remojo
Mezcla TMB/H_2O_2	100 ul
3ra incubación	**20 min**
Temperatura	t.a.*
Ácido Sulfúrico	100 ul
Lectura D.O.	450nm / 620-630nm

*t.a.*temperatura ambiente*

(*) Notas importantes:

- *El calibrador (CAL) no afecta al cálculo del valor de corte y, por lo tanto, no afecta al cálculo de los resultados de la prueba.*
- *El calibrador (CAL) se usa solo si la gestión requiere un control interno de calidad del laboratorio.*

A continuación se describe un ejemplo del esquema de dispensado:

Microplaca

	1	2	3	4	5	6	7	8	9	10	11	12
A	BL	M 3										
B	CN	M 4										
C	CN	M 5										
D	CAL(*)	M 6										
E	CAL(*)	M 7										
F	CP	M 8										
G	M 1	M 9										
H	M 2	M10										

Leyenda: BL = Blanco CN = Control Negativo
(*) CAL = Calibrador - No Obligadorio CP = Control Positivo
M = Muestra

O. CONTROL DE CALIDAD INTERNO.

Se realiza una validación sobre los controles y el calibrador cada vez que se usa el estuche, para verificar si el performance del ensayo es el esperado.
Asegurar el cumplimiento de los siguientes parámetros:

Parámetro	Exigencia
Pocillo Blanco	< 0.050 DO450nm
Control Negativo, valor medio (CN)	< 0.200 DO450nm valor después de leer el blanco Coeficiente de variación < 30%
Control Positivo	> 1000 DO450nm

Si los resultados del ensayo coinciden con lo establecido anteriormente, pase a la siguiente sección.

En caso contrario, detenga el ensayo y compruebe:

Problema	Compruebe que
Pocillo blanco > 0.050DO450nm	la solución cromógeno/substrato no se ha contaminado durante el ensayo.
Control Negativo (CN) > 0.200 DO450nm después de leer el blanco Coefficiente de variación > 30%	1. el proceso de lavado y los parámetros del lavador estén validados según los estudios previos de calificación. 2. se ha usado la solución de lavado apropiada y que el lavador ha sido cebado con la misma antes del uso. 3. no se han cometido errores en el procedimiento (dispensar el control positivo en lugar del negativo). 4. no ha existido contaminación del control negativo o de sus pocillos debido a muestras positivas derramadas, o al conjugado. 5. las micropipetas no se han contaminado con muestras positivas o con el conjugado. 6. las agujas del lavador no estén parcial o totalmente obstruidas.
Control Positivo < 1000 DO450nm	1. el procedimiento ha sido realizado correctamente. 2. no se han cometido errores en el procedimiento (dispensar el control negativo en lugar del positivo). 3. el proceso de lavado y los parámetros del lavador estén validados según los estudios previos de calificación. 4. no ha ocurrido contaminación externa del control positivo.

Si ocurre alguno de los problemas anteriores, luego de comprobar, informe al supervisor para tomar las medidas pertinentes.

***** Notas importantes:***

El análisis debe seguir el paso de lectura descrito en la sección M, punto 12.

Si se ha usado el Calibrador, comprobar los siguientes datos:

Parámetro	Exigencia
Calibrador	M/Co > 1.2

Si los resultados de la prueba no se corresponden con los requisitos indicados anteriormente, proceder del siguiente modo:

Problema	Compruebe que
Calibrador M/Co < 1.2	1. el procedimiento ha sido realizado correctamente. 2. no ha habido errores durante su distribución (dispensar el control negativo en lugar del calibrador). 3. el proceso de lavado y los parámetros del lavador estén validados según los estudios previos de calificación. 4. no ha ocurrido contaminación externa del calibrador.

En cualquier caso, si todos los demás parámetros (blanco, control negativo, control positivo) se corresponden con los requisitos establecidos, la prueba puede considerarse válida.

P. CÁLCULO DEL VALOR DE CORTE.

Los resultados de la prueba se calculan a partir de un valor medio de DO450nm/620-630nm del control Negativo (CN), mediante un valor de corte (Co) hallado con la siguiente fórmula:

Valor de corte = CN + 0.250

El valor encontrado en la prueba es utilizado para la interpretación de los resultados, según se describe a continuación.

***Nota Importante**: Cuando el cálculo de los resultados se halla mediante el sistema operativo de un equipo de ELISA automático, asegurarse de que la formulación usada para el cálculo del valor de corte, y para la interpretación de los resultados sea correcta.*

Q. INTERPRETACIÓN DE LOS RESULTADOS.
La interpretación de los resultados se realiza mediante la razón entre las DO a 450nm de las muestras (M) y el Valor de corte (Co).
Los resultados se interpretan según la siguiente tabla:

(M/Co)	Interpretación
< 1.0	Negativo
1.0 – 1.2	Equívoco
> 1.2	Positivo

Un resultado negativo indica que el paciente no está padeciendo infección aguda por el Virus Herpes Simplex tipo 2.
Cualquier paciente, cuya muestra resulte equívoca debe someterse a una nueva prueba con una segunda muestra de sangre colectada 1 ó 2 semanas después de la inicial.
Un resultado positivo es indicativo de infección por el Virus Herpes Simplex tipo 2.

A continuación, un ejemplo de los cálculos a realizar (datos obtenidos siguiendo el paso de lectura descrito en la sección M, punto 12).

Los siguientes datos no deben usarse en lugar de los valores reales obtenidos en el laboratorio.

Control Negativo: 0.090 – 0.110 – 0.070 DO 450nm
Valor medio: 0.100 DO 450nm
Menor de 0.150 – Válido
Control Positivo: 1.850 DO 450nm
Mayor de 1000 – Válido
Valor de corte = 0.100+0.250 = 0.350

Calibrador: 0.900 – 1.100 DO 450nm
Valor medio: 1.000 DO 450nm M/Co = 2.8
M/Co Mayor de 1.2 – Válido

Muestra 1: 0.070 DO 450nm
Muestra 2: 1.690 DO 450nm
Muestra 1 M/Co < 1 = negativa
Muestra 2 M/Co > 1.2 = positiva

Notas importantes:
1. La interpretación de los resultados debe hacerse bajo la vigilancia del supervisor del laboratorio para reducir el riesgo de errores de juicio y de interpretación.
2. Debe ponerse particular atención a la interpretación de los resultados ante sospecha de infección primaria por HSV en el embarazo, debido a las posibilidades de malformaciones del neonato.
3. En el monitoreo de infección por HSV durante el embarazo, se recomienda, antes de tomar cualquier decisión médica preventiva, confirmar cualquier resultado positivo, primero con el procedimiento descrito y luego con un sistema de detección de IgM anti-HSV.
4. Antes de emitir un resultado positivo, cada muestra reactiva debe someterse al exámen de confirmación reportado en la sección T, lo cual permite una correcta interpretación de los resultados ya que descarta los falsos positivos.
5. Cuando se transmiten los resultados de la prueba, del laboratorio a otras instalaciones, debe ponerse mucha atención para evitar el traslado de datos erróneos.
6. El diagnóstico de infección debe ser evaluado y comunicado al paciente por un médico calificado.

R. PERFORMANCES.

1. Límite de detección.
Hasta el momento no ha sido definido por la Comunidad Europea, un estándar internacional para la detección de anticuerpos IgM a HSV1&2. En ausencia del mismo y para garantizar una óptima sensibilidad, el límite de detección del ensayo ha sido calculado por medio de un Gold Standard Interno (IGS), a partir de una preparación "Accurun–Anti HSV 2 IgM Plasma", producida por Boston Biomedica Inc., Estados Unidos, código 9106072. Se construyó una curva de dilución limitante utilizando el Control Negativo (CN).
La siguiente tabla muestra los resultados del Control de Calidad:

Valores DO 450nm

IGS	HSV2M.CE Lote # RD1	HSV2M.CE Lote # RD2	HSV2M.CE Lote # RD3
1X	0.560	0.572	0.590
2X	0.343	0.324	0.348
4X	0.239	0.218	0.225
CN	0.145	0.132	0.139

2. Sensibilidad diagnóstica :
La sensibilidad diagnóstica se ha estudiado en un ensayo clínico utilizando paneles de 40 muestras, clasificadas como positivas mediante un estuche aprovado US FDA. El valor obtenido del análisis fue > 98%.

3. Especificidad diagnóstica :
La especificidad diagnóstica ha sido determinada en un ensayo clínico, utilizando paneles de más de 300 muestras provenientes de individuos sanos de origen europeo, clasificadas como negativas mediante un estuche de referencia.
Se emplearon además plasma sometido a métodos de tratamiento estándar (citrato, EDTA y heparina) y suero humanos para determinar la especificidad. No se ha observado falsa reactividad debida a los métodos de tratamiento de muestras.
Las muestras congeladas han sido probadas para comprobar si la colección y el almacenamiento interfiere con el procedimiento del ensayo. No se ha observado interferencia a partir de muestras limpias y libres de agregados.
Se realizó un estudio con más de 60 muestras que pudieran introducir reacción cruzada y no se observó interferencia alguna en el sistema. No se detectó reacción cruzada.
El estudio para evaluar el performance reveló un valor > 98%.
El procedimiento reportado en la sección T, permite verificar los resultados falsos positivos y de esta forma lograr una correcta interpretación de los resultados.

4. Precisión :
Ha sido calculada a partir de tres muestras, una negativa, una debilmente positiva y una positiva, examinadas en 16 réplicas en tres corridas separadas.
Los resultados son los siguientes:

HSV2M.CE: lote # RD1

Negativa (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor promedio
DO 450nm	0.092	0.113	0.097	0.101
Desviación estándar	0.011	0.019	0.010	0.013
CV %	12.25	16.83	10.24	13.11

Débil reactiva (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor promedio
DO 450nm	0.451	0.471	0.435	0.452
Desviación estándar	0.018	0.000	0.033	0.017
CV %	3.92	0.00	7.48	3.8

Altamente reactiva (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor promedio
DO 450nm	1.530	1.574	1.527	1.543
Desviación estándar	0.023	0.052	0.006	0.027
CV %	1.48	3.33	0.37	1.73

HSV2M.CE: lote # RD2

Negativa (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor promedio
DO 450nm	0.095	0.101	0.097	0.098
Desviación estándar	0.006	0.008	0.005	0.006
CV %	6.30	7.92	5.15	6.45

Débil reactiva (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor promedio
DO 450nm	0.431	0.428	0.453	0.437
Desviación estándar	0.023	0.018	0.023	0.021
CV %	5.3	4.2	5.10	4.9

Altamente reactiva (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor promedio
DO 450nm	1.558	1.552	1.541	1.550
Desviación estándar	0.031	0.025	0.039	0.032
CV %	1.98	1.61	2.53	2.04

HSV2M.CE: lote # RD3

Negativa (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor promedio
DO 450nm	0.104	0.108	0.099	0.104
Desviación estándar	0.015	0.010	0.011	0.012
CV %	14.4	9.2	11.11	11.57

Débil reactiva (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor promedio
DO 450nm	0.425	0.436	0.440	0.434
Desviación estándar	0.008	0.006	0.009	0.008
CV %	1.8	1.4	2.0	1.7

Altamente reactiva (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor promedio
DO 450nm	1.571	1.562	1.558	1.564
Desviación estándar	0.040	0.034	0.024	0.033
CV %	2.54	2.17	1.54	2.08

Nota importante:
Los datos de rendimiento se obtuvieron siguiendo el paso de lectura descrito en la sección M, punto 12.

S. LIMITACIONES.
La contaminación bacteriana o la inactivación por calor de la muestra pueden afectar los valores de DO y por tanto alterar los niveles del analito.

Las muestras que luego de ser descongeladas presentan partículas de fibrina o partículas agregadas, generan algunos resultados falsos positivos.
El ensayo es útil solo para probar muestras independientes y no mezclas.
El diagnóstico de una enfermedad infecciosa no debe establecerse en base a un solo resultado, sino que deben tenerse en consideración la historia clínica del paciente, la sintomatología, así como otros datos diagnósticos**.**

T. PRUEBA DE CONFIRMACIÓN.
Se ejecuta esta prueba con el propósito de garantizar la mayor precision del ensayo en el seguimiento del embarazo, donde un resultado falso positivo puede conducir a un aborto. La misma debe realizarse a cada una de las muestras positivas, antes de emitir un diagnóstico de infección por HSV.
Proceder para la confirmación como sigue:

1. Preparar el complejo Antígeno/Conjugado como se describe anteriormente. Este reactivo se denomina Solución A.
2. Diluir el Conjugado concentrado, 1:20 en el Diluente de Antígeno (ej: 25 ul de Conjugado concentrado en 500 ul de Diluente de Antígeno) y mezclar suavemente con ayuda del vórtex. No usar ningún vial de Ag liofilizado para este procedimiento! Este reactivo se denomina Solución B.
3. Dejar vacío el pocillo A1 para el blanco.
4. Dispensar el Control Negativo en las posiciones B1+C1, se utiliza para calcular el valor de corte y los valores M/Co.
5. Diluir 1:101 la muestra positiva para confirmar y dispensarla en las posiciones D1+E1.
6. Incubar la tira 60 minutos a +37°C.
7. Luego del lavado, el pocillo A1 para el blanco queda vacío.
8. Dispensar 100 µl de la Solución A en los pocillos B1+C1+D1.
9. Dispensar 100 µl de la Solución B en el pocillo E1.
10. Incubar la tira 60 minutos a +37°C.
11. Luego del lavado, adicionar 100 µl del Cromógeno/Substrato en todos los pocillos e incubar la tira 20 minutos a temperatura ambiente.
12. Dispensar 100µl del Acido Sulfúrico en todos los pocillos y medir la intensidad del color con el lector, según se describe en la sección I.5, utilizando un filtro de 450 nm (lectura) y otro de 620-630 nm (substracción del fondo, recomendado), calibrando el instrumento con el pocillo A1 (blanco).

La interpretación de los resultados se realiza de la siguiente forma:

1. Si la muestra en posición D1 tiene un valor de M/Co menor de 1.0, probablemente en el primer ensayo haya ocurrido un error en el dispensado o alguna contaminación. Debe repetirse el Procedimiento del Ensayo, sección M.
2. Si la muestra en posición D1 tiene un valor de M/Co mayor de 1.2 y en posición E1 el valor de M/Co es todavía mayor de 1.2, la muestra se considera un **falso positivo**. La reactividad de la muestra, en este caso, no depende de la presencia específica de HSV2, por lo tanto ha ocurrido una reacción cruzada con el conjugado.
3. Si la muestra en posición D1 tiene un valor de M/Co mayor de 1.2 y en la posición E1 el valor M/Co es menor de 1.0 se considera **realmente positiva**. La reactividad de la muestra, en este caso se debe a la presencia específica de HSV y no a reacciones cruzadas.

En la siguiente tabla se muestra la interpretación de los resultados:

Pocillo	M/Co		
D1	< 1.0	> 1.2	> 1.2
E1	< 1.0	> 1.2	< 1.0
Interpretación	Probl. de contam.	Falso positivo	Realmente positivo

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Todos los productos de diagnóstico in vitro fabricados por la empresa son controlados por un sistema certificado de control de calidad conforme a la norma ISO 13485. Cada lote se somete a un control de calidad y se libera al mercado únicamente si se ajusta a las especificaciones técnicas y criterios de aceptación de la CE.

Fabricante:
Dia.Pro Diagnostic Bioprobes S.r.l.
Via G. Carducci n° 27 – Sesto San Giovanni (Milán) – Italia

CE

Dia.Pro
Diagnostic
BioProbes

EC DECLARATION OF CONFORMITY

MANUFACTURER	DIA.PRO DIAGNOSTIC BIOPROBES S.R.L. VIA G. CARDUCCI N° 27 – 20099 SESTO SAN GIOVANNI (MILANO) – ITALY
PRODUCT	**HSV2 IgM** CODE: **HSV2M.CE** (96 tests)
CLASSIFICATION	GENERAL IVD
CONFORMITY ASSESSMENT ROUTE	SELF CERTIFICATION

WE HEREBY DECLARE THAT THE ABOVE MENTIONED PRODUCT MEETS THE PROVISIONS OF THE COUNCIL DIRECTIVE 98/79/EC FOR IN VITRO DIAGNOSTIC DEVICES.

ISO CERTIFICATE	UNE EN ISO 13485 N° 2013 11 0039 EN, RELEASED BY AEMPS (AGENCIA ESPAÑOLA DE MEDICAMENTOS Y PRODUCTOS SANITARIOS)

PLACE & DATE OF FIRST ISSUE	MILANO – OCTOBER 2004
PLACE & DATE OF CURRENT ISSUE	SESTO SAN GIOVANNI (MI) – MARCH 2019
SIGNATURE **Legal Representative** **Dr.ssa Fiorenza Scozzesi**	

Rev:	05/2018

RUB IgG

Enzyme ImmunoAssay (ELISA) for the quantitative/qualitative determination of IgG antibodies to Rubella Virus in human serum and plasma

- for “in vitro” diagnostic use only -

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REF RUBG.CE
96/192/480 Tests

Rub IgG

A. INTENDED USE
Enzyme ImmunoAssay (ELISA) for the quantitative/qualitative determination of IgG antibodies to Rubella Virus in human plasma and sera.
For "in vitro" diagnostic use only.

B. INTRODUCTION
Rubella is a small spherical enveloped virus, 55-60nm in diameter, and is the only member of the genus Rubivirus of the family Togaviridiae.
The virus contains a single positive stranded 42s RNA molecule and only one serotype is known. The virus encodes for at least three envelope glycoproteins, E1, E2a, E2b; a nucleocapsid-associated protein, C; and two nonstructural proteins.
The detection of Rubella-specific IgG and IgM antibodies is very important for the serological diagnosis of both congenital and primary postnatal rubella infections as they can lead to severe birth defects.
The absence of Rubella-specific IgG antibodies in sera, characteristically of long-term duration after primary infections, in presence of virus-specific IgM, is indicative for the risk of defects in newborn infants.
Highly specific Rubella IgG assays provide the clinician with a helpful and reliable test for the monitoring of these risks in pregnancy and for the monitoring of the immunological response upon vaccination.

C. PRINCIPLE OF THE TEST
Microplates are coated with native Rubella Virus, highly purified by sucrose gradient centrifugation and inactivated.
The solid phase is first treated with the diluted sample and IgG to Rubella Virus are captured, if present, by the antigens.
After washing out all the other components of the sample, in the 2nd incubation bound anti Rubella IgG are detected by the addition of polyclonal specific anti hIgG antibodies, labelled with peroxidase (HRP).
The enzyme captured on the solid phase, acting on the substrate/chromogen mixture, generates an optical signal that is proportional to the amount of anti Rubella Virus IgG antibodies present in the sample. A Calibration Curve, calibrated against the 1st W.H.O international standard for anti-Rubella immunoglobulin code RUBI-1-94, makes possible a quantitative determination of the IgG antibody in the patient.

D. COMPONENTS
The standard kit contains reagents to perform 96 tests.

1. Microplate: MICROPLATE
12 strips x 8 microwells coated with highly purified and UV inactivated Rubella Virus in presence of bovine proteins.
Plates are sealed into a bag with desiccant. Allow the microplate to reach room temperature before opening; reseal unused strips in the bag with desiccant and store at 2..8°C.

2. Calibration Curve: CAL N° …
Ready to use and color coded standard curve derived from human plasma positive for Rubella IgG and titrated on WHO standard ranging:
4 ml CAL1 = 0 WHO IU/ml
4 ml CAL2 = 10 WHO IU/ml
2ml CAL3 = 20 WHO IU/ml
2 ml CAL4 = 50 WHO IU/ml
2 ml CAL 5 = 100 WHO IU/ml
4 ml CAL6 = 250 WHO IU/ml.
Standards are calibrated against the 1st W.H.O international standard for anti-Rubella immunoglobulin code RUBI-1-94.
It contains human serum proteins, 2% casein, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.1% Tween 20, 0.09% Na-azide and 0.1% Kathon GC as preservatives. Standards are blue colored.

3. Control Serum: CONTROL …ml
1 vial. Lyophilized.
It contains fetal bovine serum proteins, human IgG antibodies to Rubella Virus calibrated at 20 WHO IU/ml ± 10%, 0.2 mg/ml gentamicine sulphate and 0.1% Kathon GC as preservatives.
Note: The volume necessary to dissolve the content of the vial may vary from lot to lot. Please use the right volume reported on the label.

4. Wash buffer concentrate: WASHBUF 20X
1x60ml/bottle20x concentrated solution.
Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2, 0.05% Tween 20 and 0.1% Kathon GC.

5. Enzyme conjugate : CONJ
2x8ml/vial..Ready to use and red colour coded. It contains Horseradish peroxidase conjugated polyclonal antibodies to human IgG, 5% BSA, 10 mM Tris buffer pH 6.8+/-0.1, 0.1% Kathon GC, 0.02 mg/ml gentamicine sulphate as preservatives and 0.01% red alimentary dye.

6. Chromogen/Substrate: SUBS TMB
1x16ml/vial. It contains 50 mM citrate-phosphate buffer pH 3.5-3.8, 0.03% tetra-methyl-benzidine (or TMB) and 0.02% hydrogen peroxide (or H_2O_2) and 4% dimethylsulphoxide.
Note: To be stored protected from light as sensitive to strong illumination.

7. Sulphuric Acid: H_2SO_4 0.3 M
1x15ml/vial. It contains 0.3 M H_2SO_4 solution.
Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

8. Specimen Diluent: DILSPE
2x60ml/vial. It contains 2% casein, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.1% Tween 20, 0.09% Na-azide and 0.1% Kathon GC as preservatives. The reagent is blue colour coded.

9. Plate sealing foils n°2

10. Package insert n°1

Important note: Only upon specific request, Dia.Pro can supply reagents for 192 and 480 tests, as reported below **:**

1.Microplate	n°2	n°5
2.Calibration curve	6x4.0ml/vial	6x10ml/vial
3.Control Serum	n° 2 vials	n° 5 vials
4.Wash buff conc	2x60ml/bottle	5x60ml/bottles
5.Enz. Conjugate	2x16ml/vial	2x40ml/bottles
6.Chromog/Subs	2x16ml/vial	2x40ml/bottles
7.Sulphuric Acid	1x30ml/vial	2x40ml/bottles
8.Specimen Diluent	2x120ml/vial	10x60ml/bottles
9.Plate seal foils	n° 4	n° 10
10.Pack. insert	n° 1	n° 1
Number of tests	**192**	**480**
Code	**RUBG.CE.192**	**RUBG.CE.480**

E. MATERIALS REQUIRED BUT NOT PROVIDED

1. Calibrated Micropipettes (1000 ul, 100 ul and 10 ul) and disposable plastic tips.
2. EIA grade water (double distilled or deionised, charcoal treated to remove oxidizing chemicals used as disinfectants).
3. Timer with 60 minute range or higher.
4. Absorbent paper tissues.
5. Calibrated ELISA microplate thermostatic incubator (dry or wet), set at +37°C (+/-0.5°C tolerance)..
6. Calibrated ELISA microwell reader with 450nm (reading) and with 620-630nm (blanking) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.

F. WARNINGS AND PRECAUTIONS

1. The kit has to be used by skilled and properly trained technical personnel only, under the supervision of a medical doctor responsible of the laboratory.
2. All the personnel involved in performing the assay have to wear protective laboratory clothes, talc-free gloves and glasses. The use of any sharp (needles) or cutting (blades) devices should be avoided. All the personnel involved should be trained in biosafety procedures, as recommended by the Center for Disease Control, Atlanta, U.S. and reported in the National Institute of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
3. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.
4. The laboratory environment should be controlled so as to avoid contaminants such as dust or air-born microbial agents, when opening kit vials and microplates and when performing the test. Protect the Chromogen (TMB) from strong light and avoid vibration of the bench surface where the test is undertaken.
5. Upon receipt, store the kit at 2..8°C into a temperature controlled refrigerator or cold room.
6. Do not interchange components between different lots of the kits. It is recommended that components between two kits of the same lot should not be interchanged.
7. Check that the reagents are clear and do not contain visible heavy particles or aggregates. If not, advise the laboratory supervisor to initiate the necessary procedures for kit replacement.
8. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each sample.
9. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one.
10. Do not use the kit after the expiration date stated on the external container and internal (vials) labels. A study conducted on an opened kit did not pointed out any relevant loss of activity up to six 6 uses of the device and up to 3 months.
11. Treat all specimens as potentially infective. All human serum specimens should be handled at Biosafety Level 2, as recommended by the Center for Disease Control, Atlanta, U.S. in compliance with what reported in the Institutes of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
12. The use of disposable plastic-ware is recommended in the preparation of the liquid components or in transferring components into automated workstations, in order to avoid cross contamination.
13. Waste produced during the use of the kit has to be discarded in compliance with national directives and laws concerning laboratory waste of chemical and biological substances. In particular, liquid waste generated from the washing procedure, from residuals of controls and from samples has to be treated as potentially infective material and inactivated before waste. Suggested procedures of inactivation are treatment with a 10% final concentration of household bleach for 16-18 hrs or heat inactivation by autoclave at 121°C for 20 min..
14. Accidental spills from samples and operations have to be adsorbed with paper tissues soaked with household bleach and then with water. Tissues should then be discarded in proper containers designated for laboratory/hospital waste.
15. The Sulphuric Acid is an irritant. In case of spills, wash the surface with plenty of water
16. Other waste materials generated from the use of the kit (example: tips used for samples and controls, used microplates) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

G. SPECIMEN: PREPARATION AND WARNINGS

1. Blood is drawn aseptically by venepuncture and plasma or serum is prepared using standard techniques of preparation of samples for clinical laboratory analysis. No influence has been observed in the preparation of the sample with citrate, EDTA and heparin.
2. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results. Bar code labeling and electronic reading is strongly recommended.
3. Haemolysed ("red") and visibly hyperlipemic ("milky") samples have to be discarded as they could generate false results. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they could give rise to false results.
4. Sera and plasma can be stored at +2°..8°C for up to five days after collection. For longer storage periods, samples can be stored frozen at –20°C for several months. Any frozen samples should not be frozen/thawed more than once as this may generate particles that could affect the test result.
5. If particles are present, centrifuge at 2.000 rpm for 20 min or filter using 0.2-0.8u filters to clean up the sample for testing.
6. Samples whose anti-Rubella IgG antibody concentration is expected to be higher than 250 IU/ml should be diluted before use, either 1:10 or 1:100 in the Calibrator 0 IU/ml. Dilutions have to be done in clean disposable tubes by diluting 50 ul of each specimen with 450 ul of Cal 0 (1:10). Then 50 ul of the 1:10 dilution are diluted with 450 ul of the Cal 0 (1:100). Mix tubes thoroughly on vortex and then proceed toward the dilution step reported in section M.

H. PREPARATION OF COMPONENTS AND WARNINGS

A study conducted on an opened kit has not pointed out any relevant loss of activity up to 6 re-uses of the device and up to 3 months.

Microplate:

Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant has not turned dark green, indicating a defect in manufacturing.
In this case, call Dia.Pro's customer service.
Unused strips have to be placed back into the aluminum pouch, with the desiccant supplied, firmly zipped and stored at +2°..8°C.
After first opening, remaining strips are stable until the humidity indicator inside the desiccant bag turns from yellow to green.

Calibration Curve

Ready to use component. Mix carefully on vortex before use.

Control Serum

Add the volume of ELISA grade water, reported on the label, to the lyophilised powder; let fully dissolve and then gently mix on vortex.
Note: *The control after dissolution is not stable. Store frozen in aliquots at –20°C.*

Wash buffer concentrate:

The 20x concentrated solution has to be diluted with EIA grade water up to 1200 ml and mixed gently end-over-end before use.

As some salt crystals may be present into the vial, take care to dissolve all the content when preparing the solution.
In the preparation avoid foaming as the presence of bubbles could give origin to a bad washing efficiency.
Note: *Once diluted, the wash solution is stable for 1 week at +2..8° C.*

Enzyme conjugate:
Ready to use. Mix well on vortex before use.
Be careful not to contaminate the liquid with oxidizing chemicals, air-driven dust or microbes.
If this component has to be transferred use only plastic, possibly sterile disposable containers.

Chromogen/Substrate:
Ready to use. Mix well on vortex before use.
Be careful not to contaminate the liquid with oxidizing chemicals, air-driven dust or microbes.
Do not expose to strong illumination, oxidizing agents and metallic surfaces.
If this component has to be transferred use only plastic, possible sterile disposable container

Sample Diluent
Ready to use component. Mix carefully on vortex before use.

Sulphuric Acid:
Ready to use. Mix well on vortex before use.
Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

Legenda:

Warning **H statements:**
H315 – Causes skin irritation.
H319 – Causes serious eye irritation.

Precautionary **P statements:**
P280 – Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352 – IF ON SKIN: Wash with plenty of soap and water.
P332 + P313 – If skin irritation occurs: Get medical advice/attention.
P305 + P351 + P338 – IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337 + P313 – If eye irritation persists: Get medical advice/attention.
P362 + P363 – Take off contaminated clothing and wash it before reuse.

I. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

1. Micropipettes have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (household alcohol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample. They should also be regularly maintained in order to show a precision of 1% and a trueness of +/-2%. Decontamination of spills or residues of kit components should also be carried out regularly.
2. The ELISA incubator has to be set at +37°C (tolerance of +/- 0.5°C) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water baths are suitable for the incubations, provided that the instrument is validated for the incubation of ELISA tests.
3. The ELISA washer is extremely important to the overall performances of the assay. The washer must be carefully validated and correctly optimised using the kit controls and reference panels, before using the kit for routine laboratory tests. Usually 4-5 washing cycles (aspiration + dispensation of 350ul/well of washing solution = 1 cycle) are sufficient to ensure that the assay performs as expected. A soaking time of 20-30 seconds between cycles is suggested. In order to set correctly their number, it is recommended to run an assay with the kit controls and well characterized negative and positive reference samples, and check to match the values reported below in the sections “Validation of Test” and “Assay Performances”. Regular calibration of the volumes delivered by, and maintenance (decontamination and cleaning of needles) of the washer has to be carried out according to the instructions of the manufacturer.
4. Incubation times have a tolerance of $\pm$5%.
5. The ELISA microplate reader has to be equipped with a reading filter of 450nm and with a second filter (620-630nm, strongly recommended) for blanking purposes. Its standard performances should be (a) bandwidth $\leq$ 10 nm; (b) absorbance range from 0 to $\geq$ 2.0; (c) linearity to $\geq$ 2.0; repeatability $\geq$ 1%. Blanking is carried out on the well identified in the section “Assay Procedure”. The optical system of the reader has to be calibrated regularly to ensure that the correct optical density is measured. It should be regularly maintained according to the manufacturer ‘s instructions.
6. When using an ELISA automated work station, all critical steps (dispensation, incubation, washing, reading, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the sections “Validation of Test” and “Assay Performances”. The assay protocol has to be installed in the operating system of the unit and validated as for the washer and the reader. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set. Particular attention must be paid to avoid carry over by the needles used for dispensing and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells. The use of ELISA automated work stations is recommended when the number of samples to be tested exceed 20-30 units per run.
7. When using automatic devices, in case the vial holder of the instrument does not fit with the vials supplied in the kit, transfer the solution into appropriate containers and label them with the same label peeled out from the original vial. This operation is important in order to avoid mismatching contents of vials, when transferring them. When the test is over, return the secondary labeled containers to 2..8°C, firmly capped.
8. Dia.Pro’s customer service offers support to the user in the setting and checking of instruments used in combination with the kit, in order to assure compliance with the requirements described. Support is also provided for the installation of new instruments to be used with the kit.

L. PRE ASSAY CONTROLS AND OPERATIONS

1. Check the expiration date of the kit printed on the external label (primary container). Do not use if expired.
2. Check that the liquid components are not contaminated by visible particles or aggregates.
3. Check that the Chromogen (TMB) is colourless or pale blue by aspirating a small volume of it with a sterile plastic pipette.
4. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box (primary container). Check that the aluminium pouch, containing the microplate, is not punctured or damaged.
5. Dissolve the content of the lyophilised Control Serum as reported in the proper section.
6. Dilute all the content of the 20x concentrated Wash Solution as described above.
7. Allow all the other components to reach room temperature (about 1 hr) and then mix gently on vortex all liquid reagents.

8. Set the ELISA incubator at +37°C and prepare the ELISA washer by priming with the diluted washing solution, according to the manufacturers instructions. Set the right number of washing cycles as found in the validation of the instrument for its use with the kit.
9. Check that the ELISA reader is turned on or ensure it will be turned on at least 20 minutes before reading.
10. If using an automated work station, turn on, check settings and be sure to use the right assay protocol.
11. Check that the micropipettes are set to the required volume.
12. Check that all the other equipment is available and ready to use.
13. In case of problems, do not proceed further with the test and advise the supervisor.

M. ASSAY PROCEDURE

The assay has to be carried out according to what reported below, taking care to maintain the same incubation time for all the samples in testing.
The kit may be used for quantitative and qualitative determinations as well

M1. QUANTITATIVE DETERMINATION:

Automated assay:

In case the test is carried out automatically with an ELISA system, we suggest to make the instrument aspirate 1000 µl Sample Diluent and then 10 µl sample (1:101 dilution factor). The whole content is then dispensed into a properly defined dilution tube. Before the next sample is aspirated, needles have to be duly washed to avoid any cross-contamination among samples. When all the samples have been diluted make the instrument dispense 100 µl samples into the proper wells of the microplate.
This procedure may be carried out also in two steps of dilutions of 1:10 each (90 µl Sample Diluent + 10 µl sample) into a second dilution platform. Make then the instrument aspirate first 100 µl Sample Diluent, then 10 µl liquid from the first dilution in the platform and finally dispense the whole content in the proper well of the assay microplate.
Do not dilute Calibrators and the dissolved Control Serum as they are ready to use.
Dispense 100 µl calibrators/control in the appropriate calibration/control wells.
For the next operations follow the operative instructions reported below for the Manual Assay.
It is strongly recommended to check that the time lap between the dispensation of the first and the last sample will be calculated by the instrument and taken into consideration by delaying the first washing operation accordingly.

Manual assay:

1. Dilute samples 1:101 into a properly defined dilution tube (example: 1000 µl Sample Diluent + 10 µl sample). Do not dilute the Calibration Set as calibrators are ready to use. Mix carefully all the liquid components on vortex and then proceed as described below.
2. Place the required number of microwells in the microwell holder. Leave the A1 and B1 empty for the operation of blanking.
3. Dispense 100 µl of Calibrators and 100 µl Control Serum in duplicate. Then dispense 100 µl of diluted samples in each properly identified well.
4. Incubate the microplate for **60 min at +37°C**.

Important note: *Strips have to be sealed with the adhesive sealing foil, supplied, only when the test is carried out manually. Do not cover strips when using ELISA automatic instruments.*

5. Wash the microplate with an automatic washer by delivering and aspirating 350 µl/well of diluted washing solution as reported previously (section I.3).
6. Pipette 100 µl Enzyme Conjugate into each well, except A1+B1 blanking wells, and cover with the sealer. Check that this red coloured component has been dispensed in all the wells, except A1 and B1.

Important note: *Be careful not to touch the plastic inner surface of the well with the tip filled with the Enzyme Conjugate. Contamination might occur.*

7. Incubate the microplate for **60 min at +37°C**.
8. Wash microwells as in step 5.
9. Pipette 100 µl Chromogen/Substrate mixture into each well, the blank wells A1 and B1 included. Then incubate the microplate at **room temperature (18-24°C) for 20 minutes**.

Important note: *Do not expose to strong direct illumination. High background might be generated.*

10. Pipette 100 µl Sulphuric Acid to stop the enzymatic reaction into all the wells using the same pipetting sequence as in step 9. Addition of acid will turn the positive calibrators, the control serum and the positive samples from blue to yellow.
11. Measure the colour intensity of the solution in each well, as described in section I.5, at 450nm filter (reading) and at 620-630nm (background subtraction, strongly recommended), blanking the instrument on A1 or B1 or both.

M2. QUALITATIVE DETERMINATION

If only a qualitative determination is required, proceed as described below:

Automated assay:

Proceed as described in section M1.

Manual assay:

1. Dilute samples 1:101 into a properly defined dilution tube (example: 1000 µl Sample Diluent + 10 µl sample). Do not dilute the Calibration Set as calibrators are ready to use. Mix carefully all the liquid components on vortex and then proceed as described below.
2. Place the required number of Microwells in the microwell holder. Leave A1 well empty for the operation of blanking.
3. Dispense 100 µl of Calibrator 1 (0 IU/ml) and 100 µl Calibrator 2 (10 IU/ml) in duplicate, and 100 µl Calibrator 6 (250 IU/ml) in single. Then dispense 100 µl of diluted samples in each properly identified well.
4. Incubate the microplate for **60 min at +37°C**.

Important note: *Strips have to be sealed with the adhesive sealing foil, supplied, only when the test is carried out manually. Do not cover strips when using ELISA automatic instruments.*

5. Wash the microplate with an automatic washer by delivering and aspirating 350 µl/well of diluted washing solution as reported previously (section I.3).
6. Pipette 100 µl Enzyme Conjugate into each well, except the A1 well, and cover with the sealer. Check that this red coloured component has been dispensed in all the wells, except A1.

Important note: *Be careful not to touch the plastic inner surface of the well with the tip filled with the Enzyme Conjugate. Contamination might occur.*

7. Incubate the microplate for **60 min at +37°C**.
8. Wash microwells as in step 5.
9. Pipette 100 µl Chromogen/Substrate mixture into each well, the blank well included. Then incubate the microplate at **room temperature (18-24°C) for 20 minutes**.

Important note: *Do not expose to strong direct illumination. High background might be generated.*

10. Pipette 100 µl Sulphuric Acid into all the wells using the same pipetting sequence as in step 9. Addition of acid will turn the positive calibrators, the control serum and the positive samples from blue to yellow.
11. Measure the colour intensity of the solution in each well, as described in section I.5, at 450nm filter (reading) and at 620-630nm (background subtraction, strongly recommended), blanking the instrument on A1.

General Important notes:

1. *If the second filter is not available ensure that no finger prints are present on the bottom of the microwell before reading at 450nm. Finger prints could generate false positive results on reading.*
2. *Reading has to be carried out just after the addition of the Stop Solution and anyway not any longer than 20 minutes after its addition. Some self oxidation of the chromogen can occur leading to high background.*
3. *The Control Serum (CS) does not affect the test results calculation. The Control Serum may be used only when a laboratory internal quality control a laboratory internal quality control is required by the management.*

N. ASSAY SCHEME

Method	Operations
Calibrators & Control	100 µl
Samples diluted 1:101	100 µl
1st incubation	60 min
Temperature	+37°C
Wash step	4-5 cycles
Enzyme conjugate	100 µl
2nd incubation	60 min
Temperature	+37°C
Wash step	4-5 cycles
TMB/H2O2	100 µl
3rd incubation	20 min
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm

An example of dispensation scheme for Quantitative Analysis is reported below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	CAL4	S 1									
B	BLK	CAL4	S 2									
C	CAL1	CAL5	S 3									
D	CAL1	CAL5	S 4									
E	CAL2	CAL6	S 5									
F	CAL2	CAL6	S 6									
G	CAL3	CS	S 7									
H	CAL3	CS	S 8									

Legenda: BLK = Blank CAL = Calibrator CS = Control Serum S = Sample

An example of dispensation scheme in qualitative assays is reported below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S 3	S 11									
B	CAL1	S 4	S 12									
C	CAL1	S 5	S 13									
D	CAL2	S 6	S 14									
E	CAL2	S 7	S 15									
F	CAL6	S 8	S 16									
G	S 1	S 9	S 17									
H	S 2	S 10	S 18									

Legenda: BLK = Blank CAL = Calibrator CS = Control Serum S = Sample

O. INTERNAL QUALITY CONTROL

A validation check is carried out on the controls any time the kit is used in order to verify whether the performances of the assay are as qualified. Control that the following data are matched:

Check	Requirements
Blank well	< 0.050 OD450nm value
CAL 1 0 IU/ml	< 0.150 mean OD450nm value after blanking coefficient of variation < 30%
CAL 2 10 IU/ml	OD450nm > OD450nm CAL1 + 0.100
CAL 6 250 IU/ml	OD450nm > 1.000
Control Serum	20 IU/ml ±10%

If the results of the test match the requirements stated above, proceed to the next section. If they do not, do not proceed any further and operate as follows:

Problem	Check
Blank well > 0.050 OD450nm	1. that the Chromogen/Sustrate solution has not got contaminated during the assay
CAL 1 **0 IU/ml** > 0.150 OD450nm after blanking coefficient of variation > 30%	1. that the washing procedure and the washer settings are as validated in the pre qualification study; 2. that the proper washing solution has been used and the washer has been primed with it before use; 3. that no mistake has been done in the assay procedure (dispensation of a positive calibrator instead of the negative one; 4. that no contamination of the negative calibrator or of their wells has occurred due spills of positive samples or the enzyme conjugate; 5. that micropipettes haven't got contaminated with positive samples or with the enzyme conjugate 6. that the washer needles are not blocked or partially obstructed.

CAL 2 **10 IU/ml** OD450nm < OD450nm CAL1 + 0.100	1. that the procedure has been correctly executed; 2. that no mistake has been done in its distribution (ex.: dispensation of a wrong calibrator instead); 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the calibrator has occurred.
CAL 6 **250 IU/ml** < 1.000 OD450nm	1. that the procedure has been correctly executed; 2. that no mistake has been done in its distribution (dispensation of a wrong calibrator instead) ; 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the positive control has occurred.
Control Serum Different from expected value	First verify that: 1. the procedure has been correctly performed; 2. no mistake has occurred during its distribution (ex.: dispensation of a wrong sample); 3. the washing procedure and the washer settings are correct; 4. no external contamination of the standard has occurred. 5. the Control Serum has been dissolved with the right volume reported on the label. If a mistake has been pointed out, the assay has to be repeated after eliminating the reason of this error. If no mistake has been found, proceed as follows: **a**) a value up to +/-20% is obtained: the overall Precision of the laboratory might not enable the test to match the expected value +/-10%. Report the problem to the Supervisor for acceptance or refusal of this result. **b**) a value higher than +/-20% is obtained: in this case the test is invalid and the DiaPro's customer service has to be called.

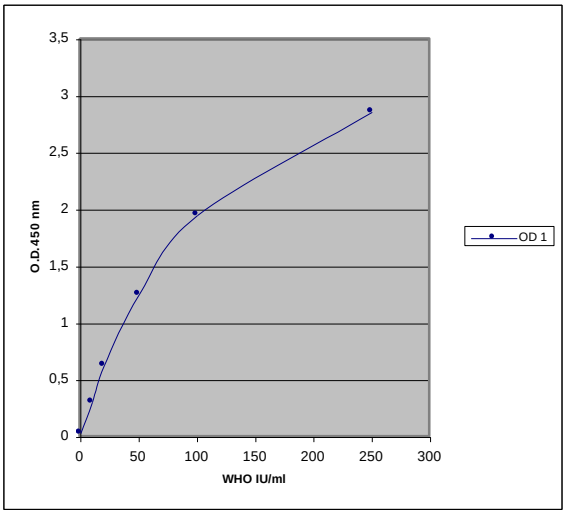
Should one of these problems have happened, after checking, report to the supervisor for further actions.

P. RESULTS

P.1 Quantitative method

If the test turns out to be valid, use for the quantitative method an approved curve fitting program to draw the calibration curve from the values obtained by reading at 450nm (4-parameters interpolation is suggested).
Then on the calibration curve calculate the concentration of anti Rubella Virus IgG antibody in samples.
An example of Calibration curve is reported in the next page.

Example of Calibration Curve :



Important Note:
Do not use the calibration curve above to make calculations.

P.2 Qualitative method

In the qualitative method, calculate the mean OD450nm values for the Calibrators 0 and 10 IU/ml and then check that the assay is valid.

Example of calculation:

The following data must not be used instead or real figures obtained by the user.

Calibrator 0 IU/ml: 0.020 – 0.024 OD450nm
Mean Value: 0.022 OD450nm
Lower than 0.150 – Accepted
Calibrator 10 IU/ml: 0.250 – 0.270 OD450nm
Mean Value: 0.260 OD450nm
Higher than Cal 0 + 0.100 – Accepted
Calibrator 250 IU/ml: 2.845 OD450nm
Higher than 1.000 – Accepted

Q. INTERPRETATION OF RESULTS

Samples with a concentration lower than 10 WHO IU/ml are considered negative for anti Rubella Virus IgG antibody by most of the international medical literature.
Samples with a concentration higher than 10 WHO IU/ml are considered positive for anti Rubella Virus IgG antibody.
This titer is considered the lowest concentration of IgG to provide an effective immunological protection against a second infection of Rubella Virus by NCCLS, USA.
Particular attention in the interpretation of results has to be used in the follow-up of pregnancy for an infection of Rubella Virus due to the risk of severe neonatal malformations.

Important notes:

1. *Interpretation of results should be done under the supervision of the laboratory supervisor to reduce the risk of judgment errors and misinterpretations.*
2. *When test results are transmitted from the laboratory to another facility, attention must be paid to avoid erroneous data transfer.*
3. *In the follow-up of pregnancy for Rubella Virus infection a positive result (presence of IgG antibody > 10 IU/ml) should be confirmed to ruled out the risk of a false positive result and a false definition of protection.*

R. PERFORMANCES

Evaluation of Performances has been conducted in accordance to what suggested in NCCLS's approved guideline for Rubella IgG testing (I/LA6-A)

1. Limit of detection

The limit of detection of the assay has been calculated by means of the 1st W.H.O international standard for anti-Rubella immunoglobulin code RUBI-1-94. The limit of detection has been calculated as mean OD450nm Calibrator 0 IU/ml + 5 SD.
The table below reports the mean OD450nm values of this standard when diluted in negative plasma and then examined in the assa for three lots.

WHO IU/ml	RUBG.CE Lot. 0303	RUBG.CE Lot. 0503	RUBG.CE Lot. 0603
50	1.292	1.301	1.354
20	0.701	0.742	0.724
10	0.402	0.451	0.425
5	0.211	0.241	0.231
Std 0	0.024	0.032	0.038

The assay shows a limit of detection far better than 10 IU/ml.

2. Diagnostic sensitivity:
The diagnostic sensitivity has been tested in an external study of performance evaluation (University Hospital, Microbiology Department, Salamanca, Spain) on panels of samples classified positive by a kit US FDA approved. Positive samples from different stage of Rubella Virus infection were tested.
The value, obtained from the analysis of more than 300 specimens, has been > 98%.

3. Diagnostic specificity:
The diagnostic specificity has been determined in the same centre on panels of negative samples from not infected individuals, classified negative with a kit US FDA approved.
Both plasma, derived with different standard techniques of preparation (citrate, EDTA and heparin), and sera have been used to determine the value of specificity.
Frozen specimens have been tested, as well, to check for interferences due to collection and storage.
No interference was observed.
Potentially interfering samples derived from patients with different pathologies (mostly ANA, AMA and RF positive) and from pregnant women were tested.
No crossreaction was observed.
An overall value > 98% of specificity was found when examined on more than 100 specimens.

4. Precision:
It has been calculated on three samples, a negative, a low positive and a positive, examined in 16 replicates in three separate runs for three lots. Results are reported as follows:

RUBG.CE: lot 0303

Calibrator 0 IU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.048	0.054	0.052	0.051
Std.Deviation	0.004	0.005	0.005	0.005
CV %	9.3	8.6	8.9	8.9

Calibrator 10 IU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.530	0.503	0.484	0.505
Std.Deviation	0.034	0.022	0.019	0.025
CV %	6.4	4.4	4.0	4.9

Calibrator 250 IU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	3.299	3.281	3.267	3.282
Std.Deviation	0.228	0.119	0.067	0.138
CV %	6.9	3.6	2.1	4.2

RUBG.CE: lot 0503

Calibrator 0 IU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.046	0.052	0.051	0.049
Std.Deviation	0.004	0.005	0.005	0.005
CV %	9.5	8.9	9	9.2

Calibrator 10 IU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.531	0.504	0.484	0.506
Std.Deviation	0.034	0.022	0.019	0.025
CV %	6.4	4.3	4	4.9

Calibrator 250 IU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	3.281	3.281	3.272	3.278
Std.Deviation	0.199	0.155	0.147	0.167
CV %	6.1	4.7	4.5	5.1

RUBG.CE: lot 0603

Calibrator 0 IU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.052	0.052	0.053	0.052
Std.Deviation	0.005	0.004	0.004	0.004
CV %	9	8.1	6.9	8

Calibrator 10 IU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.524	0.510	0.483	0.505
Std.Deviation	0.037	0.022	0.020	0.027
CV %	7.1	4.4	4.2	5.2

Calibrator 250 IU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	3.300	3.286	3.253	3.280
Std.Deviation	0.195	0.126	0.074	0.131
CV %	5.9	3.8	2.3	4

The variability shown in the tables above did not result in sample misclassification.

5. Accuracy
The assay accuracy has been checked by the dilution and recovery tests. Any “hook effect”, underestimation likely to happen at high doses of analyte, was ruled out up to 1.000 IU/ml.

S. LIMITATIONS OF THE PROCEDURE
Bacterial contamination or heat inactivation of the specimen may affect the absorbance values of the samples with consequent alteration of the level of the analyte.
Frozen samples containing fibrin particles or aggregates after thawing may generate some false results.
This test is suitable only for testing single samples and not pooled ones.
Diagnosis of an infectious disease should not be established on the basis of a single test result. The patient’s clinical history, symptomatology, as well as other diagnostic data should be considered.

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All the IVD Products manufactured by the company are under the control of a certified Quality Management System approved by an EC Notified Body. Each lot is submitted to a quality control and released into the market only if conforming with the EC technical specifications and acceptance criteria.

Manufacturer:
Dia.Pro. Diagnostic Bioprobes Srl.
Via G. Carducci n° 27 – Sesto San Giovanni (MI) - Italy

CE
0318

Dia.Pro
Diagnostic
BioProbes

EC DECLARATION OF CONFORMITY

MANUFACTURER	DIA.PRO DIAGNOSTIC BIOPROBES S.R.L. VIA G. CARDUCCI N° 27 – 20099 SESTO SAN GIOVANNI (MILANO) – ITALY
PRODUCT	**RUB IgG** CODES: **RUBG.CE (96 tests)** RUBG.CE.192 (192 tests) RUBG.CE.480 (480 tests)
CLASSIFICATION	ANNEX II – LIST B
CONFORMITY ASSESSMENT ROUTE	ANNEX IV

WE HEREBY DECLARE THAT THE ABOVE MENTIONED PRODUCT MEETS THE PROVISIONS OF THE COUNCIL DIRECTIVE 98/79/EC FOR IN VITRO DIAGNOSTIC DEVICES.

NOTIFIED BODY	AEMPS – n° 0318
(EC) CERTIFICATE(S)	• FULL QUALITY ASSURANCE SYSTEM N° 2004 05 0442 CT (in accordance with Annex IV – except Section IV) of the Directive 98/79/EC), RELEASED BY EC NOTIFIED BODY N° 0318 • UNI EN ISO 13485 N° 2013 11 0039 EN, RELEASED BY EC NOTIFIED BODY N° 0318

PLACE & DATE OF FIRST ISSUE	MILANO – MAY 2004
PLACE & DATE OF CURRENT EMISSION	SESTO SAN GIOVANNI (MI) – MAY 2018
SIGNATURE **Legal Representative** **Dr.ssa Fiorenza Scozzesi**	

Rev:	05/2018

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EC DECLARATION OF CONFORMITY

MANUFACTURER	DIA.PRO DIAGNOSTIC BIOPROBES S.R.L. VIA G. CARDUCCI N° 27 – 20099 SESTO SAN GIOVANNI (MILANO) – ITALY
PRODUCT	**Parvovirus B19 IgG** CODE: **PARVOG.CE** (96 tests)
CLASSIFICATION	GENERAL IVD
CONFORMITY ASSESSMENT ROUTE	SELF CERTIFICATION

WE HEREBY DECLARE THAT THE ABOVE MENTIONED PRODUCT MEETS THE PROVISIONS OF THE COUNCIL DIRECTIVE 98/79/EC FOR IN VITRO DIAGNOSTIC DEVICES.

ISO CERTIFICATE	UNE EN ISO 13485 N° 2013 11 0039 EN, RELEASED BY AEMPS (AGENCIA ESPAÑOLA DE MEDICAMENTOS Y PRODUCTOS SANITARIOS)

PLACE & DATE OF FIRST ISSUE	SESTO SAN GIOVANNI (MI) – MAY 2013
PLACE & DATE OF CURRENT ISSUE	SESTO SAN GIOVANNI (MI) – MARCH 2019
SIGNATURE **Legal Representative** **Dr.ssa Fiorenza Scozzesi**	

Rev:	05/2018

Parvovirus B19 IgG

Enzyme ImmunoAssay (ELISA) for the quantitative/qualitative determination of IgG antibodies to Parvovirus B19 in human serum and plasma

- for “in vitro” diagnostic use only -

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REF PARVOG.CE
96 Tests

Parvovirus B19 IgG

A. INTENDED USE
Enzyme ImmunoAssay (ELISA) for the quantitative/qualitative determination of IgG antibodies to Parvovirus B19 in human plasma and sera.
For "in vitro" diagnostic use only.

B. INTRODUCTION
The **B19 virus**, generally referred to as **parvovirus B19** was the first (and until 2005 the only) known human virus in the family of parvoviruses, genus erythrovirus. Parvovirus B19 is a non-enveloped, icosahedral virus that contains a single-stranded linear DNA genome. It is classified as erythrovirus because of its capability to invade red blood cell precursors in the bone marrow. Three genotypes (with subtypes) have been recognized. The viral capsid is composed of two structural proteins , namely VP1 (83kD) and VP2 (53 kD). Infection by Parvovirus B19 spreads through respiratory secretions but also through blood or blood products. The infection causes a mild illness characterized by an erythematous maculopapular facial rash called fifth disease or erythema infectious. It is tipical in childhood and is also seen in adults. A person usually gets sick within 4 to 14 days after getting infected with parvovirus B19 but about 20% of children and adults who get infected with this virus will not have any symptoms. Infection during pregnancy presents the risk of transmission to the fetus that may result in hydrops fetalis. People with weakened immune systems caused by leukemia, cancer, organ transplants, or HIV infection are at risk for serious complications from fifth disease. It can cause chronic anemia that requires medical treatment.
Therefore the detection of Parvovirus B19-specific antibodies becomes very important.

C. PRINCIPLE OF THE TEST
Microplates are coated with Parvovirus B19 antigen.
The solid phase is first treated with the diluted sample and IgG to Parvovirus B19 are captured, if present, by the antigens.
After washing out all the other components of the sample, in the 2nd incubation bound anti Parvovirus IgG are detected by the addition of polyclonal specific anti hIgG antibodies, labelled with peroxidase (HRP).
The enzyme captured on the solid phase, acting on the substrate/chromogen mixture, generates an optical signal that is proportional to the amount of anti Parvovirus IgG antibodies present in the sample. A Calibration Curve, calibrated against the 2nd W.H.O international standard for Anti-Parvovirus B19 code 01/602, makes possible a quantitative determination of the IgG antibody in the patient.

D. COMPONENTS
The kit contains reagents to perform 96 tests.

1. Microplate: MICROPLATE
12 strips x 8 microwells coated with Parvovirus B19 antigens.
Plates are sealed into a bag with desiccant. Allow the microplate to reach room temperature before opening; reseal unused strips in the bag with desiccant and store at 2..8°C.

2. Calibration Curve: CAL N° …
Ready to use and color coded standard curve derived from human plasma positive for Parvovirus B19 IgG and titrated on WHO standard ranging:
4ml/vial CAL1 = 0 WHO IU/ml
4ml/vial CAL2 = 3 WHO IU/ml
2ml/vial CAL3 = 6 WHO IU/ml
2ml/vial CAL4 = 12 WHO IU/ml
2ml/vial CAL5 = 20 WHO IU/ml
4ml/vial CAL6 = 40 WHO IU/ml.
Standards are calibrated against the 2nd W.H.O international standard for Anti-Parvovirus B19 code 01/602.
It contains human serum proteins, 2% casein, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.1% Tween 20, 0.09% Na-azide and 0.045% ProClin 300 as preservatives. Standards are blue colored.

3. Control Serum: CONTROL …ml
1 vial. Lyophilized.
It contains bovine serum proteins, human plasma positive to Parvovirus B19 calibrated at 12 WHO IU/ml ± 10%, 0.2 mg/ml gentamicine sulphate and 0.045% ProClin 300 as preservatives.
Note: The volume necessary to dissolve the content of the vial may vary from lot to lot. Please use the right volume reported on the label.

4. Wash buffer concentrate: WASHBUF 20X
1x60ml/bottle20x concentrated solution.
Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2, 0.05% Tween 20 and 0.045% ProClin 300.

5. Enzyme conjugate : CONJ
2x8ml/vial.Ready to use and red colour coded. It contains Horseradish peroxidase conjugated polyclonal antibodies to human IgG, 5% BSA, 10 mM Tris buffer pH 6.8+/-0.1, 0.045% ProClin 300, 0.02 mg/ml gentamicine sulphate as preservatives and 0.01% red alimentary dye.

6. Chromogen/Substrate: SUBS TMB
1x16ml/vial. It contains 50 mM citrate-phosphate buffer pH 3.5-3.8, 0.03% tetra-methyl-benzidine (or TMB) and 0.02% hydrogen peroxide (or H_2O_2) and 4% dimethylsulphoxide.
Note: To be stored protected from light as sensitive to strong illumination.

7. Sulphuric Acid: H_2SO_4 0.3 M
1x15ml/vial. It contains 0.3 M H_2SO_4 solution.
Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

8. Specimen Diluent: DILSPE
2x60ml/vial. It contains 2% casein, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.1% Tween 20, 0.09% Na-azide and 0.045% ProClin 300 as preservatives. The reagent is blue colour coded.

9. Plate sealing foils n°2

10. Package insert n°1

E. MATERIALS REQUIRED BUT NOT PROVIDED
1. Calibrated Micropipettes (1000 ul, 100 ul and 10 ul) and disposable plastic tips.
2. EIA grade water (double distilled or deionised, charcoal treated to remove oxidizing chemicals used as disinfectants).
3. Timer with 60 minute range or higher.
4. Absorbent paper tissues.
5. Calibrated ELISA microplate thermostatic incubator (dry or wet), set at +37°C (+/-0.5°C tolerance)..
6. Calibrated ELISA microwell reader with 450nm (reading) and with 620-630nm (blanking) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.

F. WARNINGS AND PRECAUTIONS

1. The kit has to be used by skilled and properly trained technical personnel only, under the supervision of a medical doctor responsible of the laboratory.
2. All the personnel involved in performing the assay have to wear protective laboratory clothes, talc-free gloves and glasses. The use of any sharp (needles) or cutting (blades) devices should be avoided. All the personnel involved should be trained in biosafety procedures, as recommended by the Center for Disease Control, Atlanta, U.S. and reported in the National Institute of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
3. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.
4. The laboratory environment should be controlled so as to avoid contaminants such as dust or air-born microbial agents, when opening kit vials and microplates and when performing the test. Protect the Chromogen (TMB) from strong light and avoid vibration of the bench surface where the test is undertaken.
5. Upon receipt, store the kit at 2..8°C into a temperature controlled refrigerator or cold room.
6. Do not interchange components between different lots of the kits. It is recommended that components between two kits of the same lot should not be interchanged.
7. Check that the reagents are clear and do not contain visible heavy particles or aggregates. If not, advise the laboratory supervisor to initiate the necessary procedures for kit replacement.
8. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each sample. Do not reuse disposable tips.
9. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one. Do not reuse disposable tips.
10. Do not use the kit after the expiration date stated on the external container and internal (vials) labels. A study conducted on an opened kit did not pointed out any relevant loss of activity up to six 6 uses of the device and up to 3 months.
11. Treat all specimens as potentially infective. All human serum specimens should be handled at Biosafety Level 2, as recommended by the Center for Disease Control, Atlanta, U.S. in compliance with what reported in the Institutes of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
12. The use of disposable plastic-ware is recommended in the preparation of the liquid components or in transferring components into automated workstations, in order to avoid cross contamination.
13. Waste produced during the use of the kit has to be discarded in compliance with national directives and laws concerning laboratory waste of chemical and biological substances. In particular, liquid waste generated from the washing procedure, from residuals of controls and from samples has to be treated as potentially infective material and inactivated before waste. Suggested procedures of inactivation are treatment with a 10% final concentration of household bleach for 16-18 hrs or heat inactivation by autoclave at 121°C for 20 min..
14. Accidental spills from samples and operations have to be adsorbed with paper tissues soaked with household bleach and then with water. Tissues should then be discarded in proper containers designated for laboratory/hospital waste.
15. The Sulphuric Acid is an irritant. In case of spills, wash the surface with plenty of water
16. Other waste materials generated from the use of the kit (example: tips used for samples and controls, used microplates) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

G. SPECIMEN: PREPARATION AND WARNINGS

1. Blood is drawn aseptically by venepuncture and plasma or serum is prepared using standard techniques of preparation of samples for clinical laboratory analysis. No influence has been observed in the preparation of the sample with citrate, EDTA and heparin.
2. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results. Bar code labeling and electronic reading is strongly recommended.
3. Haemolysed ("red") and visibly hyperlipemic ("milky") samples have to be discarded as they could generate false results. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they could give rise to false results.
4. Sera and plasma can be stored at +2°…+8°C in primary collection tubes for up to five days after collection.

Do not freeze primary tubes of collection. For longer storage periods, sera and plasma samples, carefully removed from the primary collection tube, can be stored frozen at –20°C for several months. Any frozen samples should not be frozen/thawed more than once as this may generate particles that could affect the test result.

5. If particles are present, centrifuge at 2.000 rpm for 20 min or filter using 0.2-0.8u filters to clean up the sample for testing.
6. Samples whose anti-Parvovirus B19 IgG antibody concentration is expected to be higher than 40 IU/ml should be diluted before use, either 1:10 in the Specimen Diluent. Dilutions have to be done in clean disposable tubes by diluting 50 ul of each specimen with 450 ul of Specimen Diluent (1:10). Mix tubes thoroughly on vortex and then proceed toward the dilution step reported in section M.

H. PREPARATION OF COMPONENTS AND WARNINGS

A study conducted on an opened kit has not pointed out any relevant loss of activity up to 6 re-uses of the device and up to 3 months.

Microplate:

Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant has not turned dark green, indicating a defect in manufacturing.
In this case, call Dia.Pro's customer service.
Unused strips have to be placed back into the aluminum pouch, with the desiccant supplied, firmly zipped and stored at +2°..8°C.
After first opening, remaining strips are stable until the humidity indicator inside the desiccant bag turns from yellow to green.

Calibration Curve

Ready to use component. Mix carefully on vortex before use.

Control Serum

Add the volume of ELISA grade water, reported on the label, to the lyophilised powder; let fully dissolve and then gently mix on vortex.
Note: *The control after dissolution is not stable. Store frozen in aliquots at –20°C. Do not thaw and freeze the aliquot again.*

Wash buffer concentrate:

The 20x concentrated solution has to be diluted with EIA grade water up to 1200 ml and mixed gently end-over-end before use. As some salt crystals may be present into the vial, take care to dissolve all the content when preparing the solution.
In the preparation avoid foaming as the presence of bubbles could give origin to a bad washing efficiency.
Note: *Once diluted, the wash solution is stable for 1 week at +2..8° C.*

Enzyme conjugate:
Ready to use. Mix well on vortex before use.
Be careful not to contaminate the liquid with oxidizing chemicals, air-driven dust or microbes.
If this component has to be transferred use only plastic, possibly sterile disposable containers.

Chromogen/Substrate:
Ready to use. Mix well on vortex before use.
Be careful not to contaminate the liquid with oxidizing chemicals, air-driven dust or microbes.
Do not expose to strong illumination, oxidizing agents and metallic surfaces.
If this component has to be transferred use only plastic, possible sterile disposable container
Sample Diluent
Ready to use component. Mix carefully on vortex before use.

Sulphuric Acid:
Ready to use. Mix well on vortex before use.
Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

Legenda:

Warning **H statements:**
H315 – Causes skin irritation.
H319 – Causes serious eye irritation.

Precautionary **P statements:**
P280 – Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352 – IF ON SKIN: Wash with plenty of soap and water.
P332 + P313 – If skin irritation occurs: Get medical advice/attention.
P305 + P351 + P338 – IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337 + P313 – If eye irritation persists: Get medical advice/attention.
P362 + P363 – Take off contaminated clothing and wash it before reuse.

I. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

1. **Micropipettes** have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (household alcohol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample. They should also be regularly maintained in order to show a precision of 1% and a trueness of +/-2%. Decontamination of spills or residues of kit components should also be carried out regularly.
2. The **ELISA incubator** has to be set at +37°C (tolerance of +/-0.5°C) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water baths are suitable for the incubations, provided that the instrument is validated for the incubation of ELISA tests and the right temperature of +37°C is assured to the microplate.
3. The **ELISA washer** is extremely important to the overall performances of the assay. The washer must be carefully validated in advance, checked for the delivery of the right dispensation volume and regularly submitted to maintenance according to the manufacturer's instructions for use. In particular the washer, at the end of the daily workload, has to be extensively cleaned out of salts with deionized water. Before use, the washer has to be extensively primed with the diluted Washing Solution.
   The instrument weekly has to be submitted to decontamination according to its manual (NaOH 0.1 M decontamination suggested).
   5 washing cycles (aspiration + dispensation of 350ul/well of washing solution + 20 sec soaking = 1 cycle) are sufficient to ensure the assay with the declared performances. If soaking is not possible add one more cycle of washing.
   An incorrect washing cycle or salt-blocked needles are the major cause of false positive reactions.
4. Incubation times have a tolerance of $\pm$5%.
5. The **ELISA microplate reader** has to be equipped with a reading filter of 450nm and with a second filter of 620-630nm mandatory for blanking purposes. Its standard performances should be (a) bandwidth $\leq$ 10 nm; (b) absorbance range from 0 to $\geq$ 2.0; (c) linearity to $\geq$ 2.0; repeatability $\geq$ 1%. Blanking is carried out on the well identified in the section "Assay Procedure". The optical system of the reader has to be calibrated regularly to ensure that the correct optical density is measured. It should be regularly maintained according to the manufacturer 's instructions.
6. When using an **ELISA automated work station**, all critical steps (dispensation, incubation, washing, reading, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the sections "Validation of Test" and "Assay Performances". The assay protocol has to be installed in the operating system of the unit and validated as for the washer and the reader. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set. Particular attention must be paid to avoid carry over by the needles used for dispensing and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells. The use of ELISA automated work stations is recommended when the number of samples to be tested exceed 20-30 units per run.
7. Dia.Pro's customer service offers support to the user in the setting and checking of instruments used in combination with the kit, in order to assure compliance with the requirements described. Support is also provided for the installation of new instruments to be used with the kit.

L. PRE ASSAY CONTROLS AND OPERATIONS

1. Check the expiration date of the kit printed on the external label (primary container). Do not use if expired.
2. Check that the liquid components are not contaminated by visible particles or aggregates.
3. Check that the Chromogen (TMB) is colourless or pale blue by aspirating a small volume of it with a sterile plastic pipette.
4. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box (primary container). Check that the aluminium pouch, containing the microplate, is not punctured or damaged.
5. Dissolve the content of the lyophilised Control Serum as reported in the proper section.
6. Dilute all the content of the 20x concentrated Wash Solution as described above.
7. Allow all the other components to reach room temperature (about 1 hr) and then mix gently on vortex all liquid reagents.
8. Set the ELISA incubator at +37°C and prepare the ELISA washer by priming with the diluted washing solution, according to the manufacturer instructions. Set the right number of washing cycles as reported in the specific section.
9. Check that the ELISA reader is turned on or ensure it will be turned on at least 20 minutes before reading.
10. If using an automated work station, turn on, check settings and be sure to use the right assay protocol.
11. Check that the micropipettes are set to the required volume.

12. Check that all the other equipment is available and ready to use.
13. In case of problems, do not proceed further with the test and advise the supervisor.

M. ASSAY PROCEDURE

The assay has to be carried out according to what reported below, taking care to maintain the same incubation time for all the samples in testing.
The kit may be used for quantitative and qualitative determinations as well.

M1. QUANTITATIVE DETERMINATION:

1. Dilute samples 1:101 into a properly defined dilution tube (example: 1000 µl Sample Diluent + 10 µl sample). Do not dilute the Calibration Set as calibrators are ready to use. Mix carefully all the liquid components on vortex and then proceed as described below.
2. Place the required number of microwells in the microwell holder. Leave the A1 and B1 empty for the operation of blanking.
3. Dispense 100 µl of Calibrators in duplicate. Then dispense 100 µl of diluted samples in each properly identified well. The Control Serum doesn't have to be used in every single analysis; it may be used whenever an internal quality control is required by the management to check the overall performances of the laboratory itself. In case, dispense 100 ul of the Control Serum, prepared according to instructions, in duplicate into a proper well.
4. Incubate the microplate for **60 min at +37°C**.

Important note: *Strips have to be sealed with the adhesive sealing foil, supplied, only when the test is carried out manually. Do not cover strips when using ELISA automatic instruments.*

5. Wash the microplate with an automatic washer by delivering and aspirating 350 µl/well of diluted washing solution as reported previously (section I.3).
6. Pipette 100 µl Enzyme Conjugate into each well, except A1+B1 blanking wells, and cover with the sealer. Check that this red coloured component has been dispensed in all the wells, except A1 and B1.

Important note: *Be careful not to touch the plastic inner surface of the well with the tip filled with the Enzyme Conjugate. Contamination might occur.*

7. Incubate the microplate for **60 min at +37°C**.
8. Wash microwells as in step 5.
9. Pipette 100 µl Chromogen/Substrate mixture into each well, the blank wells A1 and B1 included. Then incubate the microplate at **room temperature (18-24°C) for 20 minutes**.

Important note: *Do not expose to strong direct illumination. High background might be generated.*

10. Pipette 100 µl Sulphuric Acid to stop the enzymatic reaction into all the wells using the same pipetting sequence as in step 9. Addition of acid will turn the positive calibrators, the control serum and the positive samples from blue to yellow.
11. Measure the colour intensity of the solution in each well, as described in section I.5, at 450nm filter (reading) and at 620-630nm (background subtraction), blanking the instrument on A1 or B1 or both (mandatory).

M2. QUALITATIVE DETERMINATION

If only a qualitative determination is required, proceed as described below:

1. Dilute samples 1:101 into a properly defined dilution tube (example: 1000 µl Sample Diluent + 10 µl sample). Do not dilute the Calibrator 1 (0IU/ml) and Calibrator 5 (20IU/ml) as they are ready to use. The Control Serum doesn't have to be used in every single analysis; it may be used whenever an internal quality control is required by the management to check the overall performances of the laboratory itself. Mix carefully all the liquid components on vortex and then proceed as described below.
2. Place the required number of Microwells in the microwell holder. Leave A1 well empty for the operation of blanking.
3. Dispense 100 µl of Calibrator 1 (0 IU/ml) and 100 µl Calibrator 5 (20 IU/ml) in duplicate, and 100 µl Control Serum, prepared according to instructions, in single. Then dispense 100 µl of diluted samples in each properly identified well.
4. Incubate the microplate for **60 min at +37°C**.

Important note: *Strips have to be sealed with the adhesive sealing foil, supplied, only when the test is carried out manually. Do not cover strips when using ELISA automatic instruments.*

5. Wash the microplate with an automatic washer by delivering and aspirating 350 µl/well of diluted washing solution as reported previously (section I.3).
6. Pipette 100 µl Enzyme Conjugate into each well, except the A1 well, and cover with the sealer. Check that this red coloured component has been dispensed in all the wells, except A1.

Important note: *Be careful not to touch the plastic inner surface of the well with the tip filled with the Enzyme Conjugate. Contamination might occur.*

7. Incubate the microplate for **60 min at +37°C**.
8. Wash microwells as in step 5.
9. Pipette 100 µl Chromogen/Substrate mixture into each well, the blank well included. Then incubate the microplate at **room temperature (18-24°C) for 20 minutes**.

Important note: *Do not expose to strong direct illumination. High background might be generated.*

10. Pipette 100 µl Sulphuric Acid into all the wells using the same pipetting sequence as in step 9. Addition of acid will turn the positive calibrators, the control serum and the positive samples from blue to yellow.
11. Measure the colour intensity of the solution in each well, as described in section I.5, at 450nm filter (reading) and at 620-630nm (background subtraction), blanking the instrument on A1 or B1 or both (mandatory).

General Important notes:

1. *Ensure that no finger prints are present on the bottom of the microwell before reading. Finger prints could generate false positive results on reading.*
2. *Reading has to be carried out just after the addition of the Stop Solution and anyway not any longer than 20 minutes after its addition. Some self oxidation of the chromogen can occur leading to high background.*

N. ASSAY SCHEME

Method	Operations
Calibrators & Control(*) Samples diluted 1:101	100 µl 100 µl
1st incubation	60 min
Temperature	+37°C
Wash step	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
Enzyme conjugate	100 µl
2nd incubation	60 min
Temperature	+37°C
Wash step	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
TMB/H2O2	100 µl
3rd incubation	20 min
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm / 620-630nm

(*) Important Notes:

- *The Control Serum (CS) it does not affect the test's results calculation.*
- *The Control Serum (CS) used only if a laboratory internal quality control is required by the Management.*

An example of dispensation scheme for Quantitative Analysis is reported below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	CAL4	S 1									
B	BLK	CAL4	S 2									
C	CAL1	CAL5	S 3									
D	CAL1	CAL5	S 4									
E	CAL2	CAL6	S 5									
F	CAL2	CAL6	S 6									
G	CAL3	CS(*)	S 7									
H	CAL3	CS(*)	S 8									

Legenda: BLK = Blank CAL = Calibrator CS(*) = Control Serum - Not mandatory
S = Sample

An example of dispensation scheme in qualitative assays is reported below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S 2	S 10									
B	CAL1	S 3	S 11									
C	CAL1	S 4	S 12									
D	CAL5	S 5	S 13									
E	CAL5	S 6	S 14									
F	CAL5	S 7	S 15									
G	CS(*)	S 8	S 16									
H	S 1	S 9	S 17									

Legenda: BLK = Blank CAL = Calibrator CS(*) = Control Serum - Not mandatory
S = Sample

O. ASSAY QUALITY CONTROL

A validation check is carried out on the Calibrators any time the kit is used in order to verify whether the performances of the assay are as expected and required by the IVDD directive 98/79/EC.
The Control Serum is used only when required by the management for an internal verification of the performances of the laboratory itself.

Control that the following data are matched:

Check	Requirements
Blank well	< 0.050 OD450nm value
CAL 1 0 IU/ml	< 0.150 mean OD450nm value after blanking coefficient of variation < 30%
CAL 2 3 IU/ml	OD450nm > OD450nm CAL1 + 0.100
CAL 5 20 IU/ml	OD450nm > 0.750
CAL 6 40 IU/ml	OD450nm > 1.000

If the results of the test match the requirements stated above, proceed to the next section.

If they do not, do not proceed any further and operate as follows:

Problem	Check
Blank well > 0.050 OD450nm	1. that the Chromogen/Sustrate solution has not got contaminated during the assay
CAL 1 **0 IU/ml** > 0.150 OD450nm after blanking coefficient of variation > 30%	1. that the washing procedure and the washer settings are as validated in the pre qualification study; 2. that the proper washing solution has been used and the washer has been primed with it before use; 3. that no mistake has been done in the assay procedure (dispensation of a positive calibrator instead of the negative one; 4. that no contamination of the negative calibrator or of their wells has occurred due spills of positive samples or the enzyme conjugate; 5. that micropipettes haven't got contaminated with positive samples or with the enzyme conjugate 6. that the washer needles are not blocked or partially obstructed.

CAL 2 **3 IU/ml** OD450nm < OD450nm CAL1 + 0.100	1. that the procedure has been correctly executed; 2. that no mistake has been done in its distribution (ex.: dispensation of a wrong calibrator instead); 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the calibrator has occurred.
CAL 5 **20 IU/ml** < 0.750 OD450nm	1. that the procedure has been correctly executed; 2. that no mistake has been done in its distribution (dispensation of a wrong calibrator instead) ; 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the positive control has occurred.
CAL 6 **40 IU/ml** < 1.000 OD450nm	1. that the procedure has been correctly executed; 2. that no mistake has been done in its distribution (dispensation of a wrong calibrator instead) ; 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the positive control has occurred.

Should one of these problems have happened, after checking, report to the supervisor for further actions.

**** Note:**

If Control Serum has used, verify the following data:

Check	Requirements
Control Serum	Mean OD450nm CAL4 ± 20%

If the results of the test doesn't match the requirements stated above, operate as follows:

Problem	Check
Control Serum Different from expected value	First verify that: 1. the procedure has been correctly performed; 2. no mistake has occurred during its distribution (e.g.: dispensation of a wrong sample); 3. the washing procedure and the washer settings are correct; 4. no external contamination of the control has occurred. 5. the Control Serum has been dissolved with the right volume reported on the label. If a mistake has been pointed out, the assay has to be repeated after eliminating the reason of this error.

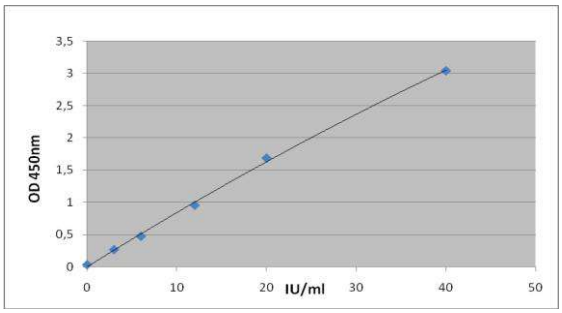
Anyway, if all other parameters (Blank, CAL1, CAL2, CAL5, CAL6), match the established requirements, the test may be considered valid.

P. RESULTS

P.1 Quantitative method

If the test turns out to be valid, use for the quantitative method an approved curve fitting program to draw the calibration curve from the values obtained by reading at 450nm (4-parameters interpolation is suggested).
Then on the calibration curve calculate the concentration of anti Parvovirus B19 IgG antibody in samples.

Example of Calibration Curve :



Important Note:
Do not use the calibration curve above to make calculations.

P.2 Qualitative method
In the qualitative method, calculate the mean OD450nm values for Calibrator 1(0 IU/ml), and for Calibrator 5(20IU/ml) and then check that the assay is valid.
In this case the results are calculated by means of a cut-off value determined with the following formula:

Cut-Off (Co) = CAL5/5

Important note*: When the calculation of results is performed by the operating system of an ELISA automated work station, ensure that the proper formulation is used to calculate the cut-off value and generate the correct interpretation of results.*

Q. INTERPRETATION OF RESULTS

Q.1 Quantitative method
Samples with a concentration lower than 3 WHO IU/ml are considered negative for anti Parvovirus B19 IgG antibody by most of the international medical literature.
Samples with a concentration between 3 and 5 WHO IU/ml are considered equivocal for anti Parvovirus B19 IgG antibody.
Samples with a concentration higher than 5 WHO IU/ml are considered positive for anti Parvovirus B19 IgG antibody.
This titer is considered the lowest concentration of IgG to provide an effective immunological protection.

Q.2 Qualitative method

Results are interpreted as ratio between the sample OD450nm and the cut-off value or S/Co.

Results are interpreted according to the following table:

S/Co	Interpretation
< 0.8	Negative
0.8 – 1.2	Equivocal
> 1.2	Positive

Any patient showing an equivocal result should be retested on a second sample taken 1-2 weeks after the initial sample.

Important notes:

1. *Interpretation of results should be done under the supervision of the laboratory supervisor to reduce the risk of judgment errors and misinterpretations.*
2. *When test results are transmitted from the laboratory to another facility, attention must be paid to avoid erroneous data transfer.*
3. *Diagnosis has to be done and released to the patient by a suitably qualified medical doctor.*

An example of calculation is reported below.

The following data must not be used instead or real figures obtained by the user.

Calibrator 0 IU/ml: 0.020 – 0.024 OD450nm
Mean Value: 0.022 OD450nm
Lower than 0.150 – Accepted
Calibrator 20 IU/ml: 1.489 - 1.545 OD450nm
Mean Value: 1.517 OD450nm
Higher than 0.750 – Accepted

Cut-Off = 1.517 / 5 = 0.303

Sample 1: 0.028 OD450nm
Sample 2: 1.890 OD450nm
Sample 1 S/Co < 0.9 negative
Sample 2 S/Co > 1.0 positive

R. PERFORMANCES

Evaluation of Performances has been conducted in accordance to what suggested in NCCLS's approved guideline C24-A2.

1. Limit of detection

The limit of detection of the assay has been calculated by means of the 2nd W.H.O international standard for Anti-Parvovirus B19 code 01/602. The limit of detection has been calculated as mean OD450nm Calibrator 0 IU/ml + 5 SD.
The table below reports the mean OD450nm values of this standard when diluted in negative plasma and then examined in the assay for two lots.

WHO IU/ml	PARVOG.CE Lot P1	PARVOG.CE Lot P2
40	3.041	3.110
20	1.686	1.570
12	0.954	0.925
6	0.473	0.549
3	0.266	0.233
1.5	0.112	0.087
Std 0	0.030	0.055

The assay shows a limit of detection far better than 3 IU/ml.

2. Diagnostic sensitivity & Specificity :

The Diagnostic **Sensitivity** was calculated on a panel of 50 samples classified positive for the IgG anti parvovirus B19 by a reference kit CE marked.
A value of ≥ 98% was observed when referring to the reference device.
The Diagnostic **Specificity** was calculated on a panel of more than 100 samples classified negative with the reference device.
A value ≥ 98% was observed.
These findings are summarized in the following table.

Sensitivity	≥ 98 %
Specificity	≥ 98 %

4. Precision:

It has been calculated on three samples, a negative, a low positive and a positive, examined in 16 replicates in three separate runs for two lots. Results are reported as follows:

PARVOG.CE: lot P1

Calibrator 0 IU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.064	0.065	0.069	0.066
Std.Deviation	0.007	0.010	0.011	0.009
CV %	10.6	15.3	15.2	13.7

Calibrator 3 IU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.313	0.305	0.352	0.323
Std.Deviation	0.031	0.036	0.024	0.030
CV %	9.9	11.7	6.9	9.5

Calibrator 20 IU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	1.790	1.799	2.077	1.888
Std.Deviation	0.085	0.084	0.082	0.084
CV %	4.7	4.6	3.9	4.4

PARVOG.CE: lot P2

Calibrator 0 IU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.099	0.089	0.087	0.092
Std.Deviation	0.017	0.012	0.009	0.013
CV %	17.4	13.8	10.3	13.8

Calibrator 3 IU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.334	0.362	0.380	0.358
Std.Deviation	0.021	0.035	0.029	0.028
CV %	6.4	9.7	7.7	7.9

Calibrator 20 IU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	2.085	2.040	2.591	2.239
Std.Deviation	0.081	0.099	0.125	0.101
CV %	3.9	4.9	4.8	4.5

The variability shown in the tables above did not result in sample misclassification.

5. Accuracy

The assay accuracy has been checked by the dilution and recovery tests. Any “hook effect”, underestimation likely to happen at high doses of analyte, was ruled out up to 77 IU/ml.

S. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or heat inactivation of the specimen may affect the absorbance values of the samples with consequent alteration of the level of the analyte.

Frozen samples containing fibrin particles or aggregates after thawing may generate some false results.

This test is suitable only for testing single samples and not pooled ones.

Diagnosis of an infectious disease should not be established on the basis of a single test result. The patient’s clinical history, symptomatology, as well as other diagnostic data should be considered.

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All the IVD Products manufactured by the company are under the control of a certified Quality Management System in compliance with ISO 13485 rule. Each lot is submitted to a quality control and released into the market only if conforming with the EC technical specifications and acceptance criteria.

Manufacturer:
Dia.Pro Diagnostic Bioprobes S.r.l.
Via G. Carducci n° 27 – Sesto San Giovanni (MI) – Italy

CE

Dia.Pro
Diagnostic
BioProbes

EC DECLARATION OF CONFORMITY

MANUFACTURER	DIA.PRO DIAGNOSTIC BIOPROBES S.R.L. VIA G. CARDUCCI N° 27 – 20099 SESTO SAN GIOVANNI (MILANO) – ITALY
PRODUCT	**Parvovirus B19 IgM** CODE: **PARVOM.CE** (96 tests)
CLASSIFICATION	GENERAL IVD
CONFORMITY ASSESSMENT ROUTE	SELF CERTIFICATION

WE HEREBY DECLARE THAT THE ABOVE MENTIONED PRODUCT MEETS THE PROVISIONS OF THE COUNCIL DIRECTIVE 98/79/EC FOR IN VITRO DIAGNOSTIC DEVICES.

ISO CERTIFICATE	UNE EN ISO 13485 N° 2013 11 0039 EN, RELEASED BY AEMPS (AGENCIA ESPAÑOLA DE MEDICAMENTOS Y PRODUCTOS SANITARIOS)

PLACE & DATE OF FIRST ISSUE	SESTO SAN GIOVANNI (MI) – MAY 2013
PLACE & DATE OF CURRENT ISSUE	SESTO SAN GIOVANNI (MI) – MARCH 2019
SIGNATURE **Legal Representative** **Dr.ssa Fiorenza Scozzesi**	

Rev:	05/2018

Parvovirus B19 IgM

Enzyme ImmunoAssay (ELISA) for the qualitative determination of IgM antibodies to Parvovirus B19 in human serum and plasma

- for “in vitro” diagnostic use only -

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REF PARVOM.CE
96 Tests

Parvovirus B19 IgM

A. INTENDED USE

Enzyme ImmunoAssay (ELISA) for the qualitative determination of IgM antibodies to Parvovirus B19 in human plasma and sera.
For "in vitro" diagnostic use only.

B. INTRODUCTION

The **B19 virus**, generally referred to as **parvovirus B19** was the first (and until 2005 the only) known human virus in the family of parvoviruses, genus erythrovirus. Parvovirus B19 is a non-enveloped, icosahedral virus that contains a single-stranded linear DNA genome. It is classified as erythrovirus because of its capability to invade red blood cell precursors in the bone marrow. Three genotypes (with subtypes) have been recognized. The viral capsid is composed of two structural proteins , namely VP1 (83kD) and VP2 (53 kD). Infection by Parvovirus B19 spreads through respiratory secretions but also through blood or blood products. The infection causes a mild illness characterized by an erythematous maculopapular facial rash called fifth disease or erythema infectious. It is tipical in childhood and is also seen in adults. A person usually gets sick within 4 to 14 days after getting infected with parvovirus B19 but about 20% of children and adults who get infected with this virus will not have any symptoms. Infection during pregnancy presents the risk of transmission to the fetus that may result in hydrops fetalis. In particular the presence of IgM antibodies is reported to be correlated to the acute phase of illness, while IgG antibodies become present at different titers shortly after primary infections and last in blood for many years.
People with weakened immune systems caused by leukemia, cancer, organ transplants, or HIV infection are at risk for serious complications from fifth disease. It can cause chronic anemia that requires medical treatment.
Therefore the detection of Parvovirus B19-specific antibodies becomes very important.

C. PRINCIPLE OF THE TEST

Microplates are coated with Parvovirus B19 antigens.
The solid phase is first treated with the diluted sample and IgM to Parvovirus B19 are captured, if present, by the antigens.
After washing out all the other components of the sample, in the 2nd incubation bound anti Parvovirus IgM are detected by the addition of polyclonal specific anti hIgM antibodies, labelled with peroxidase (HRP).
The enzyme captured on the solid phase, acting on the substrate/chromogen mixture, generates an optical signal that is proportional to the amount of anti Parvovirus IgM antibodies present in the sample. The presence of IgM in the sample may therefore be determined by means of a cut-off value able to discriminate between negative and positive samples.
Neutralization of IgG anti-Parvovirus , carried out directly in the well, is performed in the assay in order to block interferences due to this class of antibodies in the determination of IgM.

D. COMPONENTS

Each kit contains sufficient reagents to perform 96 tests.

1. Microplate: MICROPLATE
12 strips x 8 microwells coated with Parvovirus B19 antigens.
Plates are sealed into a bag with desiccant. Allow the microplate to reach room temperature before opening; reseal unused strips in the bag with desiccant and store at 2..8°C.

2. Negative Control: CONTROL -
1x4.0 ml/vial. Ready to use. It contains, human plasma negative to Parvovirus B19, 2% casein, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.1% Tween 20, 0.09% Na-azide and 0.1% Kathon GC as preservatives.
The Negative Control is pale yellow color coded.

3. Positive Control: CONTROL +
1x4.0 ml/vial. Ready to use. It contains human plasma positive to Parvovirus B19, 2% casein, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.1% Tween 20, 0.09% Na-azide and 0.1% Kathon GC as preservatives.
The Positive Control is green yellow color coded.

4. Calibrator: CAL ….
n° 1 vial. Lyophilized reagent to be dissolved with EIA grade water as reported in the label. It contains bovine serum proteins, human plasma positive to Parvovirus, 0.2 mg/ml gentamicine sulphate and 0.1% Kathon GC as preservatives.
Note: The volume necessary to dissolve the content of the vial may vary from lot to lot. Please use the right volume reported on the label .

5. Wash buffer concentrate: WASHBUF 20X
1x60ml/bottle20x concentrated solution.
Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2, 0.05% Tween 20 and 0.05% Kathon GC.

6. Enzyme conjugate : CONJ
1x16ml/vial. Ready to use and red colour coded. It contains Horseradish peroxidase conjugated polyclonal antibodies to human IgM, 5% BSA, 10 mM Tris buffer pH 6.8+/-0.1, 0.1% Kathon GC and 0.02% gentamicine sulphate as preservatives.

7. Chromogen/Substrate: SUBS TMB
1x16ml/vial. It contains 50 mM citrate-phosphate buffer pH 3.5-3.8, 4% dimethylsulphoxide, 0.03% tetra-methyl-benzidine (or TMB) and 0.02% hydrogen peroxide (or H_2O_2).
Note: To be stored protected from light as sensitive to strong illumination.

8. Sulphuric Acid: H2SO4 0.3 M
1x15ml/vialIt contains 0.3 M H_2SO_4 solution.
Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

9. Specimen Diluent: DILSPE
2x60ml/vial. It contains 2% casein, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.1% Tween 20, 0.5% NP40, 0.09% Na-azide and 0.1% Kathon GC as preservatives. To be used to dilute the sample.

10. Neutralizing Reagent: SOLN NEUT
1x8ml/vial. It contains goat anti hIgG, 2% casein, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.1% Tween 20, 0.09% Na-azide and 0.1% Kathon GC as preservatives.

11. Plate sealing foils n°2

12. Package insert n°1

E. MATERIALS REQUIRED BUT NOT PROVIDED

1. Calibrated Micropipettes (1000 ul, 100 ul and 10 ul) and disposable plastic tips.
2. EIA grade water (double distilled or deionised, charcoal treated to remove oxidizing chemicals used as disinfectants).
3. Timer with 60 minute range or higher.
4. Absorbent paper tissues.
5. Calibrated ELISA microplate thermostatic incubator (dry or wet), set at +37°C (+/-0.5°C tolerance)..
6. Calibrated ELISA microwell reader with 450nm (reading) and with 620-630nm (blanking) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.

F. WARNINGS AND PRECAUTIONS

1. The kit has to be used by skilled and properly trained technical personnel only, under the supervision of a medical doctor responsible of the laboratory.
2. All the personnel involved in performing the assay have to wear protective laboratory clothes, talc-free gloves and glasses. The use of any sharp (needles) or cutting (blades) devices should be avoided. All the personnel involved should be trained in biosafety procedures, as recommended by the Center for Disease Control, Atlanta, U.S. and reported in the National Institute of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
3. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.
4. The laboratory environment should be controlled so as to avoid contaminants such as dust or air-born microbial agents, when opening kit vials and microplates and when performing the test. Protect the Chromogen (TMB) from strong light and avoid vibration of the bench surface where the test is undertaken.
5. Upon receipt, store the kit at 2..8°C into a temperature controlled refrigerator or cold room.
6. Do not interchange components between different lots of the kits. It is recommended that components between two kits of the same lot should not be interchanged.
7. Check that the reagents are clear and do not contain visible heavy particles or aggregates. If not, advise the laboratory supervisor to initiate the necessary procedures for kit replacement.
8. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each sample.
9. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one.
10. Do not use the kit after the expiration date stated on the external container and internal (vials) labels. A study conducted on an opened kit did not pointed out any relevant loss of activity up to six 6 uses of the device and up to 3 months.
11. Treat all specimens as potentially infective. All human serum specimens should be handled at Biosafety Level 2, as recommended by the Center for Disease Control, Atlanta, U.S. in compliance with what reported in the Institutes of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
12. The use of disposable plastic-ware is recommended in the preparation of the liquid components or in transferring components into automated workstations, in order to avoid cross contamination.
13. Waste produced during the use of the kit has to be discarded in compliance with national directives and laws concerning laboratory waste of chemical and biological substances. In particular, liquid waste generated from the washing procedure, from residuals of controls and from samples has to be treated as potentially infective material and inactivated before waste. Suggested procedures of inactivation are treatment with a 10% final concentration of household bleach for 16-18 hrs or heat inactivation by autoclave at 121°C for 20 min..
14. Accidental spills from samples and operations have to be adsorbed with paper tissues soaked with household bleach and then with water. Tissues should then be discarded in proper containers designated for laboratory/hospital waste.
15. The Sulphuric Acid is an irritant. In case of spills, wash the surface with plenty of water
16. Other waste materials generated from the use of the kit (example: tips used for samples and controls, used microplates) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

G. SPECIMEN: PREPARATION AND WARNINGS

1. Blood is drawn aseptically by venepuncture and plasma or serum is prepared using standard techniques of preparation of samples for clinical laboratory analysis. No influence has been observed in the preparation of the sample with citrate, EDTA and heparin.
2. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results. Bar code labeling and electronic reading is strongly recommended.
3. Haemolysed ("red") and visibly hyperlipemic ("milky") samples have to be discarded as they could generate false results. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they could give rise to false results.
4. Sera and plasma can be stored at +2°..8°C for up to five days after collection. For longer storage periods, samples can be stored frozen at –20°C for several months. Any frozen samples should not be frozen/thawed more than once as this may generate particles that could affect the test result.
5. If particles are present, centrifuge at 2.000 rpm for 20 min or filter using 0.2-0.8u filters to clean up the sample for testing.

H. PREPARATION OF COMPONENTS AND WARNINGS

Microplate:

Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant is not turned to dark green, indicating a defect of manufacturing.
In this case call Dia.Pro's customer service.
Unused strips have to be placed back into the aluminium pouch, in presence of desiccant supplied, firmly zipped and stored at +2°..8°C. When opened the first time, residual strips are stable till the indicator of humidity inside the desiccant bag turns from yellow to green.

Negative Control

Ready to use components. Mix carefully on vortex before use.

Positive Control

Ready to use components. Mix carefully on vortex before use.

Calibrator

Add the volume of ELISA grade water, reported on the label, to the lyophilized powder; let fully dissolve and then gently mix on vortex.
Note: *The dissolved calibrator is not stable. Store it frozen in aliquots at –20°C.*

Wash buffer concentrate:

The whole content of the concentrated solution has to be diluted 20x with bidistilled water and mixed gently end-over-end before use. During preparation avoid foaming as the presence of bubbles could impact on the efficiency of the washing cycles.
Note: *Once diluted, the wash solution is stable for 1 week at +2..8° C.*

Enzyme conjugate:
Ready to use. Mix well on vortex before use.
Be careful not to contaminate the liquid with oxidizing chemicals, air-driven dust or microbes.
If this component has to be transferred use only plastic, possibly sterile disposable containers.

Chromogen/Substrate:
Ready to use. Mix well on vortex before use.
Be careful not to contaminate the liquid with oxidizing chemicals, air-driven dust or microbes.
Do not expose to strong illumination, oxidizing agents and metallic surfaces.
If this component has to be transferred use only plastic, possible sterile disposable container

Sample Diluent
Ready to use component. Mix carefully on vortex before use.

Neutralizing Reagent
Ready to use component. Mix carefully on vortex before use.

Sulphuric Acid:
Ready to use. Mix well on vortex before use.
Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

Legenda:

Warning **H statements:**
H315 – Causes skin irritation.
H319 – Causes serious eye irritation.

Precautionary **P statements:**
P280 – Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352 – IF ON SKIN: Wash with plenty of soap and water.
P332 + P313 – If skin irritation occurs: Get medical advice/attention.
P305 + P351 + P338 – IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337 + P313 – If eye irritation persists: Get medical advice/attention.
P362 + P363 – Take off contaminated clothing and wash it before reuse.

I. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

1. Micropipettes have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (household alcohol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample. They should also be regularly maintained in order to show a precision of 1% and a trueness of +/-2%. Decontamination of spills or residues of kit components should also be carried out regularly.
2. The ELISA incubator has to be set at +37°C (tolerance of +/- 0.5°C) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water baths are suitable for the incubations, provided that the instrument is validated for the incubation of ELISA tests.
3. The ELISA washer is extremely important to the overall performances of the assay. The washer must be carefully validated and correctly optimised using the kit controls and reference panels, before using the kit for routine laboratory tests. Usually 4-5 washing cycles (aspiration + dispensation of 350ul/well of washing solution = 1 cycle) are sufficient to ensure that the assay performs as expected. A soaking time of 20-30 seconds between cycles is suggested. In order to set correctly their number, it is recommended to run an assay with the kit controls and well characterized negative and positive reference samples, and check to match the values reported below in the section "Internal quality Control". Regular calibration of the volumes delivered by, and maintenance (decontamination and cleaning of needles) of the washer has to be carried out according to the instructions of the manufacturer.
4. Incubation times have a tolerance of $\pm$5%.
5. The ELISA microplate reader has to be equipped with a reading filter of 450nm and with a second filter (620-630nm, strongly recommended) for blanking purposes. Its standard performances should be (a) bandwidth $\leq$ 10 nm; (b) absorbance range from 0 to $\geq$ 2.0; (c) linearity to $\geq$ 2.0; repeatability $\geq$ 1%. Blanking is carried out on the well identified in the section "Assay Procedure". The optical system of the reader has to be calibrated regularly to ensure that the correct optical density is measured. It should be regularly maintained according to the manufacturer 's instructions.
6. When using an ELISA automated work station, all critical steps (dispensation, incubation, washing, reading, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the sections "Internal Quality Control". The assay protocol has to be installed in the operating system of the unit and validated as for the washer and the reader. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set. Particular attention must be paid to avoid carry over by the needles used for dispensing and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells. The use of ELISA automated work stations is recommended when the number of samples to be tested exceed 20-30 units per run.
7. Dia.Pro's customer service offers support to the user in the setting and checking of instruments used in combination with the kit, in order to assure compliance with the requirements described. Support is also provided for the installation of new instruments to be used with the kit.

L. PRE ASSAY CONTROLS AND OPERATIONS

1. Check the expiration date of the kit printed on the external label (primary container). Do not use if expired.
2. Check that the liquid components are not contaminated by visible particles or aggregates.
3. Check that the Chromogen (TMB) is colourless or pale blue by aspirating a small volume of it with a sterile plastic pipette.
4. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box (primary container). Check that the aluminium pouch, containing the microplate, is not punctured or damaged.
5. Dissolve the content of the Calibrator as reported.
6. Dilute all the content of the 20x concentrated Wash Solution as described above.
7. Allow all the other components to reach room temperature (about 1 hr) and then mix gently on vortex all liquid reagents.
8. Set the ELISA incubator at +37°C and prepare the ELISA washer by priming with the diluted washing solution, according to the manufacturers instructions. Set the right number of washing cycles as found in the validation of the instrument for its use with the kit.
9. Check that the ELISA reader is turned on or ensure it will be turned on at least 20 minutes before reading.
10. If using an automated work station, turn on, check settings and be sure to use the right assay protocol.
11. Check that the micropipettes are set to the required volume.

12. Check that all the other equipment is available and ready to use.
13. In case of problems, do not proceed further with the test and advise the supervisor.

M. ASSAY PROCEDURE

The assay has to be carried out according to what reported below, taking care to maintain the same incubation time for all the samples in testing.

1. Dilute samples 1:101 into a properly defined dilution tube (example: 1000 µl Sample Diluent + 10 µl sample). Do not dilute the Controls/Calibrator as they are ready to use. Mix carefully all the liquid components on vortex and then proceed as described below.

2. Place the required number of Microwells in the microwell holder. Leave A1 well empty for the operation of blanking.

3. Not dispense Neutralizing Reagent in A1 used for blanking operations and in the wells used for the Controls and the Calibrator.

4. Dispense 50 µl Neutralizing Reagent in all the samples wells.

5. Dispense 100 µl of Negative Control in duplicate, 100 µl of Positive Control in single, 100 µl of Calibrator in duplicate and 100 ul of diluted samples in each properly identified well.
6. Incubate the microplate for **60 min at +37°C**.

Important note: *Strips have to be sealed with the adhesive sealing foil, supplied, only when the test is carried out manually. Do not cover strips when using ELISA automatic instruments.*

7. Wash the microplate with an automatic as reported previously (section I.3).

8. Pipette 100 µl Enzyme Conjugate into each well, except the A1 well, and cover with the sealer. Check that this red coloured component has been dispensed in all the wells, except A1.

Important note: *Be careful not to touch the plastic inner surface of the well with the tip filled with the Enzyme Conjugate. Contamination might occur.*

9. Incubate the microplate for **60 min at +37°C**.

10. Wash microwells as in step 6.

11. Pipette 100 µl Chromogen/Substrate mixture into each well, the blank well included. Then incubate the microplate at **room temperature (18-24°C) for 20 minutes**.

Important note: *Do not expose to strong direct illumination. High background might be generated.*

12. Pipette 100 µl Sulphuric Acid into all the wells using the same pipetting sequence as in step 9. Addition of acid will turn the positive calibrators, the control serum and the positive samples from blue to yellow.

13. Measure the colour intensity of the solution in each well, as described in section I.5, at 450nm filter (reading) and at 620-630nm (background subtraction, strongly recommended), blanking the instrument on A1.

General Important notes:

1. *If the second filter is not available ensure that no finger prints are present on the bottom of the microwell before reading at 450nm. Finger prints could generate false positive results on reading.*
2. *Reading has to be carried out just after the addition of the Stop Solution and anyway not any longer than 20 minutes after its addition. Some self oxidation of the chromogen can occur leading to high background.*

N. ASSAY SCHEME

Method	Operations
Controls & Calibrator (*)	100 µl
Neutralizing Reagent (only for samples)	50 µl
Samples diluted 1:101	100 µl
1st incubation	**60 min**
Temperature	+37°C
Wash step	4-5 cycles
Enzyme conjugate	100 µl
2nd incubation	**60 min**
Temperature	+37°C
Wash step	4-5 cycles
TMB/H2O2	100 µl
3rd incubation	**20 min**
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm

(*) Important Notes:

- *The Calibrator (CAL) does not affect the Cut Off calculation, therefore it does not affect the test's results calculation.*
- *The Calibrator (CAL) used only if a laboratory internal quality control is required by the Management.*

An example of dispensation scheme is reported in the table below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S3										
B	NC	S4										
C	NC	S5										
D	CAL(*)	S6										
E	CAL(*)	S7										
F	PC	S8										
G	S1	S9										
H	S2	S10										

Legenda: BLK = Blank NC = Negative Control
CAL = Calibrator - Not mandatory PC = Positive Control
S = Sample

O. INTERNAL QUALITY CONTROL

A validation check is carried out on the controls any time the kit is used in order to verify whether the performances of the assay are as expected and required by the IVDD directive 98/79/EC. Control that the following data are matched:

Check	Requirements
Blank well	< 0.100 OD450nm value
Negative Control	< 0.150 mean OD450nm value after blanking coefficient of variation < 30%
Positive Control	OD450nm > 1.000

If the results of the test match the requirements stated above, proceed to the next section.

If they do not, do not proceed any further and operate as follows:

Problem	Check
Blank well > 0.100 OD450nm	1. that the Chromogen/Sustrate solution has not got contaminated during the assay
Negative Control > 0.150 OD450nm after blanking coefficient of variation > 30%	1. that the washing procedure and the washer settings are as validated in the pre qualification study; 2. that the proper washing solution has been used and the washer has been primed with it before use; 3. that no mistake has been done in the assay procedure (dispensation of a positive control instead of the negative one; 4. that no contamination of the negative control or of their wells has occurred due spills of positive samples or the enzyme conjugate; 5. that micropipettes haven't got contaminated with positive samples or with the enzyme conjugate 6. that the washer needles are not blocked or partially obstructed.
Positive Control < 1.000 OD450nm	1. that the procedure has been correctly executed; 2. that no mistake has been done in its distribution (dispensation of a wrong control) ; 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the positive control has occurred.

Should one of these problems have happened, after checking, report to the supervisor for further actions.

**** Note:**

If Calibrator has used, verify the following data:

Check	Requirements
Calibrator	S/Co > 1.0

If the results of the test doesn't match the requirements stated above, operate as follows:

Problem	Check
Calibrator S/Co < 1.0	1. that the procedure has been correctly executed; 2. that no mistake has been done in its distribution (dispensation of a wrong control instead); 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the calibrator has occurred.

Anyway, if all other parameters (Blank, Negative Control, Positive Control), match the established requirements, the test may be considered valid.

P. RESULTS

If the test turns out to be valid, results are calculated from the mean OD450nm value of the Negative Control (NC) by means of a cut-off value (Co) determined with the following formula:

Cut-Off = NC + 0.250

***Important note**: When the calculation of results is performed by the operating system of an ELISA automated work station, ensure that the proper formulation is used to generate the correct interpretation of results.*

Q. INTERPRETATION OF RESULTS

Test results are interpreted as a ratio of the sample OD450nm value (S) and the cut-off value (Co), or S/Co, according to the following table:

S/Co	Interpretation
< 0.9	Negative
0.9 - 1.1	Equivocal
> 1.1	Positive

A negative result indicates that the patient has not developed IgM antibodies to Parvovirus.
Any patient showing an equivocal result should be retested on a second sample taken 1-2 weeks after the initial sample.
A positive result is indicative of an ongoing Parvovirus infection and therefore the patient should be treated accordingly.

Important notes:

1. *Interpretation of results should be done under the supervision of the laboratory supervisor to reduce the risk of judgment errors and misinterpretations.*
2. *When test results are transmitted from the laboratory to another facility, attention must be paid to avoid erroneous data transfer.*
3. *Diagnosis has to be done and released to the patient by a suitably qualified medical doctor.*

An example of calculation is reported below.

The following data must not be used instead or real figures obtained by the user.

Negative Control: 0.100 – 0.120 – 0.080 OD450nm
Mean Value: 0.100 OD450nm
Lower than 0.150 – Accepted

Positive Control: 1.500 OD450nm
Higher than 0.1000 – Accepted

Cut-Off = 0.100+0.250 = 0.350

Calibrator: 0.500 – 0.540 OD450nm
Mean value: 0.520 OD450nm
S/Co higher than 1.0 – Accepted

Sample 1: 0.080 OD450nm
Sample 2: 1.800 OD450nm
Sample 1 S/Co < 1.0 = negative
Sample 2 S/Co > 1.2 = positive

R. PERFORMANCE CHARACTERISTICS

Evaluation of Performances has been conducted in accordance to what suggested in NCCLS's approved guideline C24-A2.

1. Limit of detection

No international standard for Parvovirus B19 IgM Antibody detection has been defined so far by the European Community.
In its absence, an Internal Gold Standard (or IGS), has been defined in order to provide the device with a constant and excellent sensitivity.

I.G.S. Dilution	PARVOM.CE Lot P1	PARVOM.CE Lot P2
1x	1.418	1.219
2x	0.804	0.665
4x	0.407	0.383
8x	0.225	0.212
Negative Control	0.065	0.070

2. Diagnostic Sensitivity and Specificity:

The Diagnostic **Sensitivity** was calculated on a panel of 50 samples classified positive for the IgM anti Parvovirus B19 by a reference kit CE marked.
A value of ≥ 98% was observed when referring to the reference device.
The Diagnostic **Specificity** was calculated on a panel of more than 100 samples classified negative with the reference device.
A value ≥ 98% was observed.
These findings are summarized in the following table.

Sensitivity	≥ 98 %
Specificity	≥ 98 %

4. Precision:
It has been calculated on three samples, a negative, a low positive and a positive, examined in 16 replicates in three separate runs for two lots. Results are reported as follows:

PARVOM.CE: lot P1

Negative Control (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.149	0.136	0.136	0.140
Std.Deviation	0.013	0.018	0.018	0.016
CV %	8.5	13.0	13.0	11.5

Low Positive sample (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.996	0.977	0.950	0.974
Std.Deviation	0.032	0.057	0.056	0.048
CV %	3.2	5.8	5.9	5.0

Positive Control (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	3.032	2.671	3.362	3.022
Std.Deviation	0.221	0.221	0.184	0.209
CV %	7.3	8.3	5.5	7.0

PARVOM.CE: lot P2

Negative Control (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.101	0.091	0.094	0.095
Std.Deviation	0.013	0.010	0.013	0.012
CV %	12.5	11.2	13.7	12.5

Low Positive sample (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	1.227	1.261	0.970	1.153
Std.Deviation	0.085	0.085	0.090	0.087
CV %	6.9	6.7	9.2	7.6

Positive Control (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	3.350	2.937	3.170	3.152
Std.Deviation	0.174	0.199	0.197	0.190
CV %	5.2	7.0	7.1	6.4

The variability shown in the tables above did not result in sample misclassification.

5. Accuracy
The assay accuracy has been checked by the dilution and recovery tests.

S. LIMITATIONS OF THE PROCEDURE
Bacterial contamination or heat inactivation of the specimen may affect the absorbance values of the samples with consequent alteration of the level of the analyte.
Frozen samples containing fibrin particles or aggregates after thawing may generate some false results.
This test is suitable only for testing single samples and not pooled ones.
Diagnosis of an infectious disease should not be established on the basis of a single test result. The patient's clinical history, symptomatology, as well as other diagnostic data should be considered.

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All the IVD Products manufactured by the company are under the control of a certified Quality Management System in compliance with ISO 13485 rule. Each lot is submitted to a quality control and released into the market only if conforming with the EC technical specifications and acceptance criteria.

Produced by
Dia.Pro Diagnostic Bioprobes Srl
Via G. Carducci n° 27 – Sesto San Giovanni (MI) – Italy

CE

HBcAb

Competitive Enzyme Immunoassay for the determination of antibodies to Hepatitis B core Antigen in human serum and plasma

- for “in vitro” diagnostic use only -

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REF. BCAB.CE
96 Tests

HBcAb

A. INTENDED USE

Competitive Enzyme ImmunoAssay (ELISA) for the determination of antibodies to Hepatitis B core Antigen in human plasma and sera.
The kit is intended for the screening of blood units and the follow-up of HBV-infected patients.
For "in vitro" diagnostic use only.

B. INTRODUCTION

The World Health Organization (WHO) defines Hepatitis B as follows:

"Hepatitis B is one of the major diseases of mankind and is a serious global public health problem. Hepatitis means inflammation of the liver, and the most common cause is infection with one of 5 viruses, called hepatitis A,B,C,D, and E. All of these viruses can cause an acute disease with symptoms lasting several weeks including yellowing of the skin and eyes (jaundice); dark urine; extreme fatigue; nausea; vomiting and abdominal pain. It can take several months to a year to feel fit again. Hepatitis B virus can cause chronic infection in which the patient never gets rid of the virus and many years later develops cirrhosis of the liver or liver cancer.

HBV is the most serious type of viral hepatitis and the only type causing chronic hepatitis for which a vaccine is available. Hepatitis B virus is transmitted by contact with blood or body fluids of an infected person in the same way as human immunodeficiency virus (HIV), the virus that causes AIDS. However, HBV is 50 to 100 times more infectious than HIV. The main ways of getting infected with HBV are: (a) perinatal (from mother to baby at the birth); (b) child- to-child transmission; (c) unsafe injections and transfusions; (d) sexual contact.

Worldwide, most infections occur from infected mother to child, from child to child contact in household settings, and from reuse of un-sterilized needles and syringes. In many developing countries, almost all children become infected with the virus. In many industrialized countries (e.g. Western Europe and North America), the pattern of transmission is different. In these countries, mother-to-infant and child-to-child transmission accounted for up to one third of chronic infections before childhood hepatitis B vaccination programmes were implemented. However, the majority of infections in these countries are acquired during young adulthood by sexual activity, and injecting drug use. In addition, hepatitis B virus is the major infectious occupational hazard of health workers, and most health care workers have received hepatitis B vaccine.

Hepatitis B virus is not spread by contaminated food or water, and cannot be spread casually in the workplace. High rates of chronic HBV infection are also found in the southern parts of Eastern and Central Europe. In the Middle East and Indian sub-continent, about 5% are chronically infected. Infection is less common in Western Europe and North America, where less than 1% are chronically infected.

Young children who become infected with HBV are the most likely to develop chronic infection. About 90% of infants infected during the first year of life and 30% to 50% of children infected between 1 to 4 years of age develop chronic infection. The risk of death from HBV-related liver cancer or cirrhosis is approximately 25% for persons who become chronically infected during childhood.

Chronic hepatitis B in some patients is treated with drugs called *interferon or lamivudine*, which can help some patients. Patients with cirrhosis are sometimes given liver transplants, with varying success. It is preferable to prevent this disease with vaccine than to try and cure it.

Hepatitis B vaccine has an outstanding record of safety and effectiveness. Since 1982, over one billion doses of hepatitis B vaccine have been used worldwide. The vaccine is given as a series of three intramuscular doses. Studies have shown that the vaccine is 95% effective in preventing children and adults from developing chronic infection if they have not yet been infected. In many countries where 8% to 15% of children used to become chronically infected with HBV, the rate of chronic infection has been reduced to less than 1% in immunized groups of children. Since 1991, WHO has called for all countries to add hepatitis B vaccine into their national immunization programmes."

Hepatitis B core Antigen (or HBcAg) is the major component of the core particles of HBV.
HBcAg is composed of a single polypeptide of about 17 kD that is released upon disaggregating the core particles; the antigen contains at least one immunological determinant.
Upon primary infection, anti HBcAg antibodies are one of the first markers of HBV hepatitis appearing in the serum of the patient, slightly later than HBsAg, the viral surface antigen.
Anti HBcAg antibodies are produced usually at high titers and their presence is detectable even years after infection. Isolated HBcAb, in absence of other HBV markers, have been observed in infected blood units, suggesting the use of this test for screening HBV, in addition of HBsAg.
The determination of HBcAb has become important for the classification of the viral agent, together with the detection of the other markers of HBV infection, in sera and plasma.

C. PRINCIPLE OF THE TEST

The assay is based on the principle of competition where the antibodies in the sample compete with a monoclonal antibody for a fixed amount of antigen on the solid phase.
A purified recombinant HBcAg is coated to the microwells.
The patient's serum/plasma is added to the microwell together with an additive able to block interferences present in the sample.
In the second incubation after washing, a monoclonal antibody, conjugated with Horseradish Peroxidase (HRP) and specific for HBcAg is added and binds to the free rec-HBcAg coated on the plastic.
After incubation, microwells are washed to remove any unbound conjugate and then the chromogen/substrate is added. In the presence of peroxidase enzyme the colorless substrate is hydrolyzed to a colored end-product.
The color intensity is inversely proportional to the amount of antibodies to HBcAg present in the sample.

D. COMPONENTS

Each kit contains sufficient reagents to perform 96 tests.

1. Microplate MICROPLATE
8x12 microwell strips coated with recombinant HBcAg and sealed into a bag with desiccant. Allow the microplate to reach room temperature before opening; reseal unused strips in the bag with desiccant and store at 2..8℃.

2. Negative Control CONTROL -
1x1.0ml/vial. Ready to use. Contains 5% bovine serum albumin, 10 mM phosphate buffer pH 7.4 +/-0.1, 0.09% sodium azide and 0.1% Kathon GC as preservatives. The negative control is pale yellow color coded.

3. Positive Control CONTROL +
1x1.0ml/vial. Ready to use. Contains 5% bovine serum albumin, anti HBcAg antibodies at a concentration of about 10 PEI U/ml, (calibrated on PEI HBc Reference Material 82), 10 mM phosphate buffer pH 7.4 +/-0.1, 0.09% sodium azide and 0.1% Kathon GC as preservatives. The positive control is green color coded.

4. Calibrator CAL ….
n° 1 vial. Lyophilised. To be dissolved with EIA gr ade water as reported in the label. Contains fetal bovine serum, human antibodies to HBcAg at a concentration of 2 PEI U/ml +/-10% (calibrated on PEI HBc Reference Material 82) and 0.1% Kathon GC as preservative.
Note: The volume necessary to dissolve the content of the vial may vary from lot to lot. Please use the right volume reported on the label .

5. Wash buffer concentrate WASHBUF 20X
1x60ml/bottle. 20x concentrated solution.
Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2, 0.05% Tween 20 and 0.1% Kathon GC.

6. Enzyme Conjugate CONJ
1x16ml/vial. Ready-to-use solution. Contains 5% bovine serum albumine, 10 mM tris buffer pH 6.8 +/-0.1, Horseradish peroxidase conjugated mouse monoclonal antibody to HBcAg in presence of 0.3 mg/ml gentamicine sulphate and 0.1% Kathon GC as preservatives. The component is red colour coded .

7. Chromogen/Substrate SUBS TMB
1x16ml/vial. Contains a 50 mM citrate-phosphate buffered solution at pH 3.6 +/-0.1, 0.03% tetra-methyl-benzidine (TMB), 0.02% hydrogen peroxide (H_2O_2) and 4% dimethylsulphoxide
Note: To be stored protected from light as sensitive to strong illumination.

8. Specimen Diluent DILSPE
4x3ml/vial. 10 mM tris buffered solution pH 8.0 +/-0.1 containing 0.1% Kathon GC for the pre-treatment of samples and controls in the plate, blocking interference.
Note: Use all the content of one vial before opening a second one. The reagent is sensitive to oxidation.

9. Sulphuric Acid H_2SO_4 O.3 M
1x15ml/vial. Contains 0.3 M H_2SO_4 solution.
Attention: Irritant (Xi R36/38; S2/26/30)

10. Plate sealing foil n°2

11. Instruction manual n°1

E. MATERIALS REQUIRED BUT NOT PROVIDED
1. Calibrated Micropipettes (100ul and 50ul) and disposable plastic tips.
2. EIA grade water (double distilled or deionised, charcoal treated to remove oxidizing chemicals used as disinfectants).
3. Timer with 60 minute range or higher.
4. Absorbent paper tissues.
5. Calibrated ELISA microplate thermostatic incubator (dry or wet) set at +37°C.
6. Calibrated ELISA microwell reader with 450nm (reading) and with 620-630nm (blanking) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.

F. WARNINGS AND PRECAUTIONS
1. The kit has to be used by skilled and properly trained technical personnel only, under the supervision of a medical doctor responsible of the laboratory.
2. When the kit is used for the screening of blood units and blood components, it has to be used in a laboratory certified and qualified by the national authority in that field (Ministry of Health or similar entity) to carry out this type of analysis.
3. All the personnel involved in performing the assay have to wear protective laboratory clothes, talc-free gloves and glasses. The use of any sharp (needles) or cutting (blades) devices should be avoided. All the personnel involved should be trained in biosafety procedures, as recommended by the Center for Disease Control, Atlanta, U.S. and reported in the National Institute of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
4. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.
5. The laboratory environment should be controlled so as to avoid contaminants such as dust or air-born microbial agents, when opening kit vials and microplates and when performing the test. Protect the Chromogen (TMB) from strong light and avoid vibration of the bench surface where the test is undertaken.
6. Upon receipt, store the kit at 2-8°C into a temp erature controlled refrigerator or cold room.
7. Do not interchange components between different lots of the kits. It is recommended that components between two kits of the same lot should not be interchanged.
8. Check that the reagents are clear and do not contain visible heavy particles or aggregates. If not, advise the laboratory supervisor to initiate the necessary procedures.
9. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each sample.
10. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one.
11. Do not use the kit after the expiration date stated on external (primary container) and internal (vials) labels.
12. Treat all specimens as potentially infective. All human serum specimens should be handled at Biosafety Level 2, as recommended by the Center for Disease Control, Atlanta, U.S. in compliance with what reported in the Institutes of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
13. The use of disposable plastic-ware is recommended in the preparation of the washing solution or in transferring components into other containers of automated workstations, in order to avoid contamination.
14. Waste produced during the use of the kit has to be discarded in compliance with national directives and laws concerning laboratory waste of chemical and biological substances. In particular, liquid waste generated from the washing procedure, from residuals of controls and from samples has to be treated as potentially infective material and inactivated. Suggested procedures of inactivation are treatment with a 10% final concentration of household bleach for 16-18 hrs or heat inactivation by autoclave at 121°C for 20 min..
15. Accidental spills have to be adsorbed with paper tissues soaked with household bleach and then with water. Tissues should then be discarded in proper containers designated for laboratory/hospital waste.
16. The Sulphuric Acid is an irritant. In case of spills, wash the surface with plenty of water.
17. Other waste materials generated from the use of the kit (example: tips used for samples and controls, used microplates) should be handled as potentially infective and

disposed according to national directives and laws concerning laboratory wastes.

G. SPECIMEN: PREPARATION AND RECOMMANDATIONS

1. Blood is drawn aseptically by venepuncture and plasma or serum is prepared using standard techniques of preparation of samples for clinical laboratory analysis. No influence has been observed in the preparation of the sample with citrate, EDTA and heparin.
2. Avoid any addition of preservatives to samples; especially sodium azide as this chemical would affect the enzymatic activity of the conjugate.
3. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results. When the kit is used for the screening of blood units, bar code labeling and electronic reading is strongly recommended.
4. Haemolysed (red) and visibly hyperlipemic ("milky") samples have to be discarded as they could generate false results. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they could give rise to false results.
5. Sera and plasma can be stored at +2°..8°C for up to five days after collection. For longer storage periods, samples can be stored frozen at –20°C for several months. Any frozen samples should not be frozen/thawed more than once as this may generate particles that could affect the test result.
6. If particles are present, centrifuge at 2.000 rpm for 20 min or filter using 0.2-0.8u filters to clean up the sample for testing.

H. PREPARATION OF COMPONENTS AND WARNINGS

A study conducted on an opened kit has not pointed out any relevant loss of activity up to 6 re-uses of the device and up to 6 months.

1. Microplates:
Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant has not turned dark green, indicating a defect in storage.
In this case, call Dia.Pro's customer service.
Unused strips have to be placed back inside the aluminum pouch, with the desiccant supplied, firmly zipped and stored at +2°..8°C. After first opening, remaining strips are stable until the humidity indicator inside the desiccant bag turns from yellow to green.

2. Negative Control:
Ready to use. Mix well on vortex before use.

3. Positive Control:
Ready to use. Mix well on vortex before use.

4. Calibrator:
Add the volume of ELISA grade water, reported on the label, to the lyophilised powder; let fully dissolve and then gently mix on vortex.
Note: *The dissolved calibrator is not stable. Store it frozen in aliquots at –20°C.*

5. Wash buffer concentrate:
The whole content of the concentrated solution has to be diluted 20x with bidistilled water and mixed gently end-over-end before use. During preparation avoid foaming as the presence of bubbles could impact on the efficiency of the washing cycles.
Note: *Once diluted, the wash solution is stable for 1 week at +2..8°C.*

6. Enzyme conjugate:
Ready to use. Mix well on vortex before use.
Avoid contamination of the liquid with oxidizing chemicals, dust or microbes. If this component has to be transferred, use only plastic, and if possible, sterile disposable containers.

7. Chromogen/Substrate:
Ready to use. Mix well on vortex before use.
Avoid contamination of the liquid with oxidizing chemicals, air-driven dust or microbes. Do not expose to strong light, oxidizing agents and metallic surfaces.
If this component has to be transferred use only plastic, and if possible, sterile disposable container.

8. Specimen Diluent
Ready to use solution. Mix gently on vortex before use. Use all the content of one vial before opening a second one. The reagent is sensitive to oxidation.

9. Sulphuric Acid:
Ready to use. Mix well on vortex before use.
Attention: Irritant (Xi R36/38; S2/26/30)
Legenda: R 36/38 = Irritating to eyes and skin.
S 2/26/30 = In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

I. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

1. Micropipettes have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (70% ethanol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample or the components of the kit. They should also be regularly maintained in order to show a precision of 1% and a trueness of $\pm$2%.
2. The ELISA incubator has to be set at +37°C (tole rance of $\pm$0.5°C) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water baths are suitable for the incubations, provided that the instrument is validated for the incubation of ELISA tests.
3. The ELISA washer is extremely important to the overall performances of the assay. The washer must be carefully validated and correctly optimized using the kit controls/calibrator and reference panels, before using the kit for routine laboratory tests. Usually 4-5 washing cycles (aspiration + dispensation of 350 ul/well of washing solution = 1 cycle) are sufficient to ensure that the assay performs as expected. A soaking time of 20-30 seconds between cycles is suggested. In order to set correctly their number, it is recommended to run an assay with the kit controls/calibrator and well characterized negative and positive reference samples, and check to match the values reported below in the sections "Validation of Test" and "Assay Performances". Regular calibration of the volumes delivered and maintenance (decontamination and cleaning of needles) of the washer has to be carried out according to the instructions of the manufacturer.
4. Incubation times have a tolerance of $\pm$5%.
5. The ELISA microplate reader has to be equipped with a reading filter of 450nm and with a second filter (620-630nm, strongly recommended) for blanking purposes. Its standard performances should be (a) bandwidth $\leq$ 10 nm; (b) absorbance range from 0 to $\geq$ 2.0; (c) linearity to $\geq$ 2.0; repeatability $\geq$ 1%. Blanking is carried out on the well identified in the section "Assay Procedure". The optical system of the reader has to be calibrated regularly to ensure that the correct optical density is measured. It should be regularly maintained according to the manufacturer 's instructions.

6. When using an ELISA automated work station, all critical steps (dispensation, incubation, washing, reading, shaking, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the sections "Validation of Test" and "Assay Performances". The assay protocol has to be installed in the operating system of the unit and validated as for the washer and the reader. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set. Particular attention must be paid to avoid carry over by the needles used for dispensing samples and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells due to strongly reactive samples, leading to false positive results. The use of ELISA automated work stations is recommended for blood screening and when the number of samples to be tested exceed 20-30 units per run.
7. Dia.Pro's customer service offers support to the user in the setting and checking of instruments used in combination with the kit, in order to assure full compliance with the requirements described. Support is also provided for the installation of new instruments to be used with the kit.

L. PRE ASSAY CONTROLS AND OPERATIONS

1. Check the expiration date of the kit printed on the external label (primary container). Do not use if expired.
2. Check that the liquid components are not contaminated by visible particles or aggregates. Check that the Chromogen (TMB) is colourless or pale blue by aspirating a small volume of it with a sterile plastic pipette. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box (primary container). Check that the aluminium pouch, containing the microplate, is not punctured or damaged.
3. Dilute all the content of the 20x concentrated Wash Solution as described above.
4. Dissolve the Calibrator as described above and gently mix.
5. Allow all the other components to reach room temperature (about 1 hr) and then mix gently on vortex all liquid reagents.
6. Set the ELISA incubator at +37℃ and prepare the ELISA washer by priming with the diluted washing solution, according to the manufacturers instructions. Set the right number of washing cycles as found in the validation of the instrument for its use with the kit.
7. Check that the ELISA reader is turned on or ensure it will be turned on at least 20 minutes before reading.
8. If using an automated work station, turn on, check settings and be sure to use the right assay protocol.
9. Check that the micropipettes are set to the required volume.
10. Check that all the other equipment is available and ready to use.
11. In case of problems, do not proceed further with the test and advise the supervisor.

M. ASSAY PROCEDURE

The assay has to be performed according to the procedure given below, taking care to maintain the same incubation time for all the samples being tested.

1. Place the required number of strips in the plastic holder and carefully identify the wells for controls, calibrator and samples.
2. Leave the A1 well empty for blanking purposes.
3. Dispense 50 ul Specimen Diluent into all the control and sample wells.
4. Pipette 50 µl of the Negative Control in triplicate, 50 ul of the Calibrator in duplicate and then 50 ul of the Positive Control in single. Then dispense 50 ul of each of the samples.
5. Incubate the microplate for **60 min at +37℃.**

Important note: *Strips have to be sealed with the adhesive sealing foil, only when the test is performed manually. Do not cover strips when using ELISA automatic instruments.*

6. When the first incubation is finished, wash the microwells as previously described (section I.3)
7. Pipette 100 µl Enzyme Conjugate in all the wells, except A1; incubate the microplate for **60 min at +37℃.**

Important note: *Be careful not to touch the plastic inner surface of the well with the tip filled with the Enzyme Conjugate. Contamination might occur.*

8. When the second incubation is finished, wash the microwells as previously described (section I.3)
9. Pipette 100 µl Chromogen/Substrate into all the wells, A1 included.

Important note: *Do not expose to strong direct light. as a high background might be generated.*

10. Incubate the microplate protected from light at **room temperature (18-24℃) for 20 minutes**. Wells dispensed with negative control and negative samples will turn from clear to blue (competitive method).
11. Pipette 100 µl Sulphuric Acid into all the wells using the same pipetting sequence as in step 9 to stop the enzymatic reaction. Addition of the stop solution will turn the negative control and negative samples from blue to yellow.
12. Measure the colour intensity of the solution in each well, as described in section I.5 using a 450nm filter (reading) and a 620-630nm filter (background subtraction, strongly recommended), blanking the instrument on A1.

Important notes:

1. *If the second filter is not available, ensure that no finger prints are present on the bottom of the microwell before reading at 450nm. Finger prints could generate false positive results on reading.*
2. *Reading has should ideally be performed immediately after the addition of the Stop Solution but definitely no longer than 20 minutes afterwards. Some self oxidation of the chromogen can occur leading to a higher background.*

N. ASSAY SCHEME

Specimen Diluent	50 ul
Controls&calibrator and samples	50 ul
1st incubation	**60 min**
Temperature	+37℃
Wash	n°4-5
Enzyme Conjugate	100 ul
2nd incubation	**60 min**
Temperature	+37℃
Wash	n°4-5
TMB/H2O2 mix	100 ul
3rd incubation	**20 min**
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm

An example of dispensation scheme is reported below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S2										
B	NC	S3										
C	NC	S4										
D	NC	S5										
E	CAL	S6										
F	CAL	S7										
G	PC	S8										
H	S1	S9										

Legenda: BLK = Blank NC = Negative Control
CAL = Calibrator PC = Positive Control S = Sample

O. INTERNAL QUALITY CONTROL

A check is performed on the controls/calibrator any time the kit is used in order to verify whether the expected OD450nm or Co/S values have been matched in the analysis.
Ensure that the following parameters are met:

Parameter	Requirements
Blank well	< 0.050 OD450nm value
Negative Control (NC)	> 1.000 OD450nm after blanking coefficient of variation < 20%
Calibrator (about 2 PEI U/ml)	Co/S > 1
Positive Control	< 0.200 OD450nm

If the results of the test match the requirements stated above, proceed to the next section.

If they do not, do not proceed any further and perform the following checks:

Problem	Check
Blank well > 0.050 OD450nm	that the Chromogen/Substrate solution has not become contaminated during the assay
Negative Control (NC) < 1.000 OD450nm after blanking coefficient of variation > 20%	1. that the washing procedure and the washer settings are as validated in the pre qualification study; 2. that the proper washing solution has been used and the washer has been primed with it before use; 3. that no mistake has been done in the assay procedure (dispensation of positive control instead of negative control; 4. that no contamination of the negative control or of the wells where the control was dispensed has occurred due to positive samples, to spills or to the enzyme conjugate; 5. that micropipettes have not become contaminated with positive samples or with the enzyme conjugate 6. that the washer needles are not blocked or partially obstructed.
Calibrator Co/S < 1	1. that the procedure has been correctly performed; 2. that no mistake has occurred during its distribution (ex.: dispensation of negative control instead 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the calibrator has occurred.
Positive Control > 0.200 OD450nm	1. that the procedure has been correctly performed; 2. that no mistake has occurred during the distribution of the control (dispensation of negative control instead of positive control). 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the positive control has occurred.

If any of the above problems have occurred, report the problem to the supervisor for further actions.

P. RESULTS

The results are calculated by means of a cut-off value determined with the following formula:

Cut-Off = (NC + PC) / 5

***Important note**: When the calculation of results is performed by the operating system of an ELISA automated work station, ensure that the proper formulation is used to calculate the cut-off value and generate the correct interpretation of results.*

Q. INTERPRETATION OF RESULTS

Results are interpreted as ratio between the cut-off value and the sample OD450nm or Co/S.

Results are interpreted according to the following table:

Co/S	Interpretation
< 0.9	Negative
0.9 - 1.1	Equivocal
> 1.1	Positive

A negative result indicates that the patient has not been infected by HBV.
Any patient showing an equivocal result should be re-tested on a second sample taken 1-2 weeks after the initial sample.
The blood unit should not be transfused.
A positive result is indicative of HBV infection and therefore the patient should be treated accordingly or the blood unit should be discarded.

Important notes:

1. *Interpretation of results should be done under the supervision of the laboratory supervisor to reduce the risk of judgement errors and misinterpretations.*
2. *When test results are transmitted from the laboratory to another facility, attention must be paid to avoid erroneous data transfer.*
3. *Diagnosis of viral hepatitis infection has to be taken by and released to the patient by a suitably qualified medical doctor.*

An example of calculation is reported below.

The following data must not be used instead or real figures obtained by the user.

Negative Control: 2.000 – 2.200 – 2.000 OD450nm
Mean Value: 2.100 OD450nm
Higher than 1.000 – Accepted

Positive Control: 0.100 OD450nm
Lower than 0.200 – Accepted

Cut-Off = (2.100 + 0.100) / 5 = 0.440

Calibrator: 0.400-0.360 OD450nm
Mean value: 0.380 OD450nm
Co/S>1 – Accepted

Sample 1: 0.028 OD450nm
Sample 2: 1.890 OD450nm
Sample 1 Co/S > 1.1 positive
Sample 2 Co/S < 0.9 negative

R. PERFORMANCES

Evaluation of Performances has been conducted in accordance to what reported in the Common Technical Specifications or CTS (art. 5, Chapter 3 of IVD Directive 98/79/EC).

1. LIMIT OF DETECTION:

The sensitivity of the assay has been calculated by means of the reference preparation for HBcAb supplied by Paul Erlich Institute (PEI HBc Reference Material 82). The assay shows a sensitivity of about 1.25 PEI U/ml.

The table below reports the Co/S values shown by the PEI standard diluted as suggested by the manufacturer to prepare a limiting dilution curve in Fetal Calf Serum (FCS).

PEI U/ml	Lot 1001	Lot 0702	Lot 0702/2	Lot 1202
5	**22.6**	**18.0**	**19.0**	**17.7**
2.5	**8.0**	**5.5**	**5.4**	**5.0**
1.25	**1.1**	**1.3**	**1.0**	**1.0**
0.625	0.4	0.4	0.4	0.4

In addition Accurun 1 – series 3000 – supplied by Boston Biomedica Inc., USA, was tested to determine its Co/S value. Results are reported in the table below:

Accurun 1 – series 3000

Value	Lot 1001	Lot 0702	Lot 1202
Co/S	2.9	2.3	2.2

2. DIAGNOSTIC SPECIFICITY AND SENSITIVITY

The Performance Evaluation of the device was carried out in a trial conducted on more than total 6000 samples.

2.1 Diagnostic Specificity

It is defined as the probability of the assay of scoring negative in the absence of specific analyte. A total of more 5000 unselected donors, including 1st time donors, were examined.

In a first study 2023 samples were tested against a US company as reference. A specificity of 99.5% was found. In a second study 1588 samples were examined against a European company. A specificity of 99.7% was found. In the last study 1565 samples were assayed against the same US company; a value of 99.8% was found.

In addition to the above population, 206 samples from hospitalized patients were tested against the European company. A value of 99.3% specificity was found.

Moreover, diagnostic specificity was assessed by testing 164 potentially interfering specimens (other infectious diseases, patients affected by non viral hepatic diseases, dialysis patients, pregnant women, hemolized, lipemic, etc.) against the European company. A value of specificity of 100% was assessed.

Finally, both human plasma, derived with different standard techniques of preparation (citrate, EDTA and heparin), and human sera have been used to determine the specificity.

No false reactivity due to the method of specimen preparation has been observed.

2.2 Diagnostic Sensitivity

It defined as the probability of the assay of scoring positive in the presence of specific analyte.

373 positive specimens were tested against the European company; a diagnostic sensitivity of 99.7 was found.

3. PRECISION

The mean values obtained from a study conducted on three lots and on two samples of different anti-HBcAg reactivity, examined in 16 replicates in three separate runs is reported below:

BCAB.CE: lot # 1202

Negative Control (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	1.943	1.939	1.924	1.935
Std.Deviation	0.081	0.078	0.103	0.087
CV %	4.2	4.0	5.3	4.5

Calibrator (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.143	0.147	0.148	0.146
Std.Deviation	0.014	0.017	0.018	0.016
CV %	9.8	11.4	12.1	11.1
Co/S	2.8	2.7	2.6	2.7

BCAB.CE: lot # 0702

Negative Control (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	2.163	2.110	2.106	2.126
Std.Deviation	0.105	0.088	0.139	0.111
CV %	4.9	4.2	6.6	5.2

Calibrator (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.182	0.193	0.195	0.190
Std.Deviation	0.018	0.023	0.019	0.020
CV %	10.0	12.0	9.9	10.6
Co/S	2.5	2.2	2.3	2.3

BCAB.CE: lot # 0702/2

Negative Control (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	2.278	2.098	2.130	2.169
Std.Deviation	0.135	0.126	0.159	0.140
CV %	5.9	6.0	7.5	6.5

Calibrator (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.193	0.190	0.199	0.134
Std.Deviation	0.023	0.023	0.027	0.025
CV %	12.1	12.3	13.5	12.6
Co/S	2.4	2.2	2.2	2.3

The variability shown in the tables did not result in sample misclassification.

S. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or heat inactivation of the specimen may affect the absorbance values of the samples with consequent alteration of the level of the analyte. This test is suitable only for testing single samples and not pooled ones.

Diagnosis of an infectious disease should not be established on the basis of a single test result. The patient's clinical history, symptomatology, as well as other diagnostic data should be considered.

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All the IVD Products manufactured by the company are under the control of a certified Quality Management System approved by an EC Notified Body. Each lot is submitted to a quality control and released into the market only if conforming with the EC technical specifications and acceptance criteria.

Produced by
Dia.Pro Diagnostic Bioprobes Srl
Via G. Carducci n° 27 – Sesto San Giovanni (MI) – Italy

CE
0318

HBc IgM

"Capture" Enzyme ImmunoAssay (ELISA) for the quantitative/qualitative determination of IgM class antibody to Hepatitis B Virus core Antigen in human plasma and sera

- for "in vitro" diagnostic use only -

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REF BCM.CE
96 Tests

HBc IgM

A. INTENDED USE

Enzyme ImmunoAssay (ELISA) for the quantitative/qualitative determination of IgM class antibodies to Hepatitis B Virus core Antigen in human plasma and sera with the "capture" system.
The kit is intended for the classification of the viral agent and for the follow-up of chronic patients under therapy.
For “in vitro” diagnostic use only.

B. INTRODUCTION

Hepatitis B core Antigen (or HBcAg) is the major component of the core particles of Hepatitis B virus (or HBV).
Particles have a size of 27nm and contain a circular double-stranded DNA molecule, a specific DNA-polymerase and HBcAg. HBcAg is composed of a single polypeptide of about 17 kD that is released upon disaggregation of the core particles ; the antigen contains at least one immunological determinant.
Upon primary infection, anti HBcAg IgM antibodies are one of the first markers of HBV hepatitis appearing in the serum of the patient, together or slightly later than HBsAg, the viral surface antigen.
Anti HBcAg IgM titers, very high during the acute phase, decrease along the illness, as IgG antibodies appear, down to undetectable levels in convalescent patients.
In chronic hepatitis, however, spikes of anti HBcAg IgM synthesis are present, confirming reactivation of HBV in hepatocites and giving origin to permanent IgM low titers.
The determination of anti HBcAg IgM antibodies has become very important for the fast classification of the virus, of the phase of the illness and for the monitoring of patients under treatment with interferon.

C. PRINCIPLE OF THE TEST

The assay is based on the principle of "IgM capture" where IgM class antibodies in the sample are first captured by the solid phase coated with anti hIgM antibody.
After washing out all the other components of the sample and in particular IgG antibodies, the specific IgM captured on the solid phase are detected by the addition of a purified preparation of recombinant HBcAg, labelled with a monoclonal antibody conjugated with peroxidase (HRP).
After incubation, microwells are washed to remove unbound conjugate and then the chromogen/substrate is added.
In the presence of peroxidase the colourless substrate is hydrolysed to a coloured end-product, whose optical density may be detected and is proportional to the amount of IgM antibodies to HBcAg present in the sample.

D. COMPONENTS

Each kit contains sufficient reagents to perform 96 tests.

1. Microplate: MICROPLATE
8x12 microwell strips coated with purified anti human IgM specific mouse monoclonal antibody, post-coated with bovine serum proteins and sealed into a bag with desiccant. Allow the microplate to reach room temperature before opening; reseal unused strips in the bag with desiccant and store at 4°C.

2. Calibration Curve: CAL N° …
6x2.0 ml/vial. Ready to use and color coded standard curve calibrated on the HBcIgM reference preparation supplied by Paul Erlich Institute (HBc-Referenzserum-IgM 84), ranging: CAL1 = 0 U/ml // CAL2 = 5 U/ml // CAL3 = 10 U/ml // CAL4 = 20 U/ml // CAL 5 = 50 U/ml // CAL 6 = 100 U/ml.
It contains chemical inactivated HBcIgM positive human plasma, 100 mM Tris buffer pH 7.4+/-0.1, 0.5% Tween 20, 0.09% sodium azide and 0.045% ProClin 300 as preservatives.
The Calibration Curve is coded with blue alimentary dye.
Important Note: Even if plasma has been chemically inactivated, handle this component as potentially infectious.

3. Wash buffer concentrate: WASHBUF 20X
1x60ml/bottle. 20x concentrated solution.
Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2, 0.05% Tween 20 and 0.045% ProClin 300.

4. Enzyme Conjugate (Immunocomplex) : CONJ
1x16.0 ml/vial. Ready-to-use solution. Contains an immunocomplex formed by a specific mouse monoclonal antibody, labelled with HRP, and a purified recombinant HBcAg.
The reagent is dissolved into a buffer solution 10 mM Tris buffer pH 6.8+/-0.1, 2% BSA, 0.045% ProClin 300 and 0.02% gentamicine sulphate as preservatives. The component is red colour coded.

5. Specimen Diluent : DILSPE
2x60.0 ml/vial. Buffered solution for the dilution of samples; it contains 100 mM Tris buffer pH 7.4+/-0.1, 0.5% Tween 20, 2% Casein, 0.045% ProClin 300 and 0.09% sodium azide as preservatives. The component is blue color coded.

6. Control Serum : CONTROL …ml
1 vial. Lyophilized. Contains fetal bovine serum, human HBcIgM positive human plasma calibrated at 20 ± 10% PEI U/ml. 0.2 mg/ml gentamicine sulphate and 0.045% ProClin 300 as preservatives.
Important Notes
1. The volume necessary to dissolve the content of the vial may vary from lot to lot. Please use the right volume reported on the label .
2. Important Note: Even if plasma has been chemically inactivated, handle this component as potentially infectious.

7. Chromogen/Substrate : SUBS TMB
1x16ml/vial. Contains a 50 mM citrate-phosphate buffered solution at pH 3.5-3.8, 4% dimethylsulphoxide, 0.03% tetra-methyl-benzidine or TMB and 0.02% hydrogen peroxide or H_2O_2.
Note: To be stored protected from light as sensitive to strong illumination.

8. Sulphuric Acid: H2SO4 0.3 M
1x15ml/vial. Contains 0.3 M H_2SO_4 solution.
Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

9. Plate sealing foils: n° 2

10. Package insert: n° 1

E. MATERIALS REQUIRED BUT NOT PROVIDED

1. Calibrated Micropipettes (150ul, 100ul and 50ul) and disposable plastic tips.
2. EIA grade water (double distilled or deionised, charcoal treated to remove oxidizing chemicals used as disinfectants).
3. Timer with 60 minute range or higher.
4. Absorbent paper tissues.
5. Calibrated ELISA microplate thermostatic incubator (dry or wet) set at +37°C.
6. Calibrated ELISA microwell reader with 450nm (reading) and with 620-630nm (blanking) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.

F. WARNINGS AND PRECAUTIONS

1. The kit has to be used by skilled and properly trained technical personnel only, under the supervision of a medical doctor responsible of the laboratory.
2. All the personnel involved in performing the assay have to wear protective laboratory clothes, talc-free gloves and glasses. The use of any sharp (needles) or cutting (blades) devices should be avoided. All the personnel involved should be trained in biosafety procedures, as recommended by the Center for Disease Control, Atlanta, U.S. and reported in the National Institute of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
3. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.
4. The laboratory environment should be controlled so as to avoid contaminants such as dust or air-born microbial agents, when opening kit vials and microplates and when performing the test. Protect the Chromogen/Substrate (TMB) from strong light and avoid vibration of the bench surface where the test is undertaken.
5. Upon receipt, store the kit at 2-8°C into a temperature controlled refrigerator or cold room.
6. Do not interchange components between different lots of the kits. It is recommended that components between two kits of the same lot should not be interchanged.
7. Check that the reagents are clear and do not contain visible heavy particles or aggregates. If not, advise the laboratory supervisor to initiate the necessary procedures.
8. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each sample. Do not reuse disposable tips.
9. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one. Do not reuse disposable tips.
10. Do not use the kit after the expiration date stated on external (primary container) and internal (vials) labels.
11. Treat all specimens as potentially infective. All human serum specimens should be handled at Biosafety Level 2, as recommended by the Center for Disease Control, Atlanta, U.S. in compliance with what reported in the Institutes of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
12. The use of disposable plastic-ware is recommended in the preparation of the washing solution or in transferring components into other containers of automated workstations, in order to avoid contamination.
13. Waste produced during the use of the kit has to be discarded in compliance with national directives and laws concerning laboratory waste of chemical and biological substances. In particular, liquid waste generated from the washing procedure, from residuals of controls and from samples has to be treated as potentially infective material and inactivated. Suggested procedures of inactivation are treatment with a 10% final concentration of household bleach for 16-18 hrs or heat inactivation by autoclave at 121°C for 20 min..
14. Accidental spills have to be adsorbed with paper tissues soaked with household bleach and then with water. Tissues should then be discarded in proper containers designated for laboratory/hospital waste.
15. The Stop Solution is an irritant. In case of spills, wash the surface with plenty of water
16. Other waste materials generated from the use of the kit (example: tips used for samples and controls, used microplates) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

G. SPECIMEN: PREPARATION AND RECOMMANDATIONS

1. Blood is drawn aseptically by venepuncture and plasma or serum is prepared using standard techniques of preparation of samples for clinical laboratory analysis. No influence has been observed in the preparation of the sample with citrate, EDTA and heparin.
2. Avoid any addition of preservatives; especially sodium azide as this chemical would affect the enzymatic activity of the conjugate, generating false negative results.
3. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results.
4. Haemolysed and visibly hyperlipemic ("milky") samples have to be discarded as they could generate false results. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they could give rise to false results.
5. Sera and plasma can be stored at +2°…+8°C in primary collection tubes for up to five days after collection.

Do not freeze primary tubes of collection. For longer storage periods, sera and plasma samples, carefully removed from the primary collection tube, can be stored frozen at –20°C for at least 12 months. Any frozen samples should not be frozen/thawed more than once as this may generate particles that could affect the test result.

6. If particles are present, centrifuge at 2.000 rpm for 20 min or filter using 0.2-0.8u filters to clean up the sample for testing.

H. PREPARATION OF COMPONENTS AND WARNINGS

A study conducted on an opened kit has not pointed out any relevant loss of activity up to 6 re-uses of the device and up to 3 months.

Microplate:

Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant has not turned dark green, indicating a defect in manufacturing.
In this case, call Dia.Pro's customer service.
Unused strips have to be placed back into the aluminium pouch, with the desiccant supplied, firmly zipped and stored at +2°-8°C.
When opened the first time, unused strips are stable until the humidity indicator inside the desiccant bag turns from yellow to green.

Calibration Curve:

Ready to use. Mix well on vortex before use.

Wash buffer concentrate:

The whole content of the 20x concentrated solution has to be diluted with bidistilled water up to 1200ml and mixed gently end-over-end before use.
During preparation avoid foaming as the presence of bubbles could impact on the efficiency of the washing cycles.
Note: Once diluted, the wash solution is stable for 1 week at +2..8° C.

Enzyme conjugate:

Ready to use. Mix well on vortex before use.
Avoid contamination of the liquid with oxidizing chemicals, dust or microbes. If this component has to be transferred, use only plastic, and if possible, sterile disposable containers.

Specimen Diluent

Ready to use. Mix on vortex before use.

Control Serum

Dissolve the content of the vial with EIA grade water as reported in the label. Mix well on vortex before use. The dissolved control serum is ready to use.
Note: The control after dissolution is not stable. Store frozen in aliquots at –20°C.

Chromogen/Substrate:

Ready to use. Mix well on vortex before use.

Avoid contamination of the liquid with oxidizing chemicals, air-driven dust or microbes. Do not expose to strong light, oxidizing agents and metallic surfaces.
If this component has to be transferred use only plastic, and if possible, sterile disposable container

Sulphuric Acid:
Ready to use. Mix well on vortex before use.
Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

Legenda:

Warning **H statements:**
H315 – Causes skin irritation.
H319 – Causes serious eye irritation.

Precautionary **P statements:**
P280 – Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352 – IF ON SKIN: Wash with plenty of soap and water.
P332 + P313 – If skin irritation occurs: Get medical advice/attention.
P305 + P351 + P338 – IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337 + P313 – If eye irritation persists: Get medical advice/attention.
P362 + P363 – Take off contaminated clothing and wash it before reuse.

I. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

1. Micropipettes have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (household alcohol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample. They should also be regularly maintained. Decontamination of spills or residues of kit components should also be carried out regularly. They should also be regularly maintained in order to show a precision of 1% and a trueness of ±2%.
2. The ELISA incubator has to be set at +37°C (tolerance of +/- 0.5°C) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water baths are suitable for the incubations, provided that the instrument is validated for the incubation of ELISA tests.
3. The **ELISA washer** is extremely important to the overall performances of the assay. The washer must be carefully validated in advance, checked for the delivery of the right dispensation volume and regularly submitted to maintenance according to the manufacturer's instructions for use. In particular the washer, at the end of the daily workload, has to be extensively cleaned out of salts with deionized water. Before use, the washer has to be extensively primed with the diluted Washing Solution.
   The instrument weekly has to be submitted to decontamination according to its manual (NaOH 0.1 M decontamination suggested).
   5 washing cycles (aspiration + dispensation of 350ul/well of washing solution + 20 sec soaking = 1 cycle) are sufficient to ensure the assay with the declared performances. If soaking is not possible add one more cycle of washing.
   An incorrect washing cycle or salt-blocked needles are the major cause of false positive reactions.
4. Incubation times have a tolerance of ±5%.
5. The ELISA reader has to be equipped with a reading filter of 450nm and with a second filter of 620-630nm, mandatory for blanking purposes. Blanking is carried out on the well identified in the section "Assay Procedure". The optical system of the reader has to be calibrated regularly to ensure the correct optical density is measured. It should be regularly maintained according to the manufacturer 's instructions.
6. When using an ELISA automated work station, all critical steps (dispensation, incubation, washing, reading, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the sections "Validation of Test" and "Assay Performances". The assay protocol has to be installed in the operating system of the unit and validated as for the washer and the reader. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set. Particular attention must be paid to avoid carry over by the needles used for dispensing and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells. The use of ELISA automated work stations is recommended when the number of samples to be tested exceed 20-30 units per run.
7. Dia.Pro's customer service offers support to the user in the setting and checking of instruments used in combination with the kit, in order to assure compliance with the requirements described. Support is also provided for the installation of new instruments to be used with the kit.

L. PRE ASSAY CONTROLS AND OPERATIONS

1. Check the expiration date of the kit printed on the external label (primary container). Do not use if expired.
2. Check that the liquid components are not contaminated by visible particles or aggregates. Check that the Chromogen/Substrate (TMB+H_2O_2) is colourless or pale blue by aspirating a small volume of it with a sterile plastic pipette. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box (primary container). Check that the aluminium pouch, containing the microplate, is not punctured or damaged.
3. Dilute all the content of the 20x concentrated Wash Solution as described above.
4. Dissolve the Control Serum as described above and gently mix.
5. Allow all the other components to reach room temperature (about 1 hr) and then mix gently on vortex all liquid reagents.
6. Set the ELISA incubator at +37°C and prepare the ELISA washer by priming with the diluted washing solution, according to the manufacturers instructions. Set the right number of washing cycles as reported in the specific section.
7. Check that the ELISA reader is turned on or ensure it will be turned on at least 20 minutes before reading.
8. If using an automated work station, turn on, check settings and be sure to use the right assay protocol.
9. Check that the micropipettes are set to the required volume.
10. Check that all the other equipment is available and ready to use.

In case of problems, do not proceed further with the test and advise the supervisor.

M. ASSAY PROCEDURE

The assay has to be carried out according to what reported below, taking care to maintain the same incubation time for all the samples in testing.

Two procedures can be carried out with the device according to the request of the clinician.

M.1 Quantitative analysis

1. Place the required number of strips in the plastic holder and carefully identify the wells for standards and samples.

2. Dilute samples **1:101** dispensing 1 ml Sample Diluent into a disposable tube and then 10 ul sample; mix on vortex before use. Do not dilute the Calibrators and the dissolved Control Serum as they are ready-to-use.
3. Leave the A1+B1 wells empty for blanking purposes.
4. Pipette 100 µl of the Calibrators in duplicate, 100 µl dissolved Control Serum in duplicate followed by 100 µl of diluted samples. The Control Serum is used to verify that the whole analytical system works as expected. Check that Calibrators, Control Serum and samples have been correctly added.
5. Incubate the microplate **for 60 min at +37°C**.

Important note: *Strips have to be sealed with the adhesive sealing foil, only when the test is performed manually. Do not cover strips when using ELISA automatic instruments.*

6. When the first incubation is finished, wash the microwells as previously described (section I.3)
7. In all the wells except A1+B1, pipette 100 µl Enzyme Conjugate. Incubate the microplate **for 60 min at +37°C**.

Important note: *Be careful not to touch the inner surface of the well with the pipette tip and not to immerse the top of it into samples or controls. Contamination might occur.*

8. When the second incubation is finished, wash the microwells as previously described (section I.3)
9. Pipette 100 µl Chromogen/Substrate into all the wells, A1+B1 included.

Important note: *Do not expose to strong direct light. as a high background might be generated.*

10. Incubate the microplate protected from light at **room temperature (18-24°C) for 20 minutes**. Wells dispensed with positive samples, the control serum and the positive calibrators, as well, will turn from clear to blue.
11. Pipette 100 µl Sulphuric Acid into all the wells using the same pipetting sequence as in step 9 to block the enzymatic reaction.. Addition of the stop solution will turn the positive control and positive samples from blue to yellow.
12. Measure the colour intensity of the solution in each well, as described in section I.5 using a 450nm filter (reading) and a 620-630nm filter (background subtraction, mandatory), blanking the instrument on A1 or B1 or both.

M.2 Qualitative analysis

1 Place the required number of strips in the plastic holder and carefully identify the wells for standards and samples.
2 Dilute samples **1:101** dispensing 1 ml Sample Diluent into a disposable tube and then 10 ul sample; mix on vortex before use. Do not dilute the Calibrators as they are ready-to-use.
3 Leave the A1 well empty for blanking purposes.
4 Pipette 100 µl Calibrator 0 U/ml in duplicate, 100 µl Calibrator 10 U/ml in duplicate and 100 µl Calibrator 100 U/ml in single. Then dispense 100 µl diluted samples in proper sample wells. Check that Calibrators and samples have been correctly added.
5 Incubate the microplate **for 60 min at +37°C**.

Important note: *Strips have to be sealed with the adhesive sealing foil, only when the test is performed manually. Do not cover strips when using ELISA automatic instruments.*

6 When the first incubation is finished, wash the microwells as previously described (section I.3)
7 In all the wells except A1, pipette 100 µl Enzyme Conjugate. Incubate the microplate **for 60 min at +37°C**.

Important note: *Be careful not to touch the inner surface of the well with the pipette tip and not to immerse the top of it into samples or controls. Contamination might occur.*

8 When the second incubation is finished, wash the microwells as previously described (section I.3)
9 Pipette 100 µl Chromogen/Substrate into all the wells, A1 included.

Important note: *Do not expose to strong direct light. as a high background might be generated.*

10 Incubate the microplate protected from light at **room temperature (18-24°C) for 20 minutes**. Wells dispensed with positive samples, the control serum and the positive calibrators, as well, will turn from clear to blue.
11 Pipette 100 µl Sulphuric Acid into all the wells using the same pipetting sequence as in step 9 to block the enzymatic reaction. Addition of the stop solution will turn the positive control and positive samples from blue to yellow.
12 Measure the colour intensity of the solution in each well, as described in section I.5 using a 450nm filter (reading) and a 620-630nm filter (background subtraction, mandatory), blanking the instrument on A1 or B1 or both.

Important notes:

1. *Ensure that no finger prints are present on the bottom of the microwell before reading. Finger prints could generate false positive results on reading.*
2. *Reading has should ideally be performed immediately after the addition of the Stop Solution but definitely no longer than 20 minutes afterwards. Some self oxidation of the chromogen can occur leading to a higher background.*
3. *The Control Serum (CS) does not affect the cut-off calculation and therefore the test results calculation. The Control Serum may be used only when a laboratory internal quality control is required by the management*

N. ASSAY SCHEME

The assay protocol can be summarized in the table below:

Calibrators & diluted samples & dissolved Control Serum	100 ul
1st incubation	**60 min**
Temperature	+37°C
Washing steps	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
Enzyme Conjugate	100 ul
2nd incubation	**60 min**
Temperature	+37°C
Washing steps	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
Chromogen/Substrate	100ul
3rd incubation	**20 min**
Temperature	room
Sulphuric Acid	100 ul
Reading OD	450nm /620-630nm

An example of dispensation scheme in quantitative assays is reported below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	CAL4	S1									
B	BLK	CAL4	S2									
C	CAL1	CAL5	S3									
D	CAL1	CAL5	S4									
E	CAL2	CAL6	S5									
F	CAL2	CAL6	S6									
G	CAL3	CS	S7									
H	CAL3	CS	S8									

Legenda: BLK = Blank // CAL = Calibrators
CS = Control Serum // S = Sample

An example of dispensation scheme in qualitative assays is reported below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S 3	S 11									
B	CAL1	S 4	S 12									
C	CAL1	S 5	S 13									
D	CAL3	S 6	S 14									
E	CAL3	S 7	S 15									
F	CAL6	S 8	S 16									
G	S 1	S 9	S 17									
H	S 2	S 10	S 18									

Legenda: BLK = Blank // CAL = Calibrators// S = Sample

O. INTERNAL QUALITY CONTROL

A validation check is carried out on the controls any time the kit is used in order to verify whether the performances of the assay are as qualified.
Control that the following data are matched:

Parameter	Requirements
Blank well	< 0.100 OD450nm
Calibrator 0 PEI U/ml coefficient of variation	< 0.150 OD450nm after blanking < 30%
Calibrator 5 PEI U/ml	OD450nm > OD450nm Cal 0 U/ml + 5SD and anyway > OD450nm Cal 0 U/ml + 0.100
Calibrator 10 PEI U/ml	OD450nm > OD450nm Cal 0 U/ml + 0.200
Calibrator 100 PEI U/ml	> 1.000 OD450nm
Control Serum	OD450nm = OD450nm of the Calibrator 20 U/ml ± 10%

If the results of the test match the requirements stated above, proceed to the next section.

If they do not, do not proceed any further and perform the following checks:

Problem	Check
Blank well > 0.100 OD450nm	1. that the Chromogen/Substrate solution has not become contaminated during the assay
Calibrator 0 U/ml > 0.150 OD450nm after blanking coefficient of variation > 30%	1. that the washing procedure and the washer settings are as validated in the pre qualification study; 2. that the proper washing solution has been used and the washer has been primed with it before use; 3. that no mistake has been done in the assay procedure (dispensation of positive calibrators instead of Cal 0); 4. that no contamination of the Cal O, or of the wells where this was dispensed, has occurred due to positive samples, to spills or to the enzyme conjugate; 5. that micropipettes have not become contaminated with positive samples or with the enzyme conjugate 6. that the washer needles are not blocked or partially obstructed.
Calibrator 5 U/ml < CAL 0 + 5SD or < CAL 0 + 0.100	1. that the procedure has been correctly performed; 2. that no mistake has occurred during its distribution; 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the calibrator has occurred.
Calibrator 10 U/ml < CAL 0 + 0.200	1. that the procedure has been correctly performed; 2. that no mistake has occurred during its distribution; 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the calibrator has occurred.
Calibrator 100 U/ml < 1.000 OD450nm	1. that the procedure has been correctly performed; 2. that no mistake has occurred during the distribution of the calibrator; 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the calibrator has occurred.
Control Serum Different from expected value	First verify that: 1. the procedure has been correctly performed; 2. no mistake has occurred during its distribution (ex.: dispensation of a wrong sample); 3. the washing procedure and the washer settings are correct; 4. no external contamination of the standard has occurred. 5. the Control Serum has been dissolved with the right volume reported on the label. If a mistake has been pointed out, the assay has to be repeated after eliminating the reason of this error. If no mistake has been found, proceed as follows: **a**) a value up to +/-20% is obtained: the overall Precision of the laboratory might not enable the test to match the expected value +/-10%. Report the problem to the Supervisor for acceptance or refusal of this result. **b**) a value higher than +/-20% is obtained: in this case the test is invalid and the DiaPro's customer service has to be called.

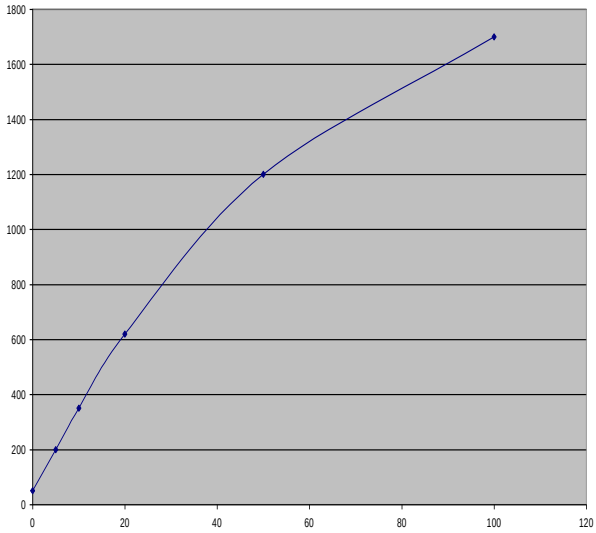
If any of the above problems have occurred, report the problem to the supervisor for further actions.

Important note:
The analysis must be done proceeding as the reading step described in the section M, point 12.

P. RESULTS

P.1 Quantitative method

If the test turns out to be valid, use for the quantitative method an approved curve fitting program to draw the calibration curve from the values obtained by reading at 450nm/620-630nm (4-parameters interpolation is suggested).
Then on the calibration curve calculate the concentration of anti HBc IgM antibody in samples.
An example of Calibration curve is reported below.



Important Note: *Do not use this example to make real calculations on samples.*

P.2 Qualitative method
In the qualitative method, calculate the mean OD450nm/620-630nm values for the Calibrators 0 and 10 U/ml and then check that the assay is valid.

Example of calculation (data obtained proceeding as the the reading step described in the section M, point 12).

The following data must not be used instead of real figures obtained by the user.

Calibrator 0 U/ml: 0.020 – 0.024 OD450nm
Mean Value: 0.022 OD450nm
Lower than 0.150 – Accepted
Calibrator 10 U/ml: 0.350 – 0.330 OD450nm
Mean Value: 0.340 OD450nm
Higher than Cal 0 + 0.200 – Accepted
Calibrator 100 U/ml: 2.845 OD450nm
Higher than 1.000 – Accepted

Q. INTERPRETATION OF RESULTS

Q.1 Qualitative results
For qualitative interpretations, the medical literature generally considers positive samples showing a concentration of HBc IgM ≥ 10 PEI U/ml.
Test results are therefore interpreted as a ratio of the sample OD450nm and the OD450nm/620-630nm of the Cal 10 PEI U/ml (or S/Co) according to the following table:

S/Co	Interpretation
< 0.9	Negative
0.9 - 1.1	Equivocal
> 1.1	Positive

Q.2 Quantitative results
The calibration curve is used to determine the concentration of IgM antibodies to HBcAg in samples.
Samples with a concentration lower than 5 PEI U/ml are considered negative for HBcIgM.
Samples with a concentration between 5 and 10 PEI U/ml are considered in a gray-zone.
In the follow up of chronic hepatitis, however, values higher of 5 PEI U/ml may be considered positive for HBcIgM, when in presence of other clinical signs.
Samples with a concentration higher than 10 PEI U/ml are considered positive for HBcIgM.

Important general notes:
1. *When the calculation of results is performed by the operating system of an ELISA automated work station, ensure that the proper formulation is used to produce the calibration curve, calculate sample concentration and generate the correct interpretation of results.*
2. *Interpretation of results should be done under the supervision of the laboratory supervisor to reduce the risk of judgement errors and misinterpretations.*
3. *A positive result is indicative of HBV infection and therefore the patient should be treated accordingly.*
4. *When test results are transmitted from the laboratory to another facility, attention must be paid to avoid erroneous data transfer.*
5. *Diagnosis of viral hepatitis infection has to be taken by and released to the patient by a suitably qualified medical doctor.*

R. PERFORMANCES
Evaluation of Performances has been conducted in accordance to what reported in the Common Technical Specifications or CTS (art. 5, Chapter 3 of IVD Directive 98/79/EC).

1. Limit of detection
The limit of detection of the assay has been calculated by means of :

1.1 the HBcIgM reference preparation supplied by Paul Erlich Institute, Germany (HBc-Referenzserum-IgM 84), on which the Standard Curve has been calibrated.
1.2 Accurun 113 (cat. N° A113-5001) supplied by Boston Biomedica Inc., USA

Results of Quality Control for three lots are given in the following tables:

BCM.CE	Lot #	0103	Lot #	0103/2	Lot #	0303
PEI U/ml	OD450nm	S/Co	OD450nm	S/Co	OD450nm	S/Co
100	2.752	**8.9**	2.883	**9,7**	2.911	**9.1**
50	1.917	**6.2**	1.972	**6.7**	2.053	**6.4**
20	0.980	**3.2**	0.914	**3.1**	1.095	**3.4**
10	0.544	**1.8**	0.513	**1.7**	0.592	**1.8**
5	0.310	**1.0**	0.296	**1.0**	0.321	**1.0**
2.5	0.155	0.5	0.149	0.5	0.161	0.5
1.25	0.084	0.3	0.084	0.3	0.093	0.3
negative	0.040		0.035		0.044	

BBI Accurun # 113 lot # 48-9999-0621

BCM.CE	Lot #	0103	Lot #	0103/2	Lot #	0303
BBI 113	OD450nm	S/Co	OD450nm	S/Co	OD450nm	S/Co
1 x	3.336	**10.8**	3.195	**10.4**	3.269	**10.3**
2 x	2.472	**8.0**	2.385	**7.8**	2.385	**7.5**
4 x	1.467	**4.7**	1.413	**4.6**	1.429	**4.5**
8 x	0.865	**2.8**	0.807	**2.6**	0.856	**2.7**
16 x	0.430	**1.4**	0.427	**1.4**	0.410	**1.3**
32 x	0.234	0.8	0.234	0.8	0.248	0.8
64 x	0.129	0.4	0.133	0.4	0.122	0.4
128 x	0.086	0.3	0.082	0.3	0.089	0.3
negative	0.040		0.040		0.052	

Moreover the BBI's panel # PHE 102 was also examined in three lots of product; data are reported below with reference to a European kit (BBI's results).

BBI – Panel code PHE 102

	Lot # 0103	Lot # 0103/2	Lot # 0303	Sorin EIA
Member	S/Co	S/Co	S/Co	S/Co
01	**6.7**	**6.3**	**6.5**	**2.0**
02	**11.3**	**10.0**	**10.7**	**6.1**
03	**9.5**	**7.2**	**8.4**	**3.0**
04	**5.8**	**3.4**	**4.1**	**2.1**
05	**11.3**	**11.4**	**11.2**	**3.1**
06	**12.1**	**11.6**	**11.8**	**4.1**
07	**0.1**	**0.1**	**0.1**	**0.2**
08	**9.2**	**8.5**	**8.8**	**2.3**
09	**12.2**	**11.7**	**11.9**	**4.2**
10	**11.7**	**10.2**	**10.8**	**2.8**
11	**5.9**	**5.8**	**5.8**	**2.1**
12	**12.7**	**11.4**	**11.7**	**5.2**
13	**11.6**	**11.0**	**11.3**	**3.6**
14	**7.0**	**6.3**	**6.6**	**2.3**
15	**12.4**	**11.5**	**11.8**	**4.5**

2. Diagnostic Sensitivity:

It is defined as the probability of the assay of scoring positive in the presence of the specific analyte.

The diagnostic sensitivity has been tested internally and externally in a qualified Clinical Laboratory on panels of samples classified positive by a US FDA approved kit.

Positive samples were collected from different patients and from different HBV pathologies (acute and chronic hepatitis).

An overall value > 98% has been found in the study conducted on a total number of more than 200 samples.

A Seroconversion panel produced by BBI, USA, code # PHM 935A, havs also been studied; results are reported below with reference to two commercial kits (BBI's results).

BBI Panel PHM 935A

	Lot # 0103	Abbott EIA	DiaSorin EIA
Member #	S/Co	S/Co	S/Co
01	0.2	0.1	0.1
02	0.2	0.1	0.1
03	0.2	0.1	0.1
04	0.1	0.1	0.1
05	0.2	0.1	0.1
06	0.2	0.1	0.1
07	0.2	0.1	0.1
08	0.1	0.1	0.1
09	0.1	0.1	0.1
10	0.1	0.1	0.1
11	0.2	0.1	0.1
12	0.2	0.1	0.1
13	**2.8**	**3.7**	0.7
14	**5.0**	**6.4**	0.9
15	**> 12**	**6.2**	**4.5**
16	**> 12**	**5.6**	**4.5**
17	**> 12**	**5.5**	**4.3**
18	**> 12**	**4.8**	**4.3**
19	**> 12**	**> 6.6**	**4.4**
20	**> 12**	**> 6.6**	**5.2**

3. Diagnostic Specificity:

It is defined as the probability of the assay of scoring negative in the absence of the specific analyte.

The diagnostic specificity has been determined internally and externally in a qualified Clinical Laboratory on panels of negative samples from normal individuals and blood donors, classified negative with a US FDA approved kit.

A total number of more than 400 negative specimens were tested. A diagnostic specificity > 98% has been found.

Moreover, the diagnostic specificity was assessed by testing more than 50 potentially interfering specimens (other infectious diseases, patients affected by non viral hepatic diseases, dialysis patients, pregnant women, hemolized, lipemic, etc.).

No interference was observed in the study.

Both plasma, derived with different standard techniques of preparation (citrate, EDTA and heparin), and sera have been used to determine the specificity. No false reactivity due to the method of specimen preparation has been observed.

Frozen specimens have also been tested to check whether this interferes with the performance of the test. No interference was observed on clean and particle free samples.

4. Precision:

It has been calculated on three samples examined in 16 replicate in three different runs, carried out on three different lots. The values found were as follows:

BCM.CE: lot # 0103

Cal 0 U/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.055	0.053	0.051	0.053
Std.Deviation	0.005	0.006	0.005	0.006
CV %	9.9	12.3	10.7	10.9

Cal 5 U/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.324	0.308	0.321	0.318
Std.Deviation	0.022	0.018	0.024	0.021
CV %	6.8	5.7	7.5	6.7

Cal 50 U/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	2.109	2.048	2.052	2.070
Std.Deviation	0.101	0.088	0.136	0.109
CV %	4.8	4.3	6.7	5.2

BCM.CE: lot # 0103/2

Cal 0 U/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.057	0.053	0.054	0.055
Std.Deviation	0.005	0.005	0.004	0.004
CV %	8.3	9.0	7.3	8.2

Cal 5 U/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.332	0.331	0.322	0.328
Std.Deviation	0.017	0.018	0.016	0.017
CV %	5.0	5.5	4.9	5.1

Cal 50 U/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	2.311	2.208	2.212	2.244
Std.Deviation	0.110	0.090	0.095	0.098
CV %	4.7	4.1	4.3	4.4

BCM.CE: lot # 0303

Cal 0 U/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.043	0.042	0.040	0.042
Std.Deviation	0.004	0.005	0.004	0.004
CV %	10.3	11.1	10.9	10.8

Cal 5 U/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.320	0.326	0.314	0.320
Std.Deviation	0.023	0.024	0.026	0.024
CV %	7.1	7.4	8.2	7.6

Cal 50 U/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	2.150	2.163	2.092	2.135
Std.Deviation	0.057	0.067	0.076	0.067
CV %	2.6	3.1	3.6	3.1

Important note:
The performance data have been obtained proceeding as the reading step described in the section M, point 12.

S. LIMITATIONS

Frozen samples containing fibrin particles or aggregates may generate false positive results.
Bacterial contamination or heat inactivation of the specimen may affect the absorbance values of the samples with consequent alteration of the level of the analyte.
This test is suitable only for testing single samples and not pooled ones.
Diagnosis of an infectious disease should not be established on the basis of a single test result. The patient's clinical history, symptomatology, as well as other diagnostic data should be considered.

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All the IVD Products manufactured by the company are under the control of a certified Quality Management System approved by an EC Notified Body. Each lot is submitted to a quality control and released into the market only if conforming with the EC technical specifications and acceptance criteria.

Manufacturer:
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CE
0318

HBc IgM

Ensayo inmunoenzimático de "Captura" (ELISA) para la determinación cualitativa/cuantitativa de anticuerpos clase IgM al Antígeno *core* del virus de la Hepatitis B en plasma y suero humanos

- Uso exclusivo para diagnóstico "in vitro"-

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REF BCM.CE
96 pruebas

HBc IgM

A. OBJETIVO DEL EQUIPO.
Ensayo inmunoenzimático (ELISA) para la determinación cualitativa/cuantitativa de anticuerpos clase IgM al Antígeno *core* del virus de la Hepatitis B (HBV) en plasma y suero humanos mediante el sistema de "Captura".
El equipo ha sido diseñado para la clasificación del agente viral y para el seguimiento de pacientes crónicos sometidos a terapia.
Uso exclusivo para diagnóstico "in vitro".

B. INTRODUCCIÓN.
El antígeno *core* del virus de la Hepatitis B (HBcAg) es el elemento principal de las partículas del núcleo del virus.
Las partículas tienen un tamaño de 27nm y contienen una mólecula de ADN circular de doble cadena, una ADN polimerasa específica y HBcAg. El antígeno del *core* está compuesto por un polipéptido simple de 17 kD, el cual es liberado en el proceso de desagregación de la partícula viral. Este antígeno contiene al menos un determinante inmunogénico. Durante la infección primaria, los anticuerpos IgM anti-HBcAg, son unos de los primeros marcadores del HBV que aparecen en el suero, conjuntamente o ligeramente antes de que aparezca el antígeno de superficie (HBsAg).
Los títulos de anticuerpos IgM al HBcAg, bastante altos durante la fase aguda, descienden en el transcurso de la enfermedad hasta alcanzar niveles no detectables en pacientes convalescientes. Sin embargo, en el caso de la hepatitis crónica, se aprecian picos de anticuerpos IgM anti-HBcAg, lo cual confirma la reactivación del virus en los hepatocitos y origina bajos títulos permanentes de IgM.
La determinación de anticuerpos IgM anti-HBcAg es de gran importancia para la rápida clasificación del virus, de las fases de la enfermedad, así como para el seguimiento de pacientes sometidos a tratamiento con interferón.

C. PRINCIPIOS DEL ENSAYO.
El ensayo se basa en el principio de "captura de IgM" donde esta clase de anticuerpos, si están presentes en la muestra, quedan capturados por la fase sólida, recubierta por anticuerpos anti-IgM humanos.
Después del lavado, mediante el cual se eliminan los restantes componentes de la muestra fundamentalmente los anticuerpos IgG, se detectan los anticuerpos IgM unidos a la fase sólida mediante la adición de una preparación de HBcAg recombinante purificada, marcada con un anticuerpo monoclonal conjugado con peroxidasa (HRP).
Después de la incubación y previo lavado, se añade la mezcla cromógeno/substrato, la cual se combina con la enzima conjugada unida a la fase sólida. El substrato es hidrolizado, en presencia de peroxidasa, a un producto coloreado final cuya densidad óptica es detectable y es proporcional a la cantidad de anticuerpos IgM al HBcAg presentes en la muestra.

D. COMPONENTES
Cada equipo contiene reactivos suficientes para realizar 96 pruebas.

1. Microplaca: MICROPLATE
8x12 tiras de pocillos recubiertos con un anticuerpo monoclonal de ratón anti-IgM humano, post-recubiertos con proteínas del suero bovino y almacenados en bolsas selladas con desecante. Se deben poner las placas a temperatura ambiente antes de abrirlas, sellar las tiras sobrantes en la bolsa con el desecante y almacenar a 4ºC.

2. Curva de Calibración: CAL N° …
6x2.0 ml/vial. Listo para el uso y curva estándar con código de color, calibrada a partir de una preparación de HBcIgM de referencia, suministrada por el Instituto Paul Erlich (HBc-Referenzserum-IgM 84), con rangos: CAL1 = 0 U/ml // CAL2 = 5 U/ml // CAL3 = 10 U/ml // CAL4 = 20 U/ml // CAL 5 = 50 U/ml // CAL 6 = 100 U/ml. Contiene plasma humano HBcIgM positivo sometido a inactivación química, tampón Tris 100 mM pH 7.4+/- 0.1, 0.5% de Tween 20, así como azida sódica 0.09% y ProClin 300 0.045% como conservantes. La Curva de Calibración está codificada con el color azul.
Nota importante: *Aunque el plasma esté inactivado por métodos químicos, se debe manipular como potencialmente infeccioso.*

3. Tampón de Lavado Concentrado: WASHBUF 20X
1x60ml/botella. Solución concentrada 20x.
Una vez diluida, la solución de lavado contiene tampón fosfato 10mM a pH 7.0+/- 0.1, Tween 20 al 0.05% y ProClin 300 al 0.045%.

4. Conjugado (Inmunocomplejo) : CONJ
1x16.0 ml/vial. Solución lista para el uso. Contiene un Inmunocomplejo formado por un anticuerpo monoclonal de ratón marcado con HRP y HBcAg recombinante purificado. El reactivo está disuelto en tampón Tris 10 mM pH 6.8+/-0.1, BSA 2%, además de sulfato de gentamicina 0.2 % y ProClin 300 0.045% como conservantes. El reactivo está codificado con el color rojo.

5. Diluente de muestras :DILSPE
2x60.0 ml/vial. Solución tamponada para disolver las muestras. Contiene tampónTris 100 mM pH 7.4 +/- 0.1, 0.5% de Tween 20, caseína al 2%, 0.045% de ProClin 300 y azida sódica al 0.09% como conservantes. El reactivo está codificado con el color azul.

6. Suero Control: CONTROL …ml
1 vial. Liofilizado.
Contiene suero bovino fetal, plasma humano positivo a HBcIgM, concentrado a 20 ±10% PEI U/ml, 0.2 mg/ml de sulfato de gentamicina y ProClin 300 0.045% como conservantes.
Notas importantes:
1. El volumen necesario para disolver el contenido del vial varía en cada lote. Se recomienda usar el volumen correcto reportado en la etiqueta.
2. Aunque el plasma esté inactivado por métodos químicos, se debe manipular como potencialmente infeccioso.

7. Cromógeno/Substrato. SUBS TMB
1x16ml/vial. Contiene una solución tamponada citrato-fosfato 50 mM pH 3.5-3.8, dimetilsulfóxido 4%, tetra-metil-benzidina (TMB) 0.03% y peróxido de hidrógeno (H_2O_2) 0.02%.
Nota: Evitar la exposición a la luz, ya que la substancia es fotosensible.

8. Ácido Sulfúrico: H_2SO_4 O.3 M
1x15ml/vial. Contiene solución de H_2SO_4 0.3M
Atención: Irritante (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

9. Sellador adhesivo, n° 2

10. Manual de instrucciones, n° 1

E. MATERIALES NECESARIOS NO SUMINISTRADOS.

1. Micropipetas calibradas (150µl, 100µl y 50µl) y puntas plásticas desechables.
2. Agua de calidad EIA (bidestilada o desionizada, tratada con carbón para remover químicos oxidantes usados como desinfectantes).
3. *Timer* con un rango de 60 minutos como mínimo.
4. Papel absorbente.
5. Incubador termostático de microplacas ELISA, calibrado (en seco o húmedo) fijo a 37ºC (tolerancia+/-1°C).
6. Lector calibrado de microplacas de ELISA con filtros de 450nm (lectura) y de 620-630 nm.
7. Lavador calibrado de microplacas ELISA.
8. Vórtex o similar.

F. ADVERTENCIAS Y PRECAUCIONES.

1. El equipo debe ser usado por personal técnico adecuadamente entrenado, bajo la supervisión de un doctor responsable del laboratorio.
2. Todas las personas encargadas de la realización de las pruebas deben llevar las ropas protectoras adecuadas de laboratorio, guantes y gafas. Evitar el uso de objetos cortantes (cuchillas) o punzantes (agujas). El personal debe ser adiestrado en procedimientos de bioseguridad, según ha sido recomendado por el Centro de Control de Enfermedades de Atlanta, Estados Unidos, y publicado por el Instituto Nacional de Salud: "Biosafety in Microbiological and Biomedical Laboratories", ed.1984.
3. Todo el personal involucrado en el manejo de muestras debe estar vacunado contra HBV y HAV, para lo cual existen vacunas disponibles, seguras y eficaces.
4. Se debe controlar el ambiente del laboratorio para evitar la contaminación de los componentes con polvo o agentes microbianos cuando se abran los equipos, así como durante la realización del ensayo. Evitar la exposición del substrato a la luz y las vibraciones de la mesa de trabajo durante el ensayo.
5. Conservar el equipo a temperaturas entre 2-8 ºC, en un refrigerador con temperatura regulada o en cámara fría.
6. No intercambiar reactivos de diferentes lotes ni tampoco de diferentes equipos.
7. Comprobar que los reactivos no contienen precipitados ni agregados en el momento del uso. De darse el caso, informar al responsable para realizar el procedimiento pertinente.
8. Evitar contaminación cruzada entre muestras de suero/ plasma usando puntas desechables y cambiándolas después de cada uso. No reutilizar puntas desechables.
9. Evitar contaminación cruzada entre los reactivos del equipo usando puntas desechables y cambiándolas después de cada uso. No reutilizar puntas desechables.
10. No usar el producto después de la fecha de caducidad indicada en el equipo e internamente en los reactivos.
11. Tratar todas las muestras como potencialmente infecciosas. Las muestras de suero humano deben ser manipuladas al nivel 2 de bioseguridad, según ha sido recomendado por el Centro de Control de Enfermedades de Atlanta, Estados Unidos y publicado por el Instituto Nacional de Salud: "Biosafety in Microbiological and Biomedical Laboratories", ed.1984.
12. Se recomienda el uso de material plástico desechable para la preparación de las soluciones de lavado y para la transferencia de los reactivos a los diferentes equipos automatizados a fin de evitar contaminaciones.
13. Los desechos producidos durante el uso del equipo deben de ser eliminados según lo establecido por las directivas nacionales y las leyes relacionadas con el tratamiento de los residuos químicos y biológicos de laboratorio. En particular, los desechos líquidos provenientes del proceso de lavado deben ser tratados como potencialmente infecciosos y deben ser inactivados. Se recomienda la inactivación con lejía al 10% de 16 a 18 horas o el uso de la autoclave a 121°C por 20 minutos.
14. En caso de derrame accidental de algún producto, se debe utilizar papel absorbente embebido en lejía y posteriormente en agua. El papel debe eliminarse en contenedores designados para este fin en hospitales y laboratorios.
15. El ácido sulfúrico es irritante. En caso de derrame, se debe lavar la superficie con abundante agua.
16. Otros materiales de desecho generados durante la utilización del equipo (por ejemplo: puntas usadas en la manipulación de las muestras y controles, microplacas usadas) deben ser manipuladas como fuentes potenciales de infección de acuerdo a las directivas nacionales y leyes para el tratamiento de residuos de laboratorio.

G. MUESTRA: PREPARACIÓN Y RECOMENDACIONES.

1. Extraer la sangre asépticamente por punción venosa y preparar el suero o plasma según la técnica estándar de los laboratorios de análisis clínico. No se ha detectado que el tratamiento con citrato, EDTA o heparina afecte las muestras.
2. Evitar la adición de conservantes, especialmente azida sódica ya que puede afectar la actividad enzimática del conjugado, generando resultados falsos negtivos.
3. Las muestras deben estar identificadas claramente mediante código de barras o nombres, a fin de evitar errores en los resultados.
4. Las muestras hemolizadas (color rojo) o hiperlipémicas (aspecto lechoso) deben ser descartadas para evitar falsos resultados, al igual que aquellas donde se observe la presencia de precipitados, restos de fibrina o filamentos microbianos.
5. El suero y el plasma pueden conservarse a una temperatura entre +2° y +8°C en tubos de recolección principales hasta cinco días después de la extracción. No congelar tubos de recolección principales. Para periodos de almacenamiento más prolongados, las muestras de plasma o suero, retiradas cuidadosamente del tubo de extracción principal, pueden almacenarse congeladas a –20°C durante almenos 12 meses. Evitar congelar/descongelar cada muestra más de una vez, ya que pueden generarse partículas que podrían afectar al resultado de la prueba.
6. Si hay presencia de agregados, la muestra se puede aclarar mediante centrifugación a 2000 rpm durante 20 minutos o por filtración con un filtro de 0,2-0,8 micras.

H. PREPARACIÓN DE LOS COMPONENTES Y PRECAUCIONES.

Según estudios realizados, no se ha detectado pérdida relevante de actividad en equipos utilizados hasta 6 veces, durante un período de hasta 3 meses.

Microplacas:

Dejar la microplaca a temperatura ambiente (aprox. 1 hora) antes de abrir el envase. Compruebe que el desecante no esté de un color verde oscuro, lo que indicaría un defecto de fabricación. De ser así, debe solicitar el servicio de Dia.Pro: Atención al cliente.

Las tiras de pocillos no utilizadas, deben guardarse herméticamente cerradas en la bolsa de aluminio con el desecante a 2-8ºC. Una vez abierto el envase, las tiras sobrantes, se mantienen estables hasta que el indicador de humedad dentro de la bolsa del desecante cambie de amarillo a verde.

Curva de Calibración:

Listo para el uso. Mezclar bien con la ayuda de un vórtex, antes de usar.

Solución de Lavado Concentrada:
Todo el contenido de la solución concentrada 20x debe diluirse con agua bidestilada hasta alcanzar 1200ml y mezclarse suavemente antes de usarse. Durante la preparación evitar la formación de espuma y burbujas, lo que podría influir en la eficiencia de los ciclos de lavado.
Nota: Una vez diluida, la solución es estable por una semana a temperaturas entre +2 y 8ºC.

Conjugado:
Listo para el uso. Mezclar bien con un vórtex antes de usar. Evitar posible contaminación del líquido con oxidantes químicos, polvo o microbios. En caso de que deba transferirse el reactivo, usar contenedores de plástico, estériles y desechables, siempre que sea posible.

Diluente de muestras :
Solución lista para el uso. Mezclar bien con un vórtex antes de usar.

Suero Control:
Añadir al polvo liofilizado el volumen de agua de calidad ELISA indicado en la etiqueta. Dejar disolver totalmente y mezclar suavemente en el vórtex. El suero disuelto está listo para el uso.
Nota: Una vez reconstituida, la solución no es estable. Se recomienda mantenerla congelada en alícuotas a –20ºC.

Cromógeno/ Substrato:
Listo para el uso. Mezclar bien con un vórtex antes de usar.
Evitar posible contaminación del líquido con oxidantes químicos, polvo o microbios. Evitar la exposición a la luz, agentes oxidantes y superficies metálicas. En caso de que deba transferirse el reactivo, usar contenedores de plástico, estériles y desechables, siempre que sea posible.

Ácido Sulfúrico:
Listo para el uso. Mezclar bien con un vórtex antes de usar.
Atención: Irritante (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

Leyenda:

Indicación de peligro, **Frases H**
H315 – Provoca irritación cutánea.
H319 – Provoca irritación ocular grave.

Consejo de prudencia, **Frases P**
P280 – Llevar guantes/prendas/gafas/máscara de protección.
P302 + P352 – EN CASO DE CONTACTO CON LA PIEL: Lavar con agua y jabón abundantes.
P332 + P313 – En caso de irritación cutánea: Consultar a un médico.
P305 + P351 + P338 – EN CASO DE CONTACTO CON LOS OJOS: Aclarar cuidadosamente con agua durante varios minutos. Quitar las lentes de contacto, si lleva y resulta fácil. Seguir aclarando.
P337 + P313 – Si persiste la irritación ocular: Consultar a un médico.
P362 + P363 – Quitarse las prendas contaminadas y lavarlas antes de volver a usarlas.

I. INSTRUMENTOS Y EQUIPAMIENTO UTILIZADOS EN COMBINACIÓN CON EL EQUIPO.

1. Las micropipetas deben ser calibradas para dispensar correctamente el volumen requerido en el ensayo y sometidas a una descontaminación periódica de las partes que pudieran entrar accidentalmente en contacto con la muestra (etanol 70%, lejía 10%, de calidad de los desinfectantes hospitalarios). Deben además, ser regularmente revisadas para mantener una precisión del 1% y una confiabilidad de +/- 2%.
2. La incubadora de ELISA debe ser ajustada a 37ºC (+/- 0.5ºC) y controlada periódicamente para mantener la temperatura correcta. Pueden emplearse incubadoras secas o baños de agua siempre que estén validados para la incubación de pruebas de ELISA.
3. El **lavador ELISA** es extremadamente importante para el rendimiento global del ensayo. El lavador debe ser validado de forma minuciosa previamente, revisado para comprobar que suministra el volumen de dispensación correcto y enviado regularmente a mantenimiento de acuerdo con las instrucciones de uso del fabricante. En particular, deben lavarse minuciosamente las sales con agua desionizada del lavador al final de la carga de trabajo diaria. Antes del uso, debe suministrarse extensivamente solución de lavado diluida al lavador. Debe enviarse el instrumento semanalmente a descontaminación según se indica en su manual (se recomienda descontaminación con NaOH 0.1 M). Para asegurar que el ensayo se realiza conforme a los rendimientos declarados, basta con 5 ciclos de lavado (aspiración + dispensado de 350 µl/pocillo de solución de lavado + 20 segundos de remojo = 1 ciclo). Si no es posible remojar, añadir un ciclo de lavado adicional. Un ciclo de lavado incorrecto o agujas obstruidas con sal son las principales causas de falsas reacciones positivas.
4. Los tiempos de incubación deben tener un margen de $\pm$5%.
5. El lector de microplacas ELISA debe estar provisto de un filtro de lectura de 450nm y de un segundo filtro de 620-630nm, obligatorio para reducir interferencias en la lectura. El procedimiento estándar debe contemplar: a) Ancho de banda <= 10 b) Rango de absorbancia de 0 a >=2.0, c) Linealidad >=2.0, reproducibilidad >=1%. El blanco se prueba en el pocillo indicado en la sección “Procedimiento del ensayo”. El sistema óptico del lector debe ser calibrado periódicamente para garantizar la correcta medición de la densidad óptica, según las normas del fabricante.
6. En caso de usar un sistema automatizado de ELISA, los pasos críticos (dispensado, incubación, lavado, lectura, agitación y procesamiento de datos) deben ser cuidadosamente fijados, calibrados, controlados y periódicamente ajustados, para garantizar los valores indicados en las secciones “Control interno de calidad” y “Procedimiento del ensayo”. El protocolo del ensayo debe ser instalado en el sistema operativo de la unidad y validado tanto para el lavador como para el lector. Por otro lado, la parte del sistema que maneja los líquidos (dispensado y lavado) debe ser validada y fijada correctamente. Debe prestarse particular atención a evitar el arrastre por las agujas de dispensación y las de lavado, a fin de minimizar la posibilidad de ocurrencia de falsos positivos por contaminación de los pocillos adyacentes por muestras fuertemente reactivas. Se recomienda el uso de sistemas automatizados para el pesquisaje en unidades de sangre y cuando la cantidad de muestras supera las 20-30 unidades por ensayo.
7. El servicio de atención al cliente en Dia.Pro, ofrece apoyo al usuario para calibrar, ajustar e instalar los equipos e instrumentos a usar en combinación con el equipo, con el propósito de asegurar el cumplimiento de los requerimientos descritos.

L. OPERACIONES Y CONTROLES PREVIOS AL ENSAYO.

1. Compruebe la fecha de caducidad indicada en la parte externa del equipo (envase primario). No usar si ha caducado.
2. Compruebe que los componentes líquidos no están contaminados con partículas o agregados visibles. Asegúrese de que el cromógeno (TMB) es incoloro o azul pálido, aspirando un pequeño volumen de este con una pipeta estéril de plástico. Compruebe que no han ocurrido rupturas ni derrames de líquido dentro de la caja (envase

primario) durante el transporte. Asegurarse de que la bolsa de aluminio que contiene la microplaca no esté rota o dañada.
3. Diluir totalmente la Solución de Lavado Concentrada 20X, como se ha descrito anteriormente y mezclar suavemente.
4. Disolver el Suero Control como se ha descrito anteriormente.
5. Dejar los componentes restantes alcanzar la temperatura ambiente (aprox. 1 hora), mezclar después suavemente en el vórtex todos los reactivos líquidos.
6. Ajustar la incubadora de ELISA a 37ºC y cebar el lavador de ELISA utilizando la solución de lavado, según las instrucciones del fabricante. Fijar el número de ciclos de lavado según se indica en la sección específica.
7. Comprobar que el lector de ELISA esté encendido al menos 20 minutos antes de realizar la lectura.
8. En caso de trabajar automáticamente, encender el equipo y comprobar que los protocolos estén correctamente programados.
9. Comprobar que las micropipetas estén fijadas en el volumen requerido.
10. Asegurarse de que el equipamiento a usar esté en perfecto estado, disponible y listo para el uso.

En caso de surgir algún problema, se debe detener el ensayo y avisar al responsable.

M. PROCEDIMIENTO DEL ENSAYO.

El ensayo debe realizarse según las instrucciones que siguen a continuación, es importante mantener en todas las muestras el mismo tiempo de incubación.
Pueden realizarse dos procedimientos acorde a los requerimientos del clínico.

M.1 Análisis Cuantitativo

1. Poner el número necesario de tiras en el soporte plástico e identificar los pocillos de las muestras y de los estándares.
2. Diluir las muestras **1:101** dispensando en un tubo desechable 1 ml de Diluente de Muestras y 10 µl de muestra, mezclar con ayuda de un vórtex, antes de usar. No diluir los Calibradores y el Suero Control disuelto ya que están listos para el uso.
3. Dejar los pocillos A1 y B1 vacíos para el blanco.
4. Dispensar 100µl de los Calibradores por duplicado, 100µl del Suero Control disuelto por duplicado y después 100µl de las muestras diluídas. El Suero Control se emplea para verificar que el sistema analítico funcione como es debido. Comprobar que el Suero Control, los Calibradores y las muestras han sido añadidos adecuadamente.
5. Incubar la microplaca durante **60 minutos a +37ºC.**

Nota importante*: Las tiras se deben sellar con el adhesivo suministrado solo cuando se hace la prueba manualmente. No sellar cuando se emplean equipos automatizados de ELISA.*

6. Después de la primera incubación, lavar los pocillos según lo descrito previamente (sección I.3).
7. Dispensar 100µl de Conjugado en todos los pocillos, excepto A1 y B1, controlar que los reactivos han sido correctamente añadidos. Incubar la microplaca durante **60 minutos a +37°C.**

Nota importante*: Tener cuidado de no tocar la pared interna del pocillo con la punta de la pipeta y no sumergir la parte superior de la misma en los controles o muestras. Podría producirse contaminación.*

8. Después de la segunda incubación, lavar los pocillos según lo descrito previamente (sección I.3).
9. Dispensar 100µl de Cromógeno/Substrato en todos los pocillos, incluidos los del blanco.

Nota importante*: No exponer directamente a fuerte iluminación, de lo contrario se generan interferencias.*

10. Incubar la microplaca, protegida de la luz, durante **20 minutos a temperatura ambiente (18-24°C).** Los pocillos correspondientes a las muestras positivas, el Suero Control y los Calibradores positivos deben cambiar de color claro a azul.
11. Dispensar 100µl de ácido sulfúrico en todos los pocillos para detener la reacción enzimática, usar la misma secuencia que en el paso 9. La adición de la Solución de parada cambia el color del control positivo y las muestras positivas de azul a amarillo.
12. Medir la intensidad del color con el lector, según se describe en la sección I.5, utilizando un filtro de 450 nm (lectura) y otro de 620-630 nm (substracción del fondo, obligatorio), calibrando el instrumento con los pocillos A1 y B1 (blanco).

M.2 Análisis Cualitativo

1. Poner el número necesario de tiras en el soporte plástico e identificar los pocillos de las muestras y de los estándares.
2. Diluir las muestras **1:101** dispensando en un tubo desechable 1 ml de Diluente de Muestras y 10 µl de muestra, mezclar con ayuda de un vórtex, antes de usar. No diluir los Calibradores disuelto ya que están listos para el uso.
3. Dejar el pocillo A1 vacío para el blanco.
4. Dispensar 100 µl del Calibrador 0 U/ml por duplicado, 100 µl del Calibrador 10 U/ml por duplicado, 100 µl del Calibrador 100 U/ml simple. Dispensar después 100 µl de las muestras diluídas en los pocillos correspondientesComprobar que el Suero Control, los Calibradores y las muestras han sido añadidos adecuadamente.
5. Incubar la microplaca durante **60 minutos a +37ºC.**

Nota importante*: Las tiras se deben sellar con el adhesivo suministrado solo cuando se hace la prueba manualmente. No sellar cuando se emplean equipos automatizados de ELISA.*

6. Después de la primera incubación, lavar los pocillos según lo descrito previamente (sección I.3).
7. Dispensar 100µl de Conjugado en todos los pocillos, excepto A1. Incubar la microplaca durante **60 minutos a +37°C.**

Nota importante*: Tener cuidado de no tocar la pared interna del pocillo con la punta de la pipeta y no sumergir la parte superior de la misma en los controles o muestras. Podría producirse contaminación.*

8. Después de la segunda incubación, lavar los pocillos según lo descrito previamente (sección I.3).
9. Dispensar 100µl de Cromógeno/Substrato en todos los pocillos, incluido el A1.

Nota importante*: No exponer directamente a fuerte iluminación, de lo contrario pudieran generarse interferencias.*

10. Incubar la microplaca, protegida de la luz, durante **20 minutos a temperatura ambiente (18-24°C).** Los pocillos correspondientes a las muestras positivas, el Suero Control y los Calibradores positivos deben cambiar de color claro a azul.
11. Dispensar 100µl de ácido sulfúrico en todos los pocillos para detener la reacción enzimática, usar la misma secuencia que en el paso 9. La adición de la Solución de parada cambia el color del control positivo y las muestras positivas de azul a amarillo.
12. Medir la intensidad del color con el lector, según se describe en la sección I.5, utilizando un filtro de 450 nm

(lectura) y otro de 620-630 nm (substracción del fondo, obligatorio), calibrando el instrumento con el pocillo A1.

Notas generales importantes:

1. *Asegurarse de que no hay impresiones digitales en el fondo de los pocillos antes de leer. Podrían generarse falsos positivos en la lectura.*

2. *La lectura debe hacerse inmediatamente después de añadir la solución de parada y, en cualquier caso, nunca transcurridos 20 minutos después de su adición. Se podría producir auto oxidación del cromógeno causando un elevado fondo.*

3. *El suero de control (CS) no afecta al cálculo del valor de corte y, por lo tanto, no afecta al cálculo de los resultados de la prueba. El suero de control (CS) se usa solo si la gestión requiere un control interno de calidad del laboratorio.*

N. ESQUEMA DEL ENSAYO

El protocolo del ensayo se resume en la siguiente tabla:

Calibradores & Muestras diluídas & Suero Control Disuelto	100 µl
1ra incubación	**60 min**
Temperatura	+37°C
Lavados	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
Conjugado	100 µl
2da incubación	**60 min**
Temperatura	+37°C
Lavados	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
Cromógeno/Substrato	100µl
3ra incubación	**20 min**
Temperatura	t.a.*
Acido Sµlfúrico	100 µl
Lectura D.O.	450nm / 620-630nm

*t.a.*temperatura ambiente*

A continuación se describe un ejemplo del esquema de dispensado en el análisis cuantitativo:

Microplaca

	1	2	3	4	5	6	7	8	9	10	11	12
A	BL	CAL4	M1									
B	BL	CAL4	M2									
C	CAL1	CAL5	M 3									
D	CAL1	CAL5	M 4									
E	CAL2	CAL6	M 5									
F	CAL2	CAL6	M 6									
G	CAL3	SC	M 7									
H	CAL3	SC	M 8									

Leyenda: BL = Blanco // CAL = Calibradores // SC= Suero Control // M = Muestra

A continuación se describe un ejemplo del esquema de dispensado en el análisis cualitativo:

Microplaca

	1	2	3	4	5	6	7	8	9	10	11	12
A	BL	M 3	M 11									
B	CAL1	M 4	M 12									
C	CAL1	M 5	M 13									
D	CAL3	M 6	M 14									
E	CAL3	M 7	M 15									
F	CAL6	M 8	M 16									
G	M1	M 9	M 17									
H	M2	M 10	M 18									

Leyenda: BL = Blanco // CAL = Calibradores // M = Muestra

O. CONTROL DE CALIDAD INTERNO.

Se realiza un grupo de pruebas con los controles cada vez que se usa el equipo para verificar si el procedimiento durante el ensayo se ha realizado correctamente.

Asegurar el cumplimiento de los siguientes parámetros:

Parámetro	Exigencia
Pocillo Blanco	< 0.100 DO450nm
Calibrador 0 PEI U/ml Coeficiente de variación	< 0.150 DO450nm después de leer el blanco < 30%
Calibrador 5 PEI U/ml	DO450nm > DO450nm Cal 0 U/ml + 5DS y > DO450nm Cal 0 U/ml + 0.100
Calibrador 10 PEI U/ml	DO450nm > DO450nm Cal 0 U/ml + 0.200
Calibrador 100 PEI U/ml	> 1.000 DO450nm
Suero Control	DO450nm = DO450nm Calibrador 20 U/ml +/-10%

Si los resultados del ensayo coinciden con lo establecido anteriormente, pase a la siguiente sección.

En caso contrario, detenga el ensayo y compruebe:

Problema	Compruebe que
Pocillo blanco > 0.100DO450nm	la solución cromógeno/substrato no se ha contaminado durante el ensayo.
Calibrador 0 U/ml > 0.150 DO 450nm después de leer el blanco Coeficiente de variación > 30%	1. el proceso de lavado y los parámetros del lavador estén validados según los estudios previos de calificación. 2. se ha usado la solución de lavado apropiada y que el lavador ha sido cebado con la misma antes del uso. 3. no se han cometido errores en el procedimiento del ensayo (dispensado de un Calibrador positivo en lugar del Cal 0) 4. no ha existido contaminación del Cal 0 o de sus pocillos debido a muestras positivas derramadas, o al conjugado. 5. las micropipetas no se han contaminado con muestras positivas o con el conjugado. 6. las agujas del lavador no estén parcial o totalmente obstruidas.

Calibrador 5 U/ml < CAL 0 + 5 DS or < CAL 0 + 0.100	1. el procedimiento ha sido realizado correctamente. 2. no ha habido errores durante su distribución (dispensar el calibrador equivocado). 3. el proceso de lavado y los parámetros del lavador estén validados según los estudios previos de calificación. 4. no ha ocurrido contaminación externa del calibrador.
Calibrador 10 U/ml < CAL 0 + 0.200	1. el procedimiento ha sido realizado correctamente. 2. no ha habido errores durante su distribución (dispensar el calibrador equivocado). 3. el proceso de lavado y los parámetros del lavador estén validados según los estudios previos de calificación. 4. no ha ocurrido contaminación externa del calibrador.
Calibrador 100 U/ml < 1.000 DO 450nm	1. el procedimiento ha sido realizado correctamente. 2. no ha habido errores durante su distribución. 3. el proceso de lavado y los parámetros del lavador estén validados según los estudios previos de calificación. 4. no ha ocurrido contaminación externa del calibrador.

Suero Control Valor distinto al esperado	1. el procedimiento ha sido realizado correctamente. 2. no ha habido errores durante su distribución (dispensar una muestra equivocada). 3. el proceso de lavado y los parámetros del lavador son correctos. 4. no ha ocurrido contaminación externa de los controles. 5. el Suero Control ha sido disuelto con el volumen correcto indicado en la etiqueta Si se indica un error, el ensayo debe repetirse tras eliminar la causa del mismo. En caso de no encontrar un error, procédase como sigue: a) si se obtiene un valor hasta +/-20%: la precisión global del laboratorio podría no permitir alcanzar +/-10% del valor esperado. Comunicar el problema al responsable para aceptar ó rechazar este resultado. b) si se obtiene un valor superior a +/-20%: en este caso el test es inválido y hay que avisar al servicio de atención al cliente de DiaPro

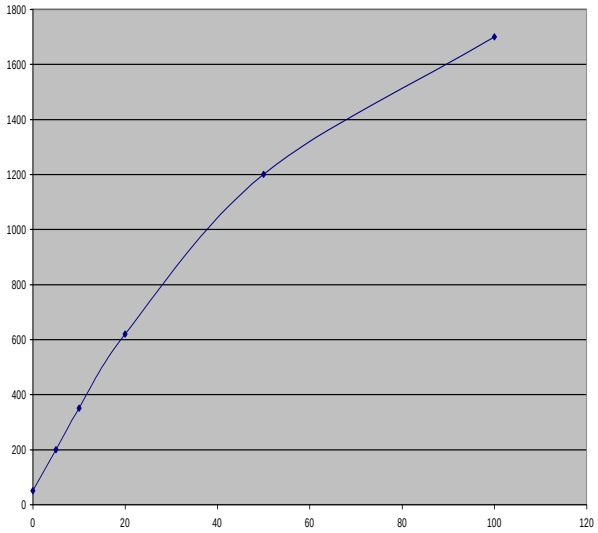
Si se presenta alguno de los problemas anteriores, avisar al responsable para tomar las medidas pertinentes.

Nota importante:
El análisis debe seguir el paso de lectura descrito en la sección M, punto 12.

P. RESULTADOS.

P.1 Método cuantitativo.
Si el ensayo resulta válido, usar para el método cuantitativo un programa de ajuste de curva para diseñar la curva de calibración con los valores obtenidos en la lectura a 450nm/620-630nm (se sugiere interpolar 4 parámetros). Después calcular sobre la curva de calibración la concentración de anticuerpos IgM anti-HBc presentes en la muestra.

A continuación, un ejemplo de curva de calibración:



Nota Importante:
No usar la curva anterior para formular los cálculos.

P.2 Método cualitativo.
En el método cualitativo, calcular los valores medios de DO450nm/620-630nm para los Calibradores 0 y 10 U/ml, después comprobar que el ensayo es válido.

A continuación, un ejemplo de los cálculos a realizar (datos obtenidos siguiendo el paso de lectura descrito en la sección M, punto 12)*:*

Los siguientes datos no deben usarse en lugar de los valores reales obtenidos en el laboratorio.

Calibrador 0 U/ml: 0.020 – 0.024 DO 450nm
Valor medio: 0.022 DO 450nm
Menor de 0.150 – Válido

Calibrador 10 U/ml: 0.350 – 0.330 DO 450nm
Valor medio: 0.340 DO 450nm
Mayor de Cal 0 + 0.200 – Válido

Calibrador 100 U/ml: 2.845 DO 450nm
Mayor de 1.000 – Válido

Q. INTERPRETACIÓN DE LOS RESULTADOS.
Q.1 Resultados cualitativos:
Para el método cualitativo, la literatura médica generalmente considera positivas aquellas muestras con una concentración de HBc IgM $\geq$ 10 PEI U/ml.
Los resultados se interpretan como la razón entre la DO 450nm /620-630nm de la muestra y la DO 450nm del Cal 10 PEI U/ml (M/Co), como se indica en la tabla:

M/Co	Interpretación
< 0.9	Negativo
0.9 - 1.1	Equívoco
> 1.1	Positivo

Q.2 Resultados Cuantitativos:
La Curva de Calibración se emplea para determinar la concentración de anticuerpos IgM anti-HBcAg, presentes en la muestra.
Las muestras con una concentración menor de 5 PEI U/ml se consideran negativas a HBcIgM.
Las muestras con una concentración entre 5 y 10 PEI U/ml se consideran en la zona gris.

En el seguimiento de hepatitis crónica, sin embargo, valores superiores a 5 PEI U/ml pueden considerarse positivos a HBcIgM si están presentes otros signos clínicos.
Las muestras con una concentración mayor de 10 PEI U/ml se consideran positivas a HBcIgM.

Notas generales importantes:

1. *Cuando el cálculo de los resultados se halla mediante el sistema operativo de un equipo de ELISA automático, asegurarse de que la formulación usada para el cálculo del valor de corte, y para la interpretación de los resultados sea correcta.*
2. *La interpretación de los resultados debe hacerse bajo la vigilancia del responsable del laboratorio para reducir el riesgo de errores de juicio y de interpretación.*
3. *Un resultado positivo indica infección por HBV por lo tanto el paciente debe ser tratado adecuadamente.*
4. *Cuando se transmiten los resultados de la prueba, del laboratorio a otras instalaciones, debe ponerse mucha atención para evitar el traslado de datos erróneos.*
5. *El diagnóstico de infección con un virus de la hepatitis debe ser evaluado y comunicado al paciente por un médico calificado.*

R. FUNCIONAMIENTO
La evaluación del funcionamiento ha sido realizada según lo reportado en las Especificaciones Técnicas Comunes (ETC) (art. 5, Capítulo 3 de las Directivas IVD 98/79/EC).

1. Límite de detección.
El límite de detección del ensayo ha sido calculado por medio de:

1.3 La preparación de referencia para HBcIgM suministrada por el Instituto Paul Erlich, Alemania (HBc-Referenzserum-IgM 84), a partir de la cual se ha calibrado la Curva Estándar.
1.4 Accurun 113 (cat. N° A113-5001) suministrada por Boston Biomedica Inc., Estados Unidos.

La siguiente tabla muestra los resultados del Control de Calidad para tres lotes analizados:

BCM.CE	Lote #	0103	Lote #	0103/2	Lote #	0303
PEI U/ml	DO450nm	M/Co	DO450nm	M/Co	DO450nm	M/Co
100	2.752	**8.9**	2.883	**9,7**	2.911	**9.1**
50	1.917	**6.2**	1.972	**6.7**	2.053	**6.4**
20	0.980	**3.2**	0.914	**3.1**	1.095	**3.4**
10	0.544	**1.8**	0.513	**1.7**	0.592	**1.8**
5	0.310	**1.0**	0.296	**1.0**	0.321	**1.0**
2.5	0.155	0.5	0.149	0.5	0.161	0.5
1.25	0.084	0.3	0.084	0.3	0.093	0.3
Negativo	0.040		0.035		0.044	

BBI Accurun # 113

BCM.CE	Lote #	0103	Lote #	0103/2	Lote #	0303
BBI 113	DO450nm	M/Co	DO450nm	M/Co	DO450nm	M/Co
1 x	3.336	**10.8**	3.195	**10.4**	3.269	**10.3**
2 x	2.472	**8.0**	2.385	**7.8**	2.385	**7.5**
4 x	1.467	**4.7**	1.413	**4.6**	1.429	**4.5**
8 x	0.865	**2.8**	0.807	**2.6**	0.856	**2.7**
16 x	0.430	**1.4**	0.427	**1.4**	0.410	**1.3**
32 x	0.234	0.8	0.234	0.8	0.248	0.8
64 x	0.129	0.4	0.133	0.4	0.122	0.4
128 x	0.086	0.3	0.082	0.3	0.089	0.3
Negativo	0.040		0.040		0.052	

Además se ha examinado el panel # PHE 102 de BBI en tres lotes del producto, los datos se reportan a continuación con referencia a un equipo europeo (resultados de BBI).

BBI – Panel código PHE 102

	Lote# 0103	Lote # 0103/2	Lote # 0303	Sorin EIA
Miembro	M/Co	M/Co	M/Co	M/Co
01	**6.7**	**6.3**	**6.5**	**2.0**
02	**11.3**	**10.0**	**10.7**	**6.1**
03	**9.5**	**7.2**	**8.4**	**3.0**
04	**5.8**	**3.4**	**4.1**	**2.1**
05	**11.3**	**11.4**	**11.2**	**3.1**
06	**12.1**	**11.6**	**11.8**	**4.1**
07	**0.1**	**0.1**	**0.1**	**0.2**
08	**9.2**	**8.5**	**8.8**	**2.3**
09	**12.2**	**11.7**	**11.9**	**4.2**
10	**11.7**	**10.2**	**10.8**	**2.8**
11	**5.9**	**5.8**	**5.8**	**2.1**
12	**12.7**	**11.4**	**11.7**	**5.2**
13	**11.6**	**11.0**	**11.3**	**3.6**
14	**7.0**	**6.3**	**6.6**	**2.3**
15	**12.4**	**11.5**	**11.8**	**4.5**

2. Sensibilidad Diagnóstica:
Se define como la probabilidad del ensayo de detectar positivos en presencia del analito específico.
La sensibilidad diagnóstica ha sido probada interna y externamente en un Laboratorio Clínico calificado, a partir de paneles de muestras clasificadas como positivas según un equipo certificado US FDA.
Las muestras positivas se obtuvieron de diferentes pacientes y a partir de diversas patologías producidas por HBV (hepatitis aguda y crónica). En un estudio realizado a más de 200 muestras, se encontró un valor > 98%.
También se realizó un estudio con un panel de Seroconversión producido por BBI, Estados Unidos, código # PHM 935A cuyos resultados se reportan a continuación con referencia a dos equipos comerciales (resultados BBI).

BBI Panel PHM 935A

	Lote # 0103	Abbott EIA	DiaSorin EIA
Miembro#	M/Co	M/Co	M/Co
01	0.2	0.1	0.1
02	0.2	0.1	0.1
03	0.2	0.1	0.1
04	0.1	0.1	0.1
05	0.2	0.1	0.1
06	0.2	0.1	0.1
07	0.2	0.1	0.1
08	0.1	0.1	0.1
09	0.1	0.1	0.1
10	0.1	0.1	0.1
11	0.2	0.1	0.1
12	0.2	0.1	0.1
13	**2.8**	**3.7**	0.7
14	**5.0**	**6.4**	0.9
15	**> 12**	**6.2**	**4.5**
16	**> 12**	**5.6**	**4.5**
17	**> 12**	**5.5**	**4.3**
18	**> 12**	**4.8**	**4.3**
19	**> 12**	**> 6.6**	**4.4**
20	**> 12**	**> 6.6**	**5.2**

3. Especificidad Diagnóstica:
Se define como la probabilidad del ensayo de detectar negativos en ausencia del analito específico.
La especificidad diagnóstica ha sido determinada interna y externamente en un Laboratorio Clínico calificado, a partir de paneles de muestras provenientes de individuos sanos y donantes de sangre, las mismas fueron clasificadas como negativas según un equipo certificado US FDA.
Se examinaron más de 400 muestras negativas, la especificidad diagnóstica encontrada fue > 98%.
También se analizaron más de 50 muestras que pudieran provocar interferencia (por ejemplo: otras enfermedades infecciosas, pacientes afectados por hepatitis no virales, pacientes sometidos a diálisis, mujeres embarazadas, hemofílicos, lipémicos, etc.). No se observaron interferencias en el estudio.
Se emplearon además plasma sometido a métodos de tratamiento estándar (citrato, EDTA y heparina) y suero humanos. No se ha observado falsa reactividad debida a los métodos de tratamiento de muestras.

Las muestras congeladas han sido probadas para comprobar si la congelación interfiere con el procedimiento del ensayo. No se ha observado interferencia a partir de muestras limpias y libres de agregados.

4. Precisión:
Se realizó un estudio con 3 muestras, examinadas en 16 réplicas, en tres corridas separadas utilizando 3 lotes diferentes. Los valores obtenidos se reportan a continuación :

BCM.CE: lote # 0103

Cal 0 U/ml (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor Promedio
DO 450nm	0.055	0.053	0.051	0.053
Desviación estándar	0.005	0.006	0.005	0.006
CV %	9.9	12.3	10.7	10.9

Cal 5 U/ml (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor Promedio
DO 450nm	0.324	0.308	0.321	0.318
Desviación estándar	0.022	0.018	0.024	0.021
CV %	6.8	5.7	7.5	6.7

Cal 50 U/ml (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor Promedio
DO 450nm	2.109	2.048	2.052	2.070
Desviación estándar	0.101	0.088	0.136	0.109
CV %	4.8	4.3	6.7	5.2

BCM.CE: lote # 0103/2

Cal 0 U/ml (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor Promedio
DO 450nm	0.057	0.053	0.054	0.055
Desviación estándar	0.005	0.005	0.004	0.004
CV %	8.3	9.0	7.3	8.2

Cal 5 U/ml (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor Promedio
DO 450nm	0.332	0.331	0.322	0.328
Desviación estándar	0.017	0.018	0.016	0.017
CV %	5.0	5.5	4.9	5.1

Cal 50 U/ml (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor Promedio
DO 450nm	2.311	2.208	2.212	2.244
Desviación estándar	0.110	0.090	0.095	0.098
CV %	4.7	4.1	4.3	4.4

BCM.CE: lote # 0303

Cal 0 U/ml (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor Promedio
DO 450nm	0.043	0.042	0.040	0.042
Desviación estándar	0.004	0.005	0.004	0.004
CV %	10.3	11.1	10.9	10.8

Cal 5 U/ml (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor Promedio
DO 450nm	0.320	0.326	0.314	0.320
Desviación estándar	0.023	0.024	0.026	0.024
CV %	7.1	7.4	8.2	7.6

Cal 50 U/ml (N = 16)

Valores medios	1ra corrida	2da corrida	3ra corrida	Valor Promedio
DO 450nm	2.150	2.163	2.092	2.135
Desviación estándar	0.057	0.067	0.076	0.067
CV %	2.6	3.1	3.6	3.1

Nota importante:
Los datos de rendimiento se obtuvieron siguiendo el paso de lectura descrito en la sección M, punto 12.

S. LIMITACIONES DEL PROCEDIMIENTO.
Las muestras que después de ser descongeladas presentan partículas de fibrina o partículas agregadas, generan algunos resultados falsos positivos.
La contaminación bacteriana de las muestras o la inactivación por calor pueden modificar los valores de absorbancia con la consiguiente alteración de los niveles del analito.
Este ensayo es adecuado solo para el análisis de muestras individuales y no para mezclas.
El diagnóstico de una enfermedad infecciosa no se debe formular en base al resultado de un solo ensayo, sino que es necesario tomar en consideración la historia clínica y la sintomatología del paciente así como otros datos diagnósticos.

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Todos los productos de diagnóstico in vitro fabricados por la empresa son controlados por un sistema certificado de control de calidad aprobado por un organismo notificado para el marcado CE. Cada lote se somete a un control de calidad y se libera al mercado únicamente si se ajusta a las especificaciones técnicas y criterios de aceptación de la CE.

Fabricante:
Dia.Pro Diagnostic Bioprobes S.r.l.
Via G. Carducci n° 27 – Sesto San Giovanni
Milán – Italia

CE
0318

HBe Ag&Ab

Enzyme Immunoassay (ELISA) for the determination of Hepatitis B Virus "e" Antigen and Antibody in human plasma and sera.

- for “in vitro” diagnostic use only -

DIA.PRO
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e-mail: info@diapro.it

REF. HBE.CE
96 Tests

HBe Ag&Ab

A. INTENDED USE

Enzyme ImmunoAssay (ELISA) for the determination of Hepatitis B Virus "e" Antigen and Antibody in human plasma and sera.

The kit is intended for the follow-up of acute infection and of chronic patients under therapy.

For “in vitro” diagnostic use only.

B. INTRODUCTION

Hepatitis B “e” Antigen or HBeAg is known to be intimately associated with Hepatitis B Virus or HBV replication and the presence of infectious Dane particles in the blood.

Recently, it has been found that HBeAg is a product of proteolytic degradation of Hepatitis B core Antigen or HBcAg, occurring in hepatocites, whose expression is under the control of the precore region of HBV genome.

If HBeAg is considered a specific marker of infectivity, the presence of anti HBeAg antibodies in blood is recognised to be a clinical sign of recovery from infection to convalescence.

The determination of these two analytes in samples from HBV patients has become important for the classification of the phase of illness and as a prognostic value in the follow up of infected patients.

C. PRINCIPLE OF THE TEST

HBeAg:

HBeAg, if present in the sample, is captured by a specific monoclonal antibody, in the 1st incubation.

In the 2nd incubation, after washing, a tracer, composed of a mix of two specific anti HBeAg monoclonal antibodies, labeled with peroxidase (HRP), is added to the microplate and binds to the captured HBeAg.

The concentration of the bound enzyme on the solid phase is proportional to the amount of HBeAg in the sample and its activity is detected by adding the chromogen/substrate in the 3rd incubation.

The presence of HBeAg in the sample is determined by means of a cut-off value that allows for the semiquantitative detection of the antigen.

HBeAb

Anti HBeAg antibodies, if present in the sample, compete with a recombinant HBeAg preparation for a fixed amount of an anti HBeAg antibody, coated on the microplate wells.

The competitive assay is carried out in two incubations, the first with the sample and recHBeAg, and the second with a tracer, composed of two anti HBeAg monoclonal antibodies, labeled with peroxidase (HRP).

The concentration of the bound enzyme on the solid phase becomes inversely proportional to the amount of anti HBeAg antibodies in the sample and its activity is detected by adding the chromogen/substrate in the third incubation.

The concentration of HBeAg specific antibodies in the sample is determined by means of a cut-off value that allows for the semi quantitative detection of anti HBeAg antibodies.

D. COMPONENTS

The kit contains reagents for total 96 tests.

1. Microplate: MICROPLATE

n° 1 coated microplate

12 strips of 8 breakable wells coated with anti HBeAg specific monoclonal antibody, postcoated with bovine serum proteins and sealed into a bag with desiccant. Allow the microplate to reach room temperature before opening; reseal unused strips in the bag with desiccant and store at 2..8°C.

2. Negative Control: CONTROL -

1x2.0ml/vial. Ready to use control. It contains bovine serum, 0.09% sodium azide and 0.045% ProClin 300 as preservatives. The negative control is colorless.

3. Antigen Positive Control: CONTROL + Ag

1x1.0ml/vial. Ready to use control. It contains 2% bovine serum albumin, non infectious recombinant HBeAg, 100 mM tris buffer pH 7.4+/-0.1, 0.09% sodium azide and 0.045% ProClin 300 as preservatives.

The positive control is green color coded.

4. Antibody Positive Control: **:** CONTROL + Ab

1x1.0ml/vial. Ready to use control. It contains 2% bovine serum albumin, human anti HbeAg positive plasma at about 10 PEI U/ml, 100 mM tris buffer pH 7.4+/-0.1, 0.09% sodium azide and 0.045% ProClin 300 as preservatives. The label is red colored.

The positive control is yellow color coded.

5. Antigen Calibrator: CALAG …ml

n° 1 vial. Lyophilised calibrator for HBeAg. To be dissolved with EIA grade water as reported in the label. It contains fetal bovine serum, non infectious recombinant HBeAg at 1 PEI U/ml +/- 10%, 0.02% gentamicine sulphate and 0.045% ProClin 300 as preservatives.

Important Note: The volume necessary to dissolve the content of the vial may vary from lot to lot. Please use the right volume reported on the label.

6. Antibody Calibrator: CALAB …ml

n° 1 vial. Lyophilized calibrator for anti HBeAg antibody. To be dissolved with EIA grade water as reported in the label. It contains fetal bovine serum, positive plasma at 0.25 PEI U/ml +/-10%, 0.02% gentamicine sulphate and 0.045% ProClin 300 as preservatives. The label is red colored.

Important Note: The volume necessary to dissolve the content of the vial may vary from lot to lot. Please use the right volume reported on the label.

7. Wash buffer concentrate: WASHBUF 20X

1x60ml/bottle. 20x concentrated solution.

Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2, 0.05% Tween 20 and 0.045% ProClin 300.

8. Enzyme conjugate: CONJ

1x16ml/vial. Ready to use conjugate. It contains Horseradish peroxidase conjugated with a mix of monoclonal antibodies to HBeAg, 10 mM Tris buffer pH 6.8+/-0.1, 2% BSA, 0.045% ProClin 300 and 0.02% gentamicine sulphate as preservatives.

The reagent is red color coded.

9. HBe Antigen: Ag-HBe

1x10ml/vial. Ready to use reagent. It contains recombinant HBeAg, fetal bovine serum, buffered solution pH 8.0+/-0.1, 0.045% ProClin 300 and 0.09% sodium azide as preservatives.

The reagent is blue color coded.

10. Chromogen/Substrate: SUBS TMB

1x16ml/vial. Ready-to-use component. It contains a 50 mM citrate-phosphate buffered solution at pH 3.5-3.8, 4% dimethylsulphoxide, 0.03% tetra-methyl-benzidine or TMB and 0.02% hydrogen peroxide or H_2O_2.

Note: To be stored protected from light as sensitive to strong illumination.

11. Sulphuric Acid: H_2SO_4 O.3 M

1x15ml/vial. It contains 0.3 M H2SO4 solution.

Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

12. Plate sealing foils n°2

13. Package insert n°1

E. MATERIALS REQUIRED BUT NOT PROVIDED

1. Calibrated Micropipettes (150ul, 100ul and 50ul) and disposable plastic tips.
2. EIA grade water (double distilled or deionised, charcoal treated to remove oxidizing chemicals used as disinfectants).
3. Timer with 60 minute range or higher.
4. Absorbent paper tissues.
5. Calibrated ELISA microplate thermostatic incubator (dry or wet) set at +37°C.
6. Calibrated ELISA microwell reader with 450nm (reading) and with 620-630nm (blanking) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.

F. WARNINGS AND PRECAUTIONS

1. The kit has to be used by skilled and properly trained technical personnel only, under the supervision of a medical doctor responsible of the laboratory.
2. All the personnel involved in performing the assay have to wear protective laboratory clothes, talc-free gloves and glasses. The use of any sharp (needles) or cutting (blades) devices should be avoided. All the personnel involved should be trained in biosafety procedures, as recommended by the Center for Disease Control, Atlanta, U.S. and reported in the National Institute of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
3. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.
4. The laboratory environment should be controlled so as to avoid contaminants such as dust or air-born microbial agents, when opening kit vials and microplates and when performing the test. Protect the Chromogen/Substrate (TMB) from strong light and avoid vibration of the bench surface where the test is undertaken.
5. Upon receipt, store the kit at 2-8°C into a temperature controlled refrigerator or cold room.
6. Do not interchange components between different lots of the kits. It is recommended that components between two kits of the same lot should not be interchanged.
7. Check that the reagents are clear and do not contain visible heavy particles or aggregates. If not, advise the laboratory supervisor to initiate the necessary procedures.
8. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each sample. Do not reuse disposable tips.
9. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one. Do not reuse disposable tips.
10. Do not use the kit after the expiration date stated on external (primary container) and internal (vials) labels.
11. Treat all specimens as potentially infective. All human serum specimens should be handled at Biosafety Level 2, as recommended by the Center for Disease Control, Atlanta, U.S. in compliance with what reported in the Institutes of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
12. The use of disposable plastic-ware is recommended in the preparation of the washing solution or in transferring components into other containers of automated workstations, in order to avoid contamination.
13. Waste produced during the use of the kit has to be discarded in compliance with national directives and laws concerning laboratory waste of chemical and biological substances. In particular, liquid waste generated from the washing procedure, from residuals of controls and from samples has to be treated as potentially infective material and inactivated. Suggested procedures of inactivation are treatment with a 10% final concentration of household bleach for 16-18 hrs or heat inactivation by autoclave at 121°C for 20 min..
14. Accidental spills have to be adsorbed with paper tissues soaked with household bleach and then with water. Tissues should then be discarded in proper containers designated for laboratory/hospital waste.
15. The Stop Solution is an irritant. In case of spills, wash the surface with plenty of water
16. Other waste materials generated from the use of the kit (example: tips used for samples and controls, used microplates) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

G. SPECIMEN: PREPARATION AND RECOMMANDATIONS

1. Blood is drawn aseptically by venepuncture and plasma or serum is prepared using standard techniques of preparation of samples for clinical laboratory analysis. No influence has been observed in the preparation of the sample with citrate, EDTA and heparin.
2. Avoid any addition of preservatives; especially sodium azide as this chemical would affect the enzymatic activity of the conjugate, generating false negative results.
3. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results.
4. Haemolysed and visibly hyperlipemic ("milky") samples have to be discarded as they could generate false results. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they could give rise to false results.
5. Sera and plasma can be stored at +2°…+8°C in primary collection tubes for up to five days after collection.
Do not freeze primary tubes of collection. For longer storage periods, sera and plasma samples, carefully removed from the primary collection tube, can be stored frozen at –20°C for at least 12 months. Any frozen samples should not be frozen/thawed more than once as this may generate particles that could affect the test result.
6. If particles are present, centrifuge at 2.000 rpm for 20 min or filter using 0.2-0.8u filters to clean up the sample for testing.

H. PREPARATION OF COMPONENTS AND WARNINGS

A study conducted on an opened kit has not pointed out any relevant loss of activity up to 6 re-uses of the device and up to 3 months.

1. Microplate:
Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant has not turned dark green, indicating a defect in manufacturing.
In this case, call Dia.Pro's customer service.
Unused strips have to be placed back into the aluminum pouch, with the desiccant supplied, firmly zipped and stored at +2°-8°C.
When opened the first time, unused strips are stable until the humidity indicator inside the desiccant bag turns from yellow to green.

2. Negative Control:
Ready to use. Mix well on vortex before use.

3. Antigen Positive Control:
Ready to use. Mix well on vortex before use.

4. Antibody Positive Control:
Ready to use. Mix well on vortex before use.

5. Antigen Calibrator:
Add the volume of ELISA grade water, reported on the label, to the lyophilized powder; let fully dissolve and then gently mix on vortex.

Note: The dissolved calibrator is not stable. Store it frozen in aliquots at –20°C.

6. Antibody Calibrator:
Add the volume of ELISA grade water, reported on the label, to the lyophilized powder; let fully dissolve and then gently mix on vortex.
Note: The dissolved calibrator is not stable. Store it frozen in aliquots at –20°C.

7. Wash buffer concentrate:
The whole content of the 20x concentrated solution has to be diluted with bidistilled water up to 1200 ml and mixed gently end-over-end before use.
During preparation avoid foaming as the presence of bubbles could impact on the efficiency of the washing cycles.
Note: Once diluted, the wash solution is stable for 1 week at +2..8° C.

8. Enzyme conjugate:
Ready to use. Mix well on vortex before use.
Avoid contamination of the liquid with oxidizing chemicals, air-driven dust or microbes. If this component has to be transferred, use only plastic, and if possible, sterile disposable containers.

9. HBe Antigen:
Ready to use. Mix well on vortex before use.
Avoid contamination of the liquid with oxidizing chemicals, air-driven dust or microbes. If this component has to be transferred, use only plastic, and if possible, sterile disposable containers.

10. Chromogen/Substrate:
Ready to use. Mix well on vortex before use.
Avoid contamination of the liquid with oxidizing chemicals, air-driven dust or microbes. Do not expose to strong light, oxidizing agents and metallic surfaces.
If this component has to be transferred use only plastic, and if possible, sterile disposable container.

11. Sulphuric Acid:
Ready to use. Mix well on vortex before use.
Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

Legenda:

Warning **H statements:**
H315 – Causes skin irritation.
H319 – Causes serious eye irritation.

Precautionary **P statements:**
P280 – Wear protective gloves/protective clothing/eye protection/face protection.

P302 + P352 – IF ON SKIN: Wash with plenty of soap and water.
P332 + P313 – If skin irritation occurs: Get medical advice/attention.
P305 + P351 + P338 – IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337 + P313 – If eye irritation persists: Get medical advice/attention.
P362 + P363 – Take off contaminated clothing and wash it before reuse.

I. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

1. Micropipettes have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (household alcohol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample. Decontamination of spills or residues of kit components should also be carried out regularly. They should also be regularly maintained in order to show a precision of 1% and a trueness of $\pm$2%.
2. The ELISA incubator has to be set at +37°C (tolerance of +/- 0.5°C) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water baths are suitable for the incubations, provided that the instrument is validated for the incubation of ELISA tests.
3. The **ELISA washer** is extremely important to the overall performances of the assay. The washer must be carefully validated in advance, checked for the delivery of the right dispensation volume and regularly submitted to maintenance according to the manufacturer's instructions for use. In particular the washer, at the end of the daily workload, has to be extensively cleaned out of salts with deionized water. Before use, the washer has to be extensively primed with the diluted Washing Solution.
   The instrument weekly has to be submitted to decontamination according to its manual (NaOH 0.1 M decontamination suggested).
   5 washing cycles (aspiration + dispensation of 350ul/well of washing solution + 20 sec soaking = 1 cycle) are sufficient to ensure the assay with the declared performances. If soaking is not possible add one more cycle of washing.
   An incorrect washing cycle or salt-blocked needles are the major cause of false positive reactions.
4. Incubation times have a tolerance of $\pm$5%.
5. The ELISA reader has to be equipped with a reading filter of 450nm and with a second filter of 620-630nm, mandatory for blanking purposes. Blanking is carried out on the well identified in the section "Assay Procedure". The optical system of the reader has to be calibrated regularly to ensure the correct optical density is measured. It should be regularly maintained according to the manufacturer 's instructions.
6. When using an ELISA automated work station, all critical steps (dispensation, incubation, washing, reading, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the section "Internal Quality Control''. The assay protocol has to be installed in the operating system of the unit and validated as for the washer and the reader. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set. Particular attention must be paid to avoid carry over by the needles used for dispensing and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells. The use of ELISA automated work stations is recommended when the number of samples to be tested exceed 20-30 units per run.
7. Dia.Pro's customer service offers support to the user in the setting and checking of instruments used in combination with the kit, in order to assure compliance with the requirements described. Support is also provided for the installation of new instruments to be used with the kit.

L. PRE ASSAY CONTROLS AND OPERATIONS

1. Check the expiration date of the kit printed on the external label (primary container). Do not use if expired.
2. Check that the liquid components are not contaminated by visible particles or aggregates. Check that the Chromogen/Substrate (TMB+H_2O_2) is colourless or pale blue by aspirating a small volume of it with a sterile plastic pipette. Check that no breakage occurred in transportation

and no spillage of liquid is present inside the box (primary container). Check that the aluminium pouch, containing the microplate, is not punctured or damaged.
3. Dilute all the content of the 20x concentrated Wash Solution as described above.
4. Dissolve the Calibrator as described above and gently mix.
5. Allow all the other components to reach room temperature (about 1 hr) and then mix gently on vortex all liquid reagents.
6. Set the ELISA incubator at +37°C and prepare the ELISA washer by priming with the diluted washing solution, according to the manufacturers instructions. Set the right number of washing cycles as reported in the specific section.
7. Check that the ELISA reader is turned on or ensure it will be turned on at least 20 minutes before reading.
8. If using an automated work station, turn on, check settings and be sure to use the right assay protocol.
9. Check that the micropipettes are set to the required volume.
10. Check that all the other equipment is available and ready to use.

In case of problems, do not proceed further with the test and advise the supervisor.

M. ASSAY PROCEDURE

The assay has to be performed according to the procedure given below, taking care to maintain the same incubation time for all the samples being tested.

A) HBe Antigen:

1. Place the required number of strips in the plastic holder and carefully identify the wells for controls, calibrator and samples.
2. Leave the A1 well empty for blanking purposes.
3. Pipette 100 µl of the Negative Control in triplicate, 100 µl of the Antigen Calibrator in duplicate and then 100 µl of the Antigen Positive Control in single.
4. Then dispense 100 µl of samples in the proper wells.
5. Check for the presence of samples in wells by naked eye (there is a marked colour difference between empty and full wells) or by reading at 450/620nm (samples show OD values higher than 0.100).
6. Incubate the microplate for **60 min at +37°C**.

Important note: *Strips have to be sealed with the adhesive sealing foil, only when the test is performed manually. Do not cover strips when using ELISA automatic instruments.*

7. When the first incubation is finished, wash the microwells as previously described (section I.3)
8. Dispense 100 µl Enzyme Conjugate in all wells, except for A1, used for blanking operations.

Important note: *Be careful not to touch the inner surface of the well with the pipette tip and not to immerse the top of it into samples or controls. Contamination might occur.*

9. Check that the reagent has been dispensed properly and then incubate the microplate for **60 min at +37°C**.
10. When the second incubation is finished, wash the microwells as previously described (section I.3)
11. Pipette 100 µl Chromogen/Substrate into all the wells, A1 included.

Important note: *Do not expose to strong direct light as a high background might be generated.*

12. Incubate the microplate protected from light at **room temperature (18-24°C) for 20 minutes**. Wells dispensed with positive control and positive samples will turn from clear to blue.
13. Pipette 100 µl Sulphuric Acid into all the wells using the same pipetting sequence as in step 11. Addition of the stop solution will turn the positive control and positive samples from blue to yellow.
14. Measure the color intensity of the solution in each well, as described in section I.5 using a 450nm filter (reading) and a 620-630nm filter (background subtraction, mandatory), blanking the instrument on A1.

B) HBe Antibody:

1. Place the required number of strips in the plastic holder and carefully identify the wells for controls, calibrator and samples.
2. Leave the A1 well empty for blanking purposes.
3. Pipette 50 µl of the Negative Control in triplicate, 50 µl of the Antibody Calibrator in duplicate and then 50 µl of the Antibody Positive Control in single.
4. Then dispense 50 µl of samples in the proper wells.
5. Check for the presence of samples in wells by naked eye (there is a marked color difference between empty and full wells) or by reading at 450/620nm (samples show OD values higher than 0.100).
6. Dispense then 50 µl of HBe Antigen in all the wells, except for A1.
7. Incubate the microplate for **60 min at +37°C**.

Important note: *Strips have to be sealed with the adhesive sealing foil, only when the test is performed manually. Do not cover strips when using ELISA automatic instruments.*

8. When the first incubation is finished, wash the microwells as previously described (section I.3)
9. Finally proceed as described for the HBeAg assay from point 8 to the last one.

Important notes:

1. *Ensure that no finger prints are present on the bottom of the microwell before reading. Finger prints could generate false positive results on reading.*
2. *Reading should ideally be performed immediately after the addition of the Stop Solution but definitely no longer than 20 minutes afterwards. Some self oxidation of the chromogen can occur leading to a higher background.*
3. *The Calibrator (CAL) does not affect the cut-off calculation and therefore the test results calculation. The Calibrator may be used only when a laboratory internal quality control is required by the management.*

N. ASSAY SCHEME

HBe antigen test

Controls and calibrator Samples	100 ul 100 ul
1st incubation	**60 min**
Temperature	+37°C
Wash step	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
Enzyme Conjugate	100 ul
2nd incubation	**60 min**
Temperature	+37°C
Wash step	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
TMB/H2O2 mix	100 ul
3rd incubation	**20 min**
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm/620-630nm

HBe antibody test

Controls and calibrator Samples	50 ul 50 ul
Neutralising antigen	50 ul
1st incubation	**60 min**
Temperature	+37°C
Wash step	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
Enzymatic conjugate	100 ul
2nd incubation	**60 min**
Temperature	+37°C
Wash step	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
TMB/H2O2 mixture	100 ul
3rd incubation	**20 min**
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm/620-630nm

An example of dispensation scheme is reported below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S2										
B	NC	S3										
C	NC	S4										
D	NC	S5										
E	CAL	S6										
F	CAL	S7										
G	PC	S8										
H	S1	S9										

Legenda: BLK = Blank // NC = Negative Control
PC = Positive Control // CAL = Calibrators // S = Sample

O. INTERNAL QUALITY CONTROL

A validation check is carried out on the controls any time the kit is used in order to verify whether the performances of the assay are as qualified.

Control that the following data are matched:

HBe Antigen

Check	OD450nm
Blank well	< 0.100 OD450nm
Negative Control (NC)	< 0.150 OD450nm after blanking coefficient of variation < 30%
Antigen Calibrator	S/Co > 2.0
Positive Control (PC)	> 1.500 OD450nm

HBe Antibody

Check	OD450nm
Blank well	< 0.100 OD450nm
Negative Control (NC)	> 1.000 OD450nm after blanking coefficient of variation < 10%
Antibody Calibrator	OD450nm < NC/1.5
Positive Control (PC)	OD450nm < NC/10

If the results of the test match the requirements stated above, proceed to the next section.

If they do not, don't proceed any further and perform the following checks:

HBeAg

Problem	Check
Blank well > 0.100 OD450nm	1. that the Chromogen/Substrate solution has not become contaminated during the assay
Negative Control (NC) > 0.150 OD450nm after blanking coefficient of variation > 30%	1. that the washing procedure and the washer settings are as validated in the pre qualification study; 2. that the proper washing solution has been used and the washer has been primed with it before use; 3. that no mistake has been done in the assay procedure (dispensation of positive control instead of negative control); 4. that no contamination of the negative control or of the wells where the control was dispensed has occurred due to positive samples, to spills or to the enzyme conjugate; 5. that micropipettes have not become contaminated with positive samples or with the enzyme conjugate 6. that the washer needles are not blocked or partially obstructed.
Calibrator S/Co < 2	1. that the procedure has been correctly performed; 2. that no mistake has occurred during its distribution (ex.: dispensation of negative control instead); 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the calibrator has occurred.
Positive Control < 1.500 OD450nm	1. that the procedure has been correctly performed; 2. that no mistake has occurred during the distribution of the control (dispensation of negative control instead of positive control); 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the positive control has occurred.

HBe antibody

Problem	Check
Blank well > 0.100 OD450nm	1. that the Chromogen/Substrate solution has not become contaminated during the assay
Negative Control (NC) < 1.000 OD450nm after blanking coefficient of variation > 10%	1. that the washing procedure and the washer settings are as validated in the pre qualification study; 2. that the proper washing solution has been used and the washer has been primed with it before use; 3. that no mistake has been done in the assay procedure (e.g.: dispensation of positive control instead of negative control; no dispensation of the Neutralizing Antigen; no dispensation of the Enzyme Conjugate); 4. that no contamination of the negative control or of the wells where the control was dispensed has occurred; 5. that micropipettes have not become contaminated with positive samples; 6. that the washer needles are not blocked or partially obstructed.

Calibrator OD450nm > NC/1.5	1. that the procedure has been correctly performed; 2. that no mistake has occurred during its distribution (ex.: dispensation of negative control instead; no dispensation of the Neutralizing Antigen; no dispensation of the Enzyme Conjugate); 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the calibrator has occurred.
Positive Control OD450nm > NC/10	1. that the procedure has been correctly performed; 2. that no mistake has occurred during the distribution of the control; 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the positive control has occurred.

If any of the above problems have occurred, report the problem to the supervisor for further actions.

Important note:
The analysis must be done proceeding as the reading step described in the section M, point 14.

P. CALCULATION OF THE CUT-OFF
The results are calculated by means of a cut-off value determined with the following formula:

HBeAg:

NC + 0.100 = Cut-Off (Co)

The value found for the test is used for the interpretation of results as described in the next paragraph.

HBeAb:

(NC + PC) / 3 = Cut-Off (Co)

***Important note**: When the calculation of results is performed by the operating system of an ELISA automated work station, ensure that the proper formulation is used to calculate the cut-off value and generate the correct interpretation of results.*

Q. INTERPRETATION OF RESULTS
Results are interpreted as follows:

HBeAg:

S/Co	Interpretation
< 0.9	Negative
0.9 - 1.1	Equivocal
> 1.1	Positive

HBeAb:

Co/S	Interpretation
< 0.9	Negative
0.9 - 1.1	Equivocal
> 1.1	Positive

Note:
S = OD450nm/620-630nm of the sample
Co = cut-off value

An example of calculation for HBeAg assay is reported below (data obtained proceeding as the the reading step described in the section M, point 14):

The following data must not be used instead or real figures obtained by the user.

Negative Control: 0.020 – 0.030 – 0.025 OD450nm
Mean Value: 0.025 OD450nm
Lower than 0.150 – Accepted

Positive Control: 2.489 OD450nm
Higher than 1.500 – Accepted
Cut-Off = 0.025+0.100 = 0.125
Calibrator: 0.520 - 0.540 OD450nm
Mean value: 0.530 OD450nm S/Co = 4.2
S/Co higher than 2.0 – Accepted

Sample 1: 0.030 OD450nm
Sample 2: 1.800 OD450nm
Sample 1 S/Co < 0.9 = negative
Sample 2 S/Co > 1.1 = positive

An example of calculation for HBeAb is reported below (data obtained proceeding as the the reading step described in the section M, point 14):

The following data must not be used instead or real figures obtained by the user.

Negative Control: 2.100 – 2.200 – 2.000 OD450nm
Mean Value: 2.100 OD450nm
Higher than 1.000 – Accepted

Positive Control: 0.100 OD450nm
Lower than NC/10 – Accepted

Cut-Off = (2.100 + 0.100) / 3 = 0.733
Calibrator: 0.720-0.760 OD450nm
Mean value: 0.740 OD450nm
OD450nm < NC/1.5 – Accepted

Sample 1: 0.020 OD450nm
Sample 2: 1.900 OD450nm
Sample 1 Co/S > 1.1 positive
Sample 2 Co/S < 0.9 negative

Important notes:
1. *Interpretation of results should be done under the supervision of the laboratory director to reduce the risk of judgment errors and misinterpretations.*
2. *The Identification of the clinical status of a HBV patient (acute, chronic, asymptomatic hepatitis) has to be done on the basis also of the other markers of HBV infection (HBsAg, HBsAb, HBcAb, HBcIgM);*
3. *When test results are transmitted from the laboratory to another facility, attention must be paid to avoid erroneous data transfer.*
4. *Diagnosis of viral hepatitis infection has to be taken by and released to the patient by a suitably qualified medical doctor.*

R. PERFORMANCE CHARACTERISTICS

A) HBeAg

1. Limit of detection
The limit of detection of the assay has been calculated by means of the International Standard for HBeAg, supplied by Paul Erlich Institute (PEI).
The data obtained by examining the limit of detection on three lots is reported in the table below.

HBE.CE Lot ID	PEI U/ml HBeAg
0103	0.25
0103/2	0.25
0303	0.25

In addition the preparation Accurun # 51, produced by Boston Biomedica Inc., USA, has been tested, upon dilution in FCS. Results are reported for three lots of products.

BBI's Accurun 51 (S/Co)

HBE.CE Lot ID	1 x	2 x	4 x	8 x	16x
0103	**4.1**	**1.6**	0.9	0.6	0.4
0103/2	**4.1**	**1.7**	0.9	0.6	0.4
0303	**4.0**	**1.6**	0.9	0.5	0.4

2. Diagnostic Sensitivity:
The diagnostic sensitivity has been tested on panels of samples classified positive by a US FDA approved kit.
Positive samples were collected from different HBV pathologies (acute, chronic) bearing HBeAg reactivity.
An overall value > 98% has been found in the study conducted on a total number of more than 200 samples.
Moreover the Panel of Seroconversion code PHM 935B, produced by BBI, was examined.
Data are reported below and compared with those reported by BBI for two other commercial products.

Sample ID	HBE.CE S/Co	Abbott EIA S/Co	Sorin EIA S/Co
21	5.4	4.5	6.3
22	3.7	4.3	5.4
23	1.9	3.2	3.1
24	1.1	2.4	1.5
25	1.0	2.1	1.2
26	0.6	1.7	0.7
27	0.2	0.8	0.3
28	0.2	0.6	0.2
29	0.2	0.4	0.2
30	0.2	0.3	0.2
31	0.1	0.3	0.2
32	0.1	0.3	0.2

Finally the Performance Panel code PHJ 201, produced by BBI, was tested. Data are reported below and compared with those reported by BBI for an other commercial product.

Member	PEI U/ml	HBE.CE	Sorin EIA
1	3	3.3	7.0
2	6	17.5	21.9
3	26	30.1	37.1
4	31	29.4	23.5
5	1	1.1	2.2
6	2	2.3	6.9
7	35	30.1	24.6
8	38	29.2	31.9
9	4	16.6	10.8
10	-	0.3	0.2
11	1	3.4	3.6
12	< 1	0.2	1.2
13	< 1	0.9	1.4
14	-	0.2	0.2
15	-	0.4	0.1
16	-	0.5	0.1
17	-	0.3	0.2
18	-	0.2	0.2
19	-	0.2	0.1
20	-	0.2	0.1
21	-	0.3	1.0
22	-	0.3	0.1
23	-	0.4	0.1
24	-	0.2	0.2
25	-	0.3	0.2

3. Diagnostic Specificity:
The diagnostic specificity has been determined on panels of negative samples from normal individuals and blood donors, classified negative with a FDA approved kit.
Both plasma, derived with different standard techniques of preparation (citrate, EDTA and heparin), and sera have been used to determine the specificity.
No false reactivity due to the method of specimen preparation has been observed.
Frozen specimens have also been tested to check whether this interferes with the performance of the test. No interference was observed on clean and particle free samples.
Samples derived from patients with different viral (HCV and HAV) and non viral pathologies of the liver that may interfere with the test were examined.
No cross reaction were observed.
The Performance Evaluation study conducted in a qualified external reference center on more than 500 samples has provided a value > 98% .

4. Precision
It has been calculated on two samples examined in 16 replicate in three different runs on three lots.
The values found were as follows:

HBE.CE: lot # 0103

Negative Control (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.030	0.027	0.032	0.029
Std.Deviation	0.002	0.002	0.003	0.002
CV %	7.4	8.2	7.9	7.8

PEI 1 U/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.569	0.559	0.575	0.568
Std.Deviation	0.027	0.029	0.028	0.028
CV %	4.7	5.3	4.9	4.9
S/Co	4.4	4.4	4.4	4.4

HBE.CE: lot # 0103/2

Negative Control (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.033	0.031	0.030	0.032
Std.Deviation	0.003	0.003	0.002	0.003
CV %	7.9	8.5	7.4	8.0

PEI 1 U/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.565	0.573	0.568	0.569
Std.Deviation	0.026	0.025	0.024	0.025
CV %	4.7	4.3	4.2	4.4
S/Co	4.2	4.4	4.4	4.3

HBE.CE: lot # 0303

Negative Control (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.029	0.034	0.038	0.034
Std.Deviation	0.003	0.003	0.004	0.003
CV %	9.7	9.8	9.2	9.6

PEI 1 U/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.579	0.573	0.564	0.572
Std.Deviation	0.023	0.028	0.025	0.025
CV %	4.1	4.8	4.5	4.5
S/Co	4.5	4.3	4.1	4.3

B) HBe Antibody

1. Limit of detection

The limit of detection of the assay has been calculated by means of the International Standard for HBeAb, supplied by Paul Erlich Institute (PEI).

The data obtained by examining the limit of detection on three lots is reported in the table below.

HBE.CE Lot ID	PEI U/ml HBeAb
0103	0.25
0103/2	0.25
0303	0.25

In addition the preparation Accurun # 52, produced by Boston Biomedica Inc., USA, has been tested, upon dilution in FCS. Results are reported for three lots of products.

Accurun 52 (Co/S)

HBE.CE Lot ID	1 x	2 x	4 x	8 x	16x
0103	1.0	0.8	0.6	0.4	0.4
0103/2	1.0	0.8	0.6	0.5	0.4
0303	1.0	0.8	0.6	0.4	0.4

2. Diagnostic sensitivity:

The diagnostic sensitivity has been tested on panels of samples classified positive for HBeAb by a US FDA approved kit.

Positive samples were collected from different HBV pathologies bearing anti HBeAg antibody reactivity.

An overall value > 98% has been found in the study conducted on a total number of more than 200 samples.

Moreover the Panel of Seroconversion code PHM 935B, produced by BBI, was examined.

Data are reported below and compared with those reported by BBI for two other commercial products.

Sample ID	HBE.CE Co/S	Abbott EIA Co/S	Sorin EIA Co/S
21	0.4	0.4	0.5
22	0.4	0.5	0.6
23	0.4	0.6	0.5
24	0.4	0.5	0.6
25	0.4	0.6	0.5
26	0.5	0.6	0.6
27	0.6	0.8	0.7
28	0.7	0.9	0.7
29	0.6	0.9	0.7
30	0.8	1.0	0.9
31	1.0	1.3	1.1
32	1.0	1.2	1.0

Finally the Performance Panel code PHJ 201, produced by BBI, was tested. Data are reported below and compared with those reported by BBI for another commercial product.

Member	PEI U/ml	HBE.CE	Sorin EIA
1	-	0.3	0.5
2	-	0.2	0.5
3	-	0.2	0.5
4	-	0.2	0.5
5	-	0.3	0.6
6	-	0.3	0.6
7	-	0.2	0.4
8	-	0.2	0.4
9	-	0.2	0.5
10	-	1.9	0.6
11	-	0.3	0.5
12	-	0.4	0.9
13	2	4.4	9.1
14	1	3.8	2.9
15	< 1	1.0	1.5
16	> 50	4.3	120.9
17	< 1	1.0	1.0
18	5	5.6	21.8
19	1	2.7	6.4
20	11	5.0	47.3
21	2	1.9	10.0
22	26	28.1	90.7
23	-	0.3	0.5
24	< 1	0.8	1.3
25	50	28.1	167.4

3. Diagnostic specificity:

The clinical specificity has been determined as described before for HBeAg.

The Performance Evaluation study conducted in a qualified external reference center on more than 500 samples has provided a value > 98% .

4. Precision:

It has been calculated on two samples examined in 16 replicate in three different runs on three lots.

The values found were as follows:

HBE.CE: lot # 0103

Negative Control (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	2.484	2.420	2.471	2.458
Std.Deviation	0.129	0.160	0.142	0.144
CV %	5.2	6.6	5.7	5.9

PEI 0.25 U/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.867	0.800	0.878	0.848
Std.Deviation	0.043	0.060	0.050	0.051
CV %	5.0	7.5	5.7	6.1
Co/S	1.0	1.0	1.0	1.0

HBE.CE: lot # 0103/2

Negative Control (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	2.316	2.361	2.413	2.363
Std.Deviation	0.127	0.144	0.146	0.139
CV %	5.5	6.1	6.0	5.9

PEI 0.25 U/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.767	0.793	0.785	0.781
Std.Deviation	0.041	0.050	0.046	0.046
CV %	5.4	6.3	5.8	5.8
Co/S	1.0	1.0	1.0	1.0

HBE.CE: lot #0303

Negative Control (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	2.334	2.415	2.437	2.395
Std.Deviation	0.146	0.155	0.158	0.153
CV %	6.3	6.4	6.5	6.4

PEI 0.25 U/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.850	0.867	0.876	0.864
Std.Deviation	0.052	0.051	0.048	0.050
CV %	6.1	5.9	5.5	5.8
Co/S	0.9	1.0	1.0	1.0

Important note:
The performance data have been obtained proceeding as the reading step described in the section M, point 14.

S. LIMITATIONS

Frozen samples containing fibrin particles or aggregates may generate false positive results.
Bacterial contamination or heat inactivation of the specimen may affect the absorbance values of the samples with consequent alteration of the level of the analyte.
This test is suitable only for testing single samples and not pooled ones.
Diagnosis of an infectious disease should not be established on the basis of a single test result. The patient's clinical history, symptomatology, as well as other diagnostic data should be considered.

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All the IVD Products manufactured by the company are under the control of a certified Quality Management System approved by an EC Notified Body. Each lot is submitted to a quality control and released into the market only if conforming with the EC technical specifications and acceptance criteria.

Manufacturer:
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CE
0318

HBsAb

Enzyme Immunoassay for qualitative/quantitative determination of antibodies to Hepatitis B surface Antigen in human serum and plasma

- for “in vitro” diagnostic use only -

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REF SAB.CE
96 Tests

HBs Ab

A. INTENDED USE

Enzyme ImmunoAssay (ELISA) for both the quantitative and qualitative determination of antibodies to the Surface Antigen of Hepatitis B Virus in human plasma and sera.
For "in vitro" diagnostic use only.

B. INTRODUCTION

The World Health Organization (WHO) defines Hepatitis B Virus infection as follows:

"Hepatitis B is one of the major diseases of mankind and is a serious global public health problem. Hepatitis means inflammation of the liver, and the most common cause is infection with one of 5 viruses, called hepatitis A,B,C,D, and E. All of these viruses can cause an acute disease with symptoms lasting several weeks including yellowing of the skin and eyes (jaundice); dark urine; extreme fatigue; nausea; vomiting and abdominal pain. It can take several months to a year to feel fit again. Hepatitis B virus can cause chronic infection in which the patient never gets rid of the virus and many years later develops cirrhosis of the liver or liver cancer.

HBV is the most serious type of viral hepatitis and the only type causing chronic hepatitis for which a vaccine is available. Hepatitis B virus is transmitted by contact with blood or body fluids of an infected person in the same way as human immunodeficiency virus (HIV), the virus that causes AIDS. However, HBV is 50 to 100 times more infectious than HIV. The main ways of getting infected with HBV are: (a) perinatal (from mother to baby at the birth); (b) child- to-child transmission; (c) unsafe injections and transfusions; (d) sexual contact.

Worldwide, most infections occur from infected mother to child, from child to child contact in household settings, and from reuse of un-sterilized needles and syringes. In many developing countries, almost all children become infected with the virus. In many industrialized countries (e.g. Western Europe and North America), the pattern of transmission is different. In these countries, mother-to-infant and child-to-child transmission accounted for up to one third of chronic infections before childhood hepatitis B vaccination programmes were implemented. However, the majority of infections in these countries are acquired during young adulthood by sexual activity, and injecting drug use. In addition, hepatitis B virus is the major infectious occupational hazard of health workers, and most health care workers have received hepatitis B vaccine.

Hepatitis B virus is not spread by contaminated food or water, and cannot be spread casually in the workplace. High rates of chronic HBV infection are also found in the southern parts of Eastern and Central Europe. In the Middle East and Indian sub-continent, about 5% are chronically infected. Infection is less common in Western Europe and North America, where less than 1% are chronically infected.

Young children who become infected with HBV are the most likely to develop chronic infection. About 90% of infants infected during the first year of life and 30% to 50% of children infected between 1 to 4 years of age develop chronic infection. The risk of death from HBV-related liver cancer or cirrhosis is approximately 25% for persons who become chronically infected during childhood.

Chronic hepatitis B in some patients is treated with drugs called *interferon or lamivudine*, which can help some patients. Patients with cirrhosis are sometimes given liver transplants, with varying success. It is preferable to prevent this disease with vaccine than to try and cure it.

Hepatitis B vaccine has an outstanding record of safety and effectiveness. Since 1982, over one billion doses of hepatitis B vaccine have been used worldwide. The vaccine is given as a series of three intramuscular doses. Studies have shown that the vaccine is 95% effective in preventing children and adults from developing chronic infection if they have not yet been infected. In many countries where 8% to 15% of children used to become chronically infected with HBV, the rate of chronic infection has been reduced to less than 1% in immunized groups of children. Since 1991, WHO has called for all countries to add hepatitis B vaccine into their national immunization programmes."

Hepatitis B surface Antigen (HBsAg) is the major structural polypeptide of the envelope of the Hepatitis B Virus (HBV).
This antigen is composed mainly of the type common determinant "a" and the type specific determinants "d" and "y", present only on the specific serotypes.
Upon infection, a strong immunological response develops firstly against the type specific determinants and in a second time against the "a" determinant.
Anti "a" antibodies are however recognised to be most effective in the neutralisation of the virus, protecting the patient from other infections and leading it to convalescence.
The detection of HBsAb has become important for the follow up of patients infected by HBV and the monitoring of recipients upon vaccination with synthetic and natural HBsAg.

C. PRINCIPLE OF THE TEST

Microplates are coated with a preparation of highly purified HBsAg that in the first incubation with sample specifically captures anti HBsAg antibodies to the solid phase.
After washing, captured antibodies are detected by an HBsAg, labelled with peroxidase (HRP), that specifically binds the second available binding site of these antibodies.
The enzyme specifically bound to wells, by acting on the substrate/chromogen mixture, generates an optical signal that is proportional to the amount of HBsAb in the sample and can be detected by an ELISA reader.
The amount of antibodies may be quantitated by means of a standard curve calibrated against the W.H.O reference preparation.
Samples are pre treated in the well with an specimen diluent able to block interference present in vaccinated individuals.

D. COMPONENTS

Each kit contains sufficient reagents to perform 96 tests.

1. Microplate: MICROPLATE
8x12 microwell strips coated with purified heat-inactivated HBsAg of both subtypes (ad and ay) from human origin and sealed into a bag with desiccant.
Allow the microplate to reach room temperature before opening; reseal unused strips in the bag with desiccant and store at 4°C.

2. Calibration Curve: CAL N° …
5x2.0 ml/vial. Ready to use and colour coded standard curve, derived from HBsAb positive plasma titrated on WHO standard for anti HBsAg (1st reference preparation 1977, lot 17-2-77), ranging: CAL1 = 0 mIU/ml // CAL2 = 10 mIU/ml // CAL3 = 50 mIU/ml // CAL4 = 100 mIU/ml // CAL 5 = 250 mIU/ml.
Contains human serum proteins, 5% BSA, 10 mM phosphate buffer pH 7.4+/-0.1, 0.09% sodium azide and 0.1% Kathon GC as preservatives. Standards are blue coloured.

3. Wash buffer concentrate: WASHBUF 20X
1x60ml/bottle. 20x concentrated solution.
Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2, 0.05% Tween 20 and 0.1% Kathon GC.

4. Enzyme conjugate : CONJ
1x16.0 ml/vial. Ready-to-use solution and red color coded.
It contains inactivated purified HBsAg of both subtypes ad and ay, labelled with HRP, 5% BSA, 10 mM Tris buffer pH 6.8+/-0.1, 0.3 mg/ml gentamicine sulphate and 0.1% Kathon GC as preservatives.

5. Chromogen/Substrate: SUBS TMB
1x16ml/vial. Contains a 50 mM citrate-phosphate buffered solution at pH 3.5-3.8, 4% dimethylsulphoxide, 0.03% tetra-methyl-benzidine (TMB) and 0.02% hydrogen peroxide (H2O2).
Note: To be stored protected from light as sensitive to strong illumination.

6. Sulphuric Acid: H2SO4 0.3 M
1x15ml/vial. Contains 0.3 M H_2SO_4 solution.
Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

7. Specimen Diluent: DILSPE
1x8ml. 10 mM Tris Buffered solution ph 7.4 +/-0.1,suggested to be used in the follow up of vaccination. It contains 0.09% sodium azide as preservatives.

8. Control Serum: CONTROL …ml
1 vial. Lyophilized.
Contains fetal bovine serum proteins, human anti HBsAg antibodies calibrated at 50 ± 10% WHO mIU/ml. 0.3 mg/ml gentamicine sulphate and 0.1% Kathon GC as preservatives.

9. Plate sealing foil n° 2

10. Package insert n° 1

E. MATERIALS REQUIRED BUT NOT PROVIDED

1. Calibrated Micropipettes (100ul and 50ul) and disposable plastic tips.
2. EIA grade water (double distilled or deionised, charcoal treated to remove oxidizing chemicals used as disinfectants).
3. Timer with 60 minute range or higher.
4. Absorbent paper tissues.
5. Calibrated ELISA microplate thermostatic incubator (dry or wet), set at +37°C (+/-1°C tolerance)..
6. Calibrated ELISA microwell reader with 450nm (reading) and with 620-630nm (blanking, strongly recommended) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.

F. WARNINGS AND PRECAUTIONS

1. The kit has to be used by skilled and properly trained technical personnel only, under the supervision of a medical doctor responsible of the laboratory.
2. All the personnel involved in performing the assay have to wear protective laboratory clothes, talc-free gloves and glasses. The use of any sharp (needles) or cutting (blades) devices should be avoided. All the personnel involved should be trained in biosafety procedures, as recommended by the Center for Disease Control, Atlanta, U.S. and reported in the National Institute of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
3. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.
4. The laboratory environment should be controlled so as to avoid contaminants such as dust or air-born microbial agents, when opening kit vials and microplates and when performing the test. Protect the Chromogen (TMB) from strong light and avoid vibration of the bench surface where the test is undertaken.
5. Upon receipt, store the kit at 2..8°C into a temperature controlled refrigerator or cold room.
6. Do not interchange components between different lots of the kits. It is recommended that components between two kits of the same lot should not be interchanged.
7. Check that the reagents are clear and do not contain visible heavy particles or aggregates. If not, advise the laboratory supervisor to initiate the necessary procedures for kit replacement.
8. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each sample.
9. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one.
10. Do not use the kit after the expiration date stated on the external container and internal (vials) labels. A study conducted on an opened kit did not pointed out any relevant loss of activity up to six 6 uses of the device and up to 6 months.
11. Treat all specimens as potentially infective. All human serum specimens should be handled at Biosafety Level 2, as recommended by the Center for Disease Control, Atlanta, U.S. in compliance with what reported in the Institutes of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
12. The use of disposable plastic-ware is recommended in the preparation of the liquid components or in transferring components into automated workstations, in order to avoid cross contamination.
13. Waste produced during the use of the kit has to be discarded in compliance with national directives and laws concerning laboratory waste of chemical and biological substances. In particular, liquid waste generated from the washing procedure, from residuals of controls and from samples has to be treated as potentially infective material and inactivated before waste. Suggested procedures of inactivation are treatment with a 10% final concentration of household bleach for 16-18 hrs or heat inactivation by autoclave at 121°C for 20 min..
14. Accidental spills from samples and operations have to be adsorbed with paper tissues soaked with household bleach and then with water. Tissues should then be discarded in proper containers designated for laboratory/hospital waste.
15. The Sulphuric Acid is an irritant. In case of spills, wash the surface with plenty of water
16. Other waste materials generated from the use of the kit (example: tips used for samples and controls, used microplates) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

G. SPECIMEN: PREPARATION AND WARNINGS

1. Blood is drawn aseptically by venipuncture and plasma or serum is prepared using standard techniques of preparation of samples for clinical laboratory analysis. No influence has been observed in the preparation of the sample with citrate, EDTA and heparin.

2. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results. Bar code labeling and electronic reading is strongly recommended.
3. Haemolysed ("red") and visibly hyperlipemic ("milky") samples have to be discarded as they could generate false results. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they could give rise to false results.
4. Sera and plasma can be stored at +2°..8°C for up to five days after collection. For longer storage periods, samples can be stored frozen at –20°C for several months. Any frozen samples should not be freezed/thawed more than once as this may generate particles that could affect the test result.
5. If particles are present, centrifuge at 2.000 rpm for 20 min or filter using 0.2-0.8u filters to clean up the sample for testing.
6. Samples whose anti-HBsAg antibody concentration is expected to be higher than 250 mIU/ml should be diluted before use either 1:10 or 1:100 in the Calibrator 0 mIU/ml. Dilutions have to be done in clean disposable tubes by diluting 50 ul of each specimen with 450 ul of Cal 0 (1:10). Then 50 ul of the 1:10 dilution are diluted with 450 ul of the Cal 0 (1:100). Mix tubes thoroughly on vortex when preparing the diluted samples.

H. PREPARATION OF COMPONENTS AND WARNINGS

1. Microplate:
Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant has not turned green, indicating a defect in conservation.
In this case, call Dia.Pro's customer service.
Unused strips have to be placed back into the aluminum pouch, with the desiccant supplied, firmly zipped and stored at +2°-8°C. After first opening, remaining strips are stable until the humidity indicator inside the desiccant bag turns from yellow to green.

2. Calibration Curve
Ready to use. Mix well on vortex before use.

3. Control Serum
Add the volume of ELISA grade water, reported on the label, to the lyophilised powder; let fully dissolve and then gently mix on vortex.
Note: *The control after dissolution is not stable. Store frozen in aliquots at –20°C.*

4. Wash buffer concentrate:
The whole content of the concentrated solution has to be diluted 20x with bidistilled water and mixed gently end-over-end before use. During preparation avoid foaming as the presence of bubbles could impact on the efficiency of the washing cycles.
Note: Once diluted, the wash solution is stable for 1 week at +2..8° C.

5. Enzyme conjugate:
Ready to use. Mix well on vortex before use.
Avoid contamination of the liquid with oxidising chemicals, dust or microbes. If this component has to be transferred, use only plastic, and if possible, sterile disposable containers.

6. Specimen Diluent:
Ready to use. Mix well on vortex before use.

7. Chromogen/Substrate:
Ready to use. Mix well on vortex before use.
Avoid contamination of the liquid with oxidising chemicals, air-driven dust or microbes. Do not expose to strong light, oxidising agents and metallic surfaces.
If this component has to be transferred use only plastic, and if possible, sterile disposable container

8. Sulphuric Acid:
Ready to use. Mix well on vortex before use.

Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

Legenda:

Warning **H statements:**
H315 – Causes skin irritation.
H319 – Causes serious eye irritation.

Precautionary **P statements:**
P280 – Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352 – IF ON SKIN: Wash with plenty of soap and water.
P332 + P313 – If skin irritation occurs: Get medical advice/attention.
P305 + P351 + P338 – IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337 + P313 – If eye irritation persists: Get medical advice/attention.
P362 + P363 – Take off contaminated clothing and wash it before reuse.

I. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

1. Micropipettes have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (70% ethanol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample or the components of the kit. They should also be regularly maintained in order to show a precision of 1% and a trueness of $\pm$2%.
2. The ELISA incubator has to be set at +37°C (tolerance of $\pm$1°C) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water baths are suitable for the incubations, provided that the instrument is validated for the incubation of ELISA tests.
3. The ELISA washer is extremely important to the overall performances of the assay. The washer must be carefully validated and correctly optimized using the kit controls/calibrator and reference panels, before using the kit for routine laboratory tests. Usually 4-5 washing cycles (aspiration + dispensation of 350ul/well of washing solution = 1 cycle) are sufficient to ensure that the assay performs as expected. A soaking time of 20-30 seconds between cycles is suggested. In order to set correctly their number, it is recommended to run an assay with the kit controls/calibrator and well-characterized negative and positive reference samples, and check to match the values reported below in the sections "Validation of Test" and "Assay Performances". Regular calibration of the volumes delivered and maintenance (decontamination and cleaning of needles) of the washer has to be carried out according to the instructions of the manufacturer.
4. Incubation times have a tolerance of $\pm$5%.
5. The ELISA microplate reader has to be equipped with a reading filter of 450nm and with a second filter (620-630nm, strongly recommended) for blanking purposes. Its standard performances should be (a) bandwidth $\leq$ 10 nm; (b) absorbance range from 0 to $\geq$ 2.0; (c) linearity to $\geq$ 2.0; repeatability $\geq$ 1%. Blanking is carried out on the well identified in the section "Assay Procedure". The optical system of the reader has to be calibrated regularly to ensure that the correct optical density is measured. It should be regularly maintained according to the manufacturer 's instructions.
6. When using an ELISA automated workstation, all critical steps (dispensation, incubation, washing, reading, shaking, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the

values reported in the sections “Validation of Test” and “Assay Performances”. The assay protocol has to be installed in the operating system of the unit and validated as for the washer and the reader. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set. Particular attention must be paid to avoid carry over by the needles used for dispensing samples and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells due to strongly reactive samples, leading to false positive results. The use of ELISA automated work stations is recommended for blood screening and when the number of samples to be tested exceed 20-30 units per run.

7. Dia.Pro’s customer service offers support to the user in the setting and checking of instruments used in combination with the kit, in order to assure full compliance with the requirements described. Support is also provided for the installation of new instruments to be used with the kit.

L. PRE ASSAY CONTROLS AND OPERATIONS

1. Check the expiration date of the kit printed on the external label of the kit box. Do not use if expired.
2. Check that the liquid components are not contaminated by naked-eye visible particles or aggregates. Check that the Chromogen/Substrate is colorless or pale blue by aspirating a small volume of it with a sterile transparent plastic pipette. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box. Check that the aluminum pouch, containing the microplate, is not punctured or damaged.
3. Dilute all the content of the 20x concentrated Wash Solution as described above.
4. Dissolve the Control Serum as described above.
5. Allow all the other components to reach room temperature (about 1 hr) and then mix as described.
6. Set the ELISA incubator at +37°C and prepare the ELISA washer by priming with the diluted washing solution, according to the manufacturers instructions. Set the right number of washing cycles as found in the validation of the instrument for its use with the kit.
7. Check that the ELISA reader has been turned on at least 20 minutes before reading.
8. If using an automated workstation, turn it on, check settings and be sure to use the right assay protocol.
9. Check that the micropipettes are set to the required volume.
10. Check that all the other equipments are available and ready to use.

In case of problems, do not proceed further with the test and advise the supervisor.

M. ASSAY PROCEDURE

The assay has to be carried out according to what reported below, taking care to maintain the same incubation time for all the samples in testing.

Two procedures can be carried out with the device according to the request of the clinician.

M.1 Quantitative analysis

1. Place the required number of strips in the microplate holder. Leave A1 and B1 wells empty for the operation of blanking.
Store the other strips into the bag in presence of the desiccant at 2..8°C, sealed.
Then Dispense in all the wells to be used for the test, except for A1 and B1, 50µl of the Specimen Diluent.

Important note: *This additive is added before distributing samples and controls into specific wells and is particularly intended for blocking some substances present in people undergoing vaccination and capable to mask antibodies.*

2. Pipette 100µl of all the Calibrators, 100µl of Control Serum in duplicate and then 100ul of samples. The Control Serum is used to verify that the whole analytical system works as expected. Check that Calibrators, Control Serum and samples have been correctly added. Then incubate the microplate at **+37°C for 60 min**.

Important note: *Strips have to be sealed with the adhesive sealing foil only when the test is performed manually. Do not cover strips when using ELISA automatic instruments.*

3. Wash the microplate as reported in section I.3.

4. In all the wells except A1 and B1, pipette 100 µl Enzyme Conjugate. Check that the reagent has been correctly added. Incubate the microplate at **+37°C for 60 minutes.**

Important note:

1) *Be careful not to touch the inner surface of the well with the pipette tip when dispensing the Enzyme Conjugate. Contamination might occur.*
2) *Mix thoroughly the Enzyme Conjugate on vortex before use.*

5. Wash the microplate as described.
6. Pipette 100µl TMB/H_2O_2 mixture in each well, the blank wells included. Check that the reagent has been correctly added. Then incubate the microplate at **room temperature for 20 minutes**.

Important note: *Do not expose to strong direct light as a high background might be generated.*

7. Stop the enzymatic reaction by pipette 100µl Sulphuric Acid into each well and using the same pipetting sequence as in step 6. Then measure the colour intensity with a microplate reader at 450nm (reading) and at 620-630nm (blanking, strongly recommended), blanking the instrument on A1 and B1 wells.

M.2 Qualitative analysis

1. Place the required number of strips in the microplate holder. Leave A1 well empty for the operation of blanking.
Store the other strips into the bag in presence of the desiccant at 2..8°C, sealed.
2. Dispense 50 ul Specimen Diluent in all the wells, except for the blank A1. Then pipette 100µl of the Calibrator 0 mIU/ml in duplicate, 100µl of the Calibrator 10 mIU/ml in duplicate, 100µl of the Calibrator 250 mIU/ml in single, and then 100ul of samples. Check that Calibrators and samples have been correctly added. Then incubate the microplate at **+37°C for 60 min**.
3. Wash the microplate as reported in section I.3.
4. In all the wells except A1, pipette 100 µl Enzyme Conjugate. Check that the reagent has been correctly added. Incubate the microplate at **+37°C for 60 minutes.**

Important note:

3) *Be careful not to touch the inner surface of the well with the pipette tip when dispensing the Enzyme Conjugate. Contamination might occur.*
4) *Mix thoroughly the Enzyme Conjugate on vortex before use.*

5. Wash the microplate as described.
6. Pipette 100µl TMB/H_2O_2 mixture in each well, the blank wells included. Check that the reagent has been correctly added. Then incubate the microplate at **room temperature for 20 minutes**.

Important note: *Do not expose to strong direct light as a high background might be generated.*

7. Stop the enzymatic reaction by pipette 100µl Sulphuric Acid into each well and using the same pipetting sequence as in step 6. Then measure the colour intensity with a microplate reader at 450nm (reading) and at 620-630nm (blanking, strongly recommended), blanking the instrument on A1 and B1 wells.

Important general notes:

1. *If the second filter is not available, ensure that no finger prints are present on the bottom of the microwell before reading at 450nm. Finger prints could generate false positive results on reading.*
2. *Reading has should ideally be performed immediately after the addition of the Stop Solution but definitely no longer than 20 minutes afterwards. Some self oxidation of the chromogen can occur leading to a higher background.*
3. *The Control Serum (CS) does not affect the cut-off calculation and therefore the test results calculation. The Control Serum may be used only when a laboratory internal quality control is required by the management.*

N. ASSAY SCHEME (standard procedure)

Specimen Diluent Calibrators Control Serum Samples	50 ul 100 ul 100 ul 100 ul
1st incubation	**60 min**
Temperature	+37°C
Wash step	*4-5 cycles*
Enzyme Conjugate	100 ul
2nd incubation	**60 min**
Temperature	+37°C
Wash step	*4-5 cycles*
TMB/H2O2 mix	100 ul
3rd incubation	**20 min**
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm & 620nm

An example of dispensation scheme in quantitative assays is reported below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	CAL4	S3									
B	BLK	CAL4	S4									
C	CAL1	CAL5	S5									
D	CAL1	CAL5	S6									
E	CAL2	CS	S7									
F	CAL2	CS	S8									
G	CAL3	S1	S9									
H	CAL3	S2	S10									

Legenda: BLK = Blank // CAL = Calibrators // CS = Control Serum // S = Sample

An example of dispensation scheme in qualitative assays is reported below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S 3	S 11									
B	CAL1	S 4	S 12									
C	CAL1	S 5	S 13									
D	CAL2	S 6	S 14									
E	CAL2	S 7	S 15									
F	CAL5	S 8	S 16									
G	S1	S 9	S 17									
H	S2	S 10	S 18									

Legenda: BLK = Blank // CAL = Calibrators // S = Sample

O. INTERNAL QUALITY CONTROL

A validation check is carried out on the controls any time the kit is used in order to verify whether the performances of the assay are as qualified.
Control that the following data are matched:

Parameters	Requirements
Blank well	< 0.100 OD450nm
Calibrator 0 WHO mIU/ml	< 0.200 OD450nm after blanking
Calibrator 10 WHO mIU/ml	OD450nm higher than the OD450nm of the Calibrator 0 mIU/ml + 0.100
Calibrator 250 WHO mIU/ml	> 1.500 OD450nm
Control Serum	OD450nm = OD450nm CAL 50 mIU/ml ± 10%
Coefficient of variation	< 30% for the Calibrator 0 mIU/ml

If the results of the test match the requirements stated above, proceed to the next section.
If they do not, do not proceed any further and perform the following checks:

Problem	Check
Blank well > 0.100 OD450nm	1. that the Chromogen/Substrate solution has not become contaminated during the assay
Calibrator 0 mIU/ml > 0.200 coefficient of variation > 30%	1. that the washing procedure and the washer settings are as validated in the pre qualification study; 2. that the proper washing solution has been used and the washer has been primed with it before use; 3. that no mistake has been done in the assay procedure when the dispensation of standards is carried out; 4. that no contamination of the Cal 0 mIU/ml or of the wells where it was dispensed has occurred due to positive samples, to spills or to the enzyme conjugate; 5. that micropipettes have not become contaminated with positive samples or with the enzyme conjugate 6. that the washer needles are not blocked or partially obstructed.

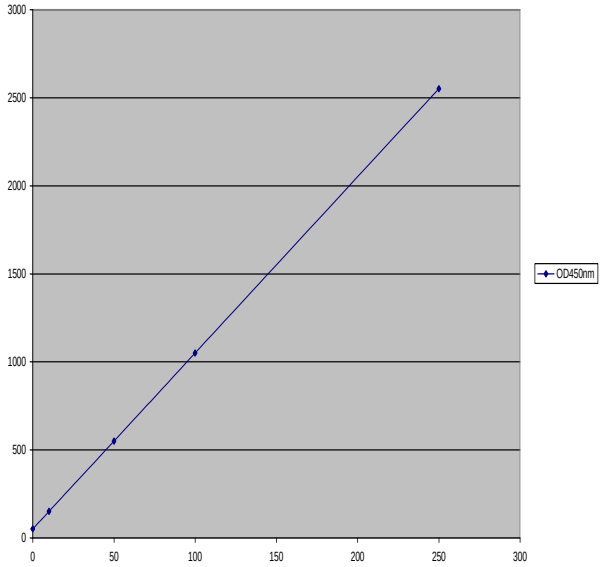
Calibrator 10 mIU/ml OD450nm < Cal 0 + 0.100	1. that the procedure has been correctly performed; 2. that no mistake has occurred during its distribution (ex.: dispensation of a wrong calibrator); 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the standard has occurred.
Calibrator 250 mIU/ml < 1.500 OD450nm	1. that the procedure has been correctly performed; 2. that no mistake has occurred during its distribution; 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the standard has occurred.
Control Serum Different from expected value	First verify that: 1. the procedure has been correctly performed; 2. no mistake has occurred during its distribution (ex.: dispensation of a wrong sample); 3. the washing procedure and the washer settings are correct; 4. no external contamination of the standard has occurred. 5. the Control Serum has been dissolved with the right volume reported on the label. If a mistake has been pointed out, the assay has to be repeated after eliminating the reason of this error. If no mistake has been found, proceed as follows: **a**) a value up to +/-20% is obtained: the overall Precision of the laboratory might not enable the test to match the expected value +/-10%. Report the problem to the Supervisor for acceptance or refusal of this result. **b**) a value higher than +/-20% is obtained: in this case the test is invalid and the DiaPro's customer service has to be called.

P. RESULTS

P.1 Quantitative method

If the test turns out to be valid, use for the quantitative method an approved curve fitting program to draw the calibration curve from the values obtained by reading at 450nm (4-parameters interpolation is suggested).
Then on the calibration curve calculate the concentration of anti HBsAg antibody in samples.
An example of Calibration curve is reported in the next page.

Example of Calibration Curve :



Important Note:
Do not use the calibration curve above to make calculations.

P.2 Qualitative method

In the qualitative method, calculate the mean OD450nm values for the Calibrators 0 and 10 mIU/ml and then check that the assay is valid.

Example of calculation:

The following data must not be used instead or real figures obtained by the user.

Calibrator 0 mIU/ml: 0.020 – 0.024 OD450nm
Mean Value: 0.022 OD450nm
Lower than 0.200 – Accepted

Calibrator 10 mIU/ml: 0.250 – 0.270 OD450nm
Mean Value: 0.260 OD450nm
Higher than Cal 0 + 0.100 – Accepted

Calibrator 250 mIU/ml: 2.845 OD450nm
Higher than 1.500 – Accepted

Q. INTERPRETATION OF RESULTS

Samples with a concentration lower than 10 WHO mIU/ml are considered negative for anti HBsAg antibody by most of the international medical literature.
Samples with a concentration higher than 10 WHO mIU/ml are considered positive for anti HBsAg antibody.
In the follow up of vaccination recipients, however, the value of 20 WHO mIU/ml is usually accepted by the medical literature as the minimum concentration at which the patient is considered clinically protected against HBV infection.

Important notes:

1. *Interpretation of results should be done under the supervision of the laboratory supervisor to reduce the risk of judgement errors and misinterpretations.*
2. *When test results are transmitted from the laboratory to another facility, attention must be paid to avoid erroneous data transfer.*
3. *Diagnosis has to be done and released to the patient by a suitably qualified medical doctor.*

R. PERFORMANCES

Evaluation of Performances has been conducted in accordance to what reported in the Common Technical Specifications or CTS (art. 5, Chapter 3 of IVD Directive 98/79/EC).

1. LIMIT OF DETECTION:

The limit of detection of the assay has been calculated by means of the HBsAb international preparation supplied by CLB on behalf of WHO (1st reference preparation 1977, lot 17-2-77), on which Calibration Curve has been calibrated. HBV negative serum was used as diluent, as recommended by the supplier.
Results of Quality Control are given in the following table:

WHO mIU/ml	SAB.CE Lot # 1002	SAB.CE Lot # 1001	SAB.CE Lot # 1002/2
50	0.933	0.812	0.846
10	0.219	0.192	0.194
5	0.110	0.096	0.104
2.5	0.057	0.058	0.067
Std 0	0.021	0.015	0.023

2. DIAGNOSTIC SPECIFICITY AND SENSITIVITY

A Performance Evaluation has been conducted on a total number of more than 700 samples.

2.1 Diagnostic Specificity

It is defined as the probability of the assay of scoring negative in the absence of specific analyte.
More than 500 negative specimens were tested, internally and externally, against a European company.
A diagnostic specificity of 98.8% was assessed. .
Moreover, diagnostic specificity was assessed by testing 113 potentially interfering specimens (other infectious diseases, patients affected by non viral hepatic diseases, dialysis patients, pregnant women, hemolized, lipemic, etc.) against the European company. A value of specificity of 100% was assessed.
Finally, both human plasma, derived with different standard techniques of preparation (citrate, EDTA and heparin), and human sera have been used to determine the specificity.
No false reactivity due to the method of specimen preparation has been observed.

2.2 Diagnostic Sensitivity

It defined as the probability of the assay of scoring positive in the presence of specific analyte.
106 vaccinated patients were evaluated providing a diagnostic sensitivity of 100%.
More than 100 HBV naturally infected patients were tested, internally and externally, against the European company; a diagnostic sensitivity of 100% was found.

3. PRECISION:

The mean values obtained from a study conducted on three samples of different anti-HBsAg reactivity, examined in 16 replicates in three separate runs is reported below:

SAB.CE: lot # 1202

Calibrator 0 mIU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.038	0.038	0.039	0.039
Std.Deviation	0.003	0.004	0.005	0.004
CV %	8.8	9.5	11.8	10.0

Calibrator 10 mIU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.250	0.243	0.244	0.246
Std.Deviation	0.020	0.023	0.017	0.020
CV %	8.0	9.3	7.0	8.1

Calibrator 250 mIU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	2.998	3.000	3.259	3.085
Std.Deviation	0.152	0.151	0.158	0.153
CV %	5.1	5.0	4.8	5.0

SAB.CE: lot # 1002

Calibrator 0 mIU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.048	0.048	0.050	0.049
Std.Deviation	0.005	0.004	0.006	0.005
CV %	9.4	8.4	11.5	9.8

Calibrator 10 mIU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.249	0.252	0.242	0.248
Std.Deviation	0.021	0.020	0.023	0.021
CV %	8.3	7.9	9.6	8.6

Calibrator 250 mIU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	3.544	3.653	3.612	3.603
Std.Deviation	0.153	0.176	0.138	0.156
CV %	4.3	4.8	3.8	4.3

SAB.CE: lot # 1002/2

Calibrator 0 mIU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.050	0.051	0.050	0.050
Std.Deviation	0.005	0.006	0.006	0.005
CV %	10.0	10.9	11.9	10.9

Calibrator 10 mIU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.226	0.238	0.239	0.234
Std.Deviation	0.015	0.017	0.018	0.016
CV %	6.5	7.0	7.5	7.0

Calibrator 250 mIU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	3.526	3.457	3.499	3.494
Std.Deviation	0.137	0.143	0.162	0.147
CV %	3.9	4.1	4.6	4.2

The variability shown in the tables did not result in sample misclassification.

4. ACCURACY

The assay accuracy has been checked by the dilution and recovery tests. Any “hook effect”, underestimation likely to happen at high doses of analyte, was ruled out up to 10.000 mIU/ml.

S. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or heat inactivation of the specimen may affect the absorbance values of the samples with consequent alteration of the level of the analyte.
This test is suitable only for testing single samples and not pooled ones.
Diagnosis of an infectious disease should not be established on the basis of a single test result. The patient’s clinical history, symptomatology, as well as other diagnostic data should be considered.

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All the IVD Products manufactured by the company are under the control of a certified Quality Management System approved by an EC Notified Body. Each lot is submitted to a quality control and released into the market only if conforming with the EC technical specifications and acceptance criteria.

Manufacturer:
Dia.Pro Diagnostic Bioprobes Srl
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HBsAg one

Version ULTRA

Fourth generation Enzyme Immunoassay (ELISA) for the determination of Hepatitis B surface Antigen or HBsAg in human serum and plasma

- for "in vitro" diagnostic use only -

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REF SAG1ULTRA.CE
96/192/480/960 Tests

HBsAg One version ULTRA

A. INTENDED USE

Fourth generation Enzyme Immunoassay (ELISA) for the one-step determination of Hepatitis B surface Antigen or HBsAg in human plasma and sera.
The kit is intended for the screening of blood units, is able to detect HBsAg mutants and finds application in the follow-up of HBV-infected patients.
For "in vitro" diagnostic use only.

B. INTRODUCTION

The World Health Organization (WHO) defines Hepatitis B Virus infection as follows:

"Hepatitis B is one of the major diseases of mankind and is a serious global public health problem. Hepatitis means inflammation of the liver, and the most common cause is infection with one of 5 viruses, called hepatitis A,B,C,D, and E. All of these viruses can cause an acute disease with symptoms lasting several weeks including yellowing of the skin and eyes (jaundice); dark urine; extreme fatigue; nausea; vomiting and abdominal pain. It can take several months to a year to feel fit again. Hepatitis B virus can cause chronic infection in which the patient never gets rid of the virus and many years later develops cirrhosis of the liver or liver cancer.

HBV is the most serious type of viral hepatitis and the only type causing chronic hepatitis for which a vaccine is available. Hepatitis B virus is transmitted by contact with blood or body fluids of an infected person in the same way as human immunodeficiency virus (HIV), the virus that causes AIDS. However, HBV is 50 to 100 times more infectious than HIV. The main ways of getting infected with HBV are: (a) perinatal (from mother to baby at the birth); (b) child- to-child transmission; (c) unsafe injections and transfusions; (d) sexual contact.

Worldwide, most infections occur from infected mother to child, from child to child contact in household settings, and from reuse of un-sterilized needles and syringes. In many developing countries, almost all children become infected with the virus. In many industrialized countries (e.g. Western Europe and North America), the pattern of transmission is different. In these countries, mother-to-infant and child-to-child transmission accounted for up to one third of chronic infections before childhood hepatitis B vaccination programmes were implemented. However, the majority of infections in these countries are acquired during young adulthood by sexual activity, and injecting drug use. In addition, hepatitis B virus is the major infectious occupational hazard of health workers, and most health care workers have received hepatitis B vaccine.

Hepatitis B virus is not spread by contaminated food or water, and cannot be spread casually in the workplace. High rates of chronic HBV infection are also found in the southern parts of Eastern and Central Europe. In the Middle East and Indian sub-continent, about 5% are chronically infected. Infection is less common in Western Europe and North America, where less than 1% are chronically infected.

Young children who become infected with HBV are the most likely to develop chronic infection. About 90% of infants infected during the first year of life and 30% to 50% of children infected between 1 to 4 years of age develop chronic infection. The risk of death from HBV-related liver cancer or cirrhosis is approximately 25% for persons who become chronically infected during childhood. Chronic hepatitis B in some patients is treated with drugs called *interferon or lamivudine*, which can help some patients. Patients with cirrhosis are sometimes given liver transplants, with varying success. It is preferable to prevent this disease with vaccine than to try and cure it.

Hepatitis B vaccine has an outstanding record of safety and effectiveness. Since 1982, over one billion doses of hepatitis B vaccine have been used worldwide. The vaccine is given as a series of three intramuscular doses. Studies have shown that the vaccine is 95% effective in preventing children and adults from developing chronic infection if they have not yet been infected. In many countries where 8% to 15% of children used to become chronically infected with HBV, the rate of chronic infection has been reduced to less than 1% in immunized groups of children. Since 1991, WHO has called for all countries to add hepatitis B vaccine into their national immunization programs."

Hepatitis B surface Antigen or HBsAg is the most important protein of the envelope of Hepatitis B Virus, responsible for acute and chronic viral hepatitis.
The surface antigen contains the determinant "a", common to all the known viral subtypes, immunologically distinguished by two distinct subgroups (ay and ad).
The ability to detect HBsAg with high sensitive immunoassays in the last years has led to an understanding of its distribution and epidemiology worldwide and to radically decrease the risk of infection in transfusion.

C. PRINCIPLE OF THE TEST

A mix of mouse monoclonal antibodies specific to the determinants "a", "d" and "y" of HBsAg is fixed to the surface of microwells. Patient's serum/plasma is added to the microwell together with a second mix of mouse monoclonal antibodies, conjugated with Horseradish Peroxidase (HRP) and directed against a different epitope of the determinant "a" and against "preS".
The specific immunocomplex, formed in the presence of HBsAg in the sample, is captured by the solid phase.
At the end of the one-step incubation, microwells are washed to remove unbound serum proteins and HRP conjugate.
The chromogen/substrate is then added and, in the presence of captured HBsAg immunocomplex, the colorless substrate is hydrolyzed by the bound HRP conjugate to a colored end-product. After blocking the enzymatic reaction, its optical density is measured by an ELISA reader.
The color intensity is proportional to the amount of HBsAg present in the sample.
The version ULTRA is particularly suitable for automated screenings and is able to detect "s" mutants.

D. COMPONENTS

The standard configuration contains reagents to perform 192 tests and is made of the following components:

1. Microplate MICROPLATE
n° 2. 12 strips of 8 breakable wells coated with anti HBsAg, affinity purified mouse monoclonal antibodies, specific to "a", "y" and "d" determinants, and sealed into a bag with desiccant.

2. Negative Control CONTROL -
1x4.0ml/vial. Ready to use control. It contains goat serum, 10 mM phosphate buffer pH 7.4+/-0.1, 0.09% Na-azide and 0.1% Kathon GC as preservatives. The negative control is pale yellow color coded.

3. Positive Control CONTROL +
1x4.0ml/vial. Ready to use control. It contains goat serum, non infectious recombinant HBsAg, 10 mM phosphate buffer pH 7.4+/-0.1, 0.02% gentamicine sulphate and 0.1% Kathon GC as preservatives. The positive control is color coded green.

4. Calibrator CAL ….
n° 2 vials. Lyophilized calibrator. To be dissolved with EIA grade water as reported in the label. Contains fetal bovine serum, non infectious recombinant HBsAg at 0.5 IU/ml (2nd WHO international standard for HBsAg, NIBSC code 00/588), 10 mM phosphate buffer pH 7.4+/-0.1, 0.02% gentamicine sulphate and 0.1% Kathon GC as preservatives.
Note: The volume necessary to dissolve the content of the vial may vary from lot to lot. Please use the right volume reported on the label .

5. Wash buffer concentrate WASHBUF 20X
2x60ml/bottle. 20X concentrated solution. Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2, 0.05% Tween 20 and 0.1% Kathon GC.

6. Enzyme Conjugate Diluent CONJ DIL
2x16ml/vial. Ready to use and pink/red color coded regent. It contains 10 mM Tris buffer pH 6.8+/-0.1, 1% normal mouse serum, 5% BSA, 0.1% Kathon GC and 0.02% gentamicine sulphate as preservatives. The solution is normally opalescent.

7. Enzyme Conjugate CONJ 20X
2x1ml/vial. 20X concentrated reagent. It contains Horseradish Peroxidase (HRP) labeled mouse monoclonal antibodies to HBsAg, determinant "a" and "preS", 10 mM Tris buffer pH 6.8+/-0.1, 5% BSA, 0.1% Kathon GC and 0.02% gentamicine sulphate as preservatives.

8. Chromogen/Substrate SUBS TMB
2x25ml/bottle. It contains a 50 mM citrate-phosphate buffered solution at pH 3.5-3.8, 4% dimethylsulphoxide, 0.03% tetra-methyl-benzidine (TMB) and 0.02% hydrogen peroxide (H2O2).
Note: To be stored protected from light as sensitive to strong illumination.

9. Sulphuric Acid H_2SO_4 O.3 M
1x25ml/bottle. It contains 0.3 M H2SO4 solution.
Note: Attention: Irritant (H315; H319; P280; P302+P352; P332+P313; P305+P351+P338; P337+P313; P362+P363)

10. Plate sealing foils n° 4

11. Package insert

Important note:
Only upon specific request , Dia.Pro can supply reagents for 96, 480, 960 tests , as reported below**:**

Microplates	N°1	N°5	N°10
Negative Control	1x2ml/vial	1x10ml/vial	1x20ml/vial
Positive Control	1x2ml/vial	1x10ml/vial	1x20ml/vial
Calibrator	N° 1 vial	N° 5 vials	N° 10 vials
Wash buffer concentrate	1x60ml/vial	5x60ml/vial	4x150ml/vial
Enzyme conjugate	1x0.8ml/vial	1x4ml/vial	2x4ml/vial
Conjugate Diluent	1x16ml/vial	2x40ml/vial	2x80ml/vial
Chromogen/Substrate	1x25ml/vial	3x42ml/vial	2x125ml/vial
Sulphuric Acid	1x15ml/vial	2x40ml/vial	2x80ml/vial
Plate sealing foils	N° 2	N° 10	N° 20
Package insert	N° 1	N° 1	N° 1
Number of tests	96	480	960
Code SAG1ULTRA.CE	96	480	960

E. MATERIALS REQUIRED BUT NOT PROVIDED
1. Calibrated Micropipettes (150ul, 100ul and 50ul) and disposable plastic tips.
2. EIA grade water (double distilled or deionised, charcoal treated to remove oxidizing chemicals used as disinfectants).
3. Timer with 60 minute range or higher.
4. Absorbent paper tissues.
5. Calibrated ELISA microplate thermostatic incubator (dry or wet), capable to provide shaking at 1300 rpm+/-150, set at +37°C.
6. Calibrated ELISA microwell reader with 450nm (reading) and with 620-630nm (blanking) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.

F. WARNINGS AND PRECAUTIONS
1. The kit has to be used by skilled and properly trained technical personnel only, under the supervision of a medical doctor responsible of the laboratory.
2. When the kit is used for the screening of blood units and blood components, it has to be used in a laboratory certified and qualified by the national authority in that field (Ministry of Health or similar entity) to carry out this type of analysis.
3. All the personnel involved in performing the assay have to wear protective laboratory clothes, talc-free gloves and glasses. The use of any sharp (needles) or cutting (blades) devices should be avoided. All the personnel involved should be trained in biosafety procedures, as recommended by the Center for Disease Control, Atlanta, U.S. and reported in the National Institute of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
4. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.
5. The laboratory environment should be controlled so as to avoid contaminants such as dust or air-born microbial agents, when opening kit vials and microplates and when performing the test. Protect the Chromogen (TMB) from strong light and avoid vibration of the bench surface where the test is undertaken.
6. Upon receipt, store the kit at 2..8°C into a temperature controlled refrigerator or cold room.
7. Do not interchange components between different lots of the kits. It is recommended that components between two kits of the same lot should not be interchanged.
8. Check that the reagents are clear and do not contain visible heavy particles or aggregates. If not, advise the laboratory supervisor to initiate the necessary procedures for kit replacement.
9. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each sample.
10. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one.
11. Do not use the kit after the expiration date stated on the external container and internal (vials) labels. A study conducted on an opened kit has not pointed out any relevant loss of activity up to 6 re-use of the device and up to 6 months.
12. Treat all specimens as potentially infective. All human serum specimens should be handled at Biosafety Level 2, as recommended by the Center for Disease Control, Atlanta, U.S. in compliance with what reported in the Institutes of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
13. The use of disposable plastic-ware is recommended in the preparation of the liquid components or in transferring components into automated workstations, in order to avoid cross contamination.
14. Waste produced during the use of the kit has to be discarded in compliance with national directives and laws concerning laboratory waste of chemical and biological substances. In particular, liquid waste generated from the washing procedure, from residuals of controls and from samples has to be treated as potentially infective material and inactivated before waste. Suggested procedures of inactivation are treatment with a 10% final concentration of household bleach for 16-18 hrs or heat inactivation by autoclave at 121°C for 20 min..
15. Accidental spills from samples and operations have to be adsorbed with paper tissues soaked with household bleach and then with water. Tissues should then be discarded in proper containers designated for laboratory/hospital waste.
16. The Stop Solution is an irritant. In case of spills, wash the surface with plenty of water
17. Other waste materials generated from the use of the kit (example: tips used for samples and controls, used microplates) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

G. SPECIMEN: PREPARATION AND WARNINGS

1. Blood is drawn aseptically by venepuncture and plasma or serum is prepared using standard techniques of preparation of samples for clinical laboratory analysis. No influence has been observed in the preparation of the sample with citrate, EDTA and heparin.
2. Avoid any addition of preservatives to samples; especially sodium azide as this chemical would affect the enzymatic activity of the conjugate, generating false negative results.
3. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results. When the kit is used for the screening of blood units, bar code labeling and electronic reading is strongly recommended.
4. Haemolysed (red) and lipemic ("milky") samples have to be discarded as they could generate false results. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as well as they could give rise to false positive results. Specimens with an altered pathway of coagulation, presenting particles after blood collection and preparation of serum/plasma as those coming from hemodialized patients, could give origin to false positive results.
5. Sera and plasma can be stored at +2°..8°C for up to seven days after collection. For longer storage periods, samples can be stored frozen at –20°C for several months. Any frozen sample should not be frozen/thawed more than once as this may generate particles that could affect the test result. If some turbidity is present or presence of microparticles is suspected after thawing, filter the sample on a disposable 0.2-0.8u filter to clean it up for testing or use the two-steps alternative method.

H. PREPARATION OF COMPONENTS AND WARNINGS

A study conducted on an opened kit has not pointed out any relevant loss of activity up to 6 re-uses of the device and up to 6 months.

1. Microplates:
Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant has not turned green, indicating a defect in conservation.
In this case, call Dia.Pro's customer service.
Unused strips have to be placed back inside the aluminum pouch, with the desiccant supplied, firmly zipped and stored at +2°..8°C. After first opening, remaining strips are stable until the humidity indicator inside the desiccant bag turns from yellow to green.

2. Negative Control:
Ready to use. Mix well on vortex before use.

3. Positive Control:
Ready to use. Mix well on vortex before use. The positive control does not contain any infective HBV as it is composed of recombinant synthetic HBsAg.

4. Calibrator:
Add the volume of ELISA grade water, reported on the label, to the lyophilized powder; let fully dissolve and then gently mix on vortex. The solution is not stable. Store the Calibrator frozen in aliquots at –20°C.

5. Wash buffer concentrate:
The 20x concentrated solution has to be diluted with EIA grade water up to 1200 ml and mixed gently end-over-end before use. As some salt crystals may be present into the vial, take care to dissolve all the content when preparing the solution.
In the preparation avoid foaming as the presence of bubbles could give origin to a bad washing efficiency.
Note: *Once diluted, the wash solution is stable for 1 week at +2..8° C.*

6. Enzyme conjugate:
The working solution is prepared by diluting the 20X concentrated reagent into the Conjugate
Mix well on vortex before use.
Avoid any contamination of the liquid with oxidizing chemicals, dust or microbes. If this component has to be transferred, use only plastic sterile disposable containers.
Important note*: The working solution is not stable. Prepare only the volume necessary for the work of the day. As an example when the kit is used in combination with other instruments or manually, dilute 0.1 ml 20X Conjugate with 1.9 ml Conjugate Diluent into a disposable plastic vial and mix carefully before use.*

7. Chromogen/Substrate:
Ready to use. Mix well by end-over-end mixing.
Avoid contamination of the liquid with oxidizing chemicals, air-driven dust or microbes. Do not expose to strong light, oxidizing agents and metallic surfaces.
If this component has to be transferred use only plastic, and if possible, sterile disposable container.

8. Sulphuric Acid:
Ready to use. Mix well by end-over-end mixing.
Attention*: Irritant* (H315; H319; P280; P302+P352; P332+P313; P305+P351+P338; P337+P313; P362+P363).

Legenda:

*Warning **H statements:***
***H315** – Causes skin irritation.*
***H319** – Causes serious eye irritation.*

*Precautionary **P statements:***
***P280** – Wear protective gloves/protective clothing/eye protection/face protection.*
***P302 + P352** – IF ON SKIN: Wash with plenty of soap and water.*
***P332 + P313** – If skin irritation occurs: Get medical advice/attention.*
***P305 + P351 + P338** – IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.*
***P337 + P313** – If eye irritation persists: Get medical advice/attention.*
***P362 + P363** - Take off contaminated clothing and wash it before reuse*.

I. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

1. **Micropipettes** have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (70% ethanol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample or the components of the kit. They should also be regularly maintained in order to show a precision of 1% and a trueness of ±2%.
2. The **ELISA incubator** has to be set at +37°C (tolerance of ±1°C) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water baths are suitable for the incubations, provided that the instrument is validated for the incubation of ELISA tests.
3. In case of **shaking** during incubations, the instrument has to ensure 350 rpm ±150. Amplitude of shaking is very important as a wrong one could give origin to splashes and therefore to some false positive result.
4. The **ELISA washer** is extremely important to the overall performances of the assay. The washer must be carefully validated and correctly optimized using the kit controls/calibrator and reference panels, before using the kit for routine laboratory tests. Usually 4-5 washing cycles (aspiration + dispensation of 350ul/well of washing solution = 1 cycle) are sufficient to ensure that the assay performs as expected. A soaking time of 20-30 seconds between cycles is suggested. In order to set correctly their number, it

is recommended to run an assay with the kit controls/calibrator and well-characterized negative and positive reference samples, and check to match the values reported below in the section "Internal Quality Control". Regular calibration of the volumes delivered and maintenance (decontamination and cleaning of needles) of the washer has to be carried out according to the instructions of the manufacturer.

5. **Incubation times** have a tolerance of ±5%.
6. The **microplate reader** has to be equipped with a reading filter of 450nm and with a second filter (620-630nm, strongly recommended) for blanking purposes. Its standard performances should be (a) bandwidth ≤ 10 nm; (b) absorbance range from 0 to ≥ 2.0; (c) linearity to ≥ 2.0; (d) repeatability ≥ 1%. Blanking is carried out on the well identified in the section "Assay Procedure". The optical system of the reader has to be calibrated regularly to ensure that the correct optical density is measured. It should be regularly maintained according to the manufacturer 's instructions.
7. When using **ELISA automated workstations**, all critical steps (dispensation, incubation, washing, reading, shaking, data handling, etc.) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the sections "Internal Quality Control". The assay protocol has to be installed in the operating system of the unit and validated by checking full matching the declared performances of the kit. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set paying particular attention to avoid carry over by the needles used for dispensing samples and for washing. The carry over effect must be studied and controlled to minimize the possibility of contamination of adjacent wells due to strongly reactive samples, leading to false positive results. The use of ELISA automated work stations is recommended for blood screening and when the number of samples to be tested exceed 20-30 units per run.
8. When using automatic devices, in case the vial holder of the instrument does not fit with the vials supplied in the kit, transfer the solution into appropriate containers and label them with the same label peeled out from the original vial. This operation is important in order to avoid mismatching contents of vials, when transferring them. When the test is over, return the secondary labeled containers to 2..8°C, firmly capped.
9. **Dia.Pro's customer service** offers support to the user in the setting and checking of instruments used in combination with the kit, in order to assure full compliance with the essential requirements of the assay. Support is also provided for the installation of new instruments to be used in combination with the kit.

L. PRE ASSAY CONTROLS AND OPERATIONS

1. Check the expiration date of the kit printed on the external label of the kit box. Do not use if expired.
2. Check that the liquid components are not contaminated by naked-eye visible particles or aggregates. Check that the Chromogen/Substrate is colorless or pale blue. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box. Check that the aluminum pouch, containing the microplate, is not punctured or damaged.
3. Dilute all the content of the 20x concentrated Wash Solution as described above.
4. Dilute the 20X concentrated Enzyme Conjugate with its Diluent as reported.
5. Dissolve the Calibrator as described above.
6. Allow all the other components to reach room temperature (about 1 hr) and then mix as described.
7. Set the ELISA incubator at +37°C and prepare the ELISA washer by priming with the diluted washing solution, according to the manufacturers instructions. Set the right number of washing cycles as found in the validation of the instrument for its use with the kit.
8. Check that the ELISA reader has been turned on at least 20 minutes before reading.
9. If using an automated workstation, turn it on, check settings and be sure to use the right assay protocol.
10. Check that the micropipettes are set to the required volume.
11. Check that all the other equipment is available and ready to use.
12. In case of problems, do not proceed further with the test and advise the supervisor.

M. ASSAY PROCEDURE

The assay has to be carried out according to what reported below, taking care to maintain the same incubation time for all the samples in testing.

Automated assay:

In case the test is carried out automatically with an ELISA system, we suggest to make the instrument dispense first 150 ul controls & calibrator, then all the samples and finally 100 ul diluted Enzyme Conjugate.
For the pre-washing step (point 1 of the assay procedure) and all the next operations follow the operative instructions reported below for the Manual Assay.
It is strongly recommended to check that the time lap between the dispensation of the first and the last sample will be calculated by the instrument and taken into consideration by delaying the first washing operation accordingly.

Manual Assay:

1. Place the required number of strips in the plastic holder and wash them once to hydrate wells. Carefully identify the wells for controls, calibrator and samples.

Important note: *Pre washing (1 cycle: dispensation of 350ul/well of washing solution+ aspiration) is fundamental to obtain reliable and specific results both in the manual and in the automatic procedures. Do not omit it !*

2. Leave the A1 well empty for blanking purposes.
3. Pipette 150µl of the Negative Control in triplicate, 150ul of the Calibrator in duplicate and then 150ul of the Positive Control in single followed by 150ul of each of the samples.
4. Check for the presence of samples in wells by naked eye (there is a marked color difference between empty and full wells) or by reading at 450/620nm. (samples show OD values higher than 0.100).
5. Dispense 100ul diluted Enzymatic Conjugate in all wells, except for A1, used for blanking operations.

Important note: *Be careful not to touch the inner surface of the well with the pipette tip when the conjugate is dispensed. Contamination might occur.*

6. Following addition of the conjugate, check that the color of the samples have changed from yellowish to pink/red and then incubate the microplate for **120 min at +37°C**.

Important notes:

a. Strips have to be sealed with the adhesive sealing foil, only when the test is performed manually. Do not cover strips when using ELISA automatic instruments.
b. If the procedure is carried out on shaking, be sure to deliver the rpm reported for in Section I.3 as otherwise intra-well contamination could occur.

7. When the first incubation is over, wash the microwells as previously described (section I.4)

8. Pipette 200 µl Chromogen/Substrate into all the wells, A1 included.

Important note: *Do not expose to strong direct light as a high background might be generated.*

9. Incubate the microplate protected from light at **18-24°C for 30 min.** Wells dispensed with the positive control, the calibrator and positive samples will turn from clear to blue.
10. Pipette 100 µl Sulphuric Acid into all the wells to stop the enzymatic reaction, using the same pipetting sequence as in step 8. Addition of the acid solution will turn the positive control, the calibrator and positive samples from blue to yellow/brown.
11. Measure the color intensity of the solution in each well, as described in section I.6 using a 450nm filter (reading) and a 620-630nm filter (background subtraction, strongly recommended), blanking the instrument on A1.

Important general notes:

1. *If the second filter is not available, ensure that no fingerprints or dust are present on the external bottom of the microwell before reading at 450nm. They could generate false positive results on reading.*
2. *Reading should ideally be performed immediately after the addition of the acid solution but definitely no longer than 20 minutes afterwards. Some self-oxidation of the chromogen can occur leading to a higher background.*
3. *When samples to be tested are not surely clean or have been stored frozen, the assay procedure reported below is recommended as long as it is far less sensitive to interferences due to hemolysis, hyperlipaemia, bacterial contamination and fibrin microparticles. The assay is carried out in two-steps at +37°C on shaking at* 350 rpm ±150 *as follows:*
   - *dispense 100 ul of controls, calibrator and samples*
   - *incubate 60 min at +37°C on shaking*
   - *wash according to instructions (section I.4)*
   - *dispense 100 ul diluted enzyme tracer*
   - *incubate 30 min at +37°C on shaking*
   - *wash*
   - *dispense 100 ul TMB&H2O2 mix*
   - *incubate 30 min at r.t. on shaking*
   - *stop and read*

   In this procedure the pre-wash can be omitted.
   This method shows performances similar to the standard one and therefore can be used in alternative.
4. *The Calibrator (CAL) does not affect the cut-off calculation and therefore the test results calculation. The Calibrator may be used only when a laboratory internal quality control is required by the management.*

N. ASSAY SCHEME

Operations	Procedure
Pre-Washing step	n° 1 cycle
Controls&Calibrator&samples Diluted Enzyme Conjugate	150 ul 100 ul
1st incubation	**120 min**
Temperature	+37°C
Washing steps	n° 4-5
Chromogen/Substrate	200ul
2nd incubation	**30 min**
Temperature	room
Sulphuric Acid	100 ul
Reading OD	450nm

An example of dispensation scheme is reported in the following section:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S2										
B	NC	S3										
C	NC	S4										
D	NC	S5										
E	CAL	S6										
F	CAL	S7										
G	PC	S8										
H	S1	S9										

Legenda: BLK = Blank NC = Negative Control
CAL = Calibrator PC = Positive Control S = Sample

O. INTERNAL QUALITY CONTROL

A check is performed on the controls/calibrator any time the kit is used in order to verify whether the expected OD450nm or S/Co values have been matched in the analysis.

Ensure that the following results are met:

Parameter	Requirements
Blank well	< 0.100 OD450nm value
Negative Control (NC)	< 0.050 mean OD450nm value after blanking
Calibrator 0.5 IU/ml	S/Co ≥ 2
Positive Control	> 1.000 OD450nm value

If the results of the test match the requirements stated above, proceed to the next section.
If they do not, do not proceed any further and perform the following checks:

Problem	Check
Blank well > 0.100 OD450nm	1. that the Chromogen/Substrate solution has not become contaminated during the assay
Negative Control (NC) > 0.050 OD450nm after blanking	1. that the washing procedure and the washer settings are as validated in the pre qualification study; 2. that the proper washing solution has been used and the washer has been primed with it before use; 3. that no mistake has been done in the assay procedure (dispensation of positive control instead of the negative one); 4. that no contamination of the negative control or of the wells where the control was dispensed has occurred due to spills of positive samples or of the enzyme conjugate; 5. that micropipettes have not become contaminated with positive samples or with the enzyme conjugate 6. that the washer needles are not blocked or partially obstructed.

Calibrator S/Co < 2	1. that the procedure has been correctly performed; 2. that no mistake has occurred during its distribution (ex.: dispensation of negative control instead of calibrator) 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the calibrator has occurred.
Positive Control < 1.000 OD450nm	1. that the procedure has been correctly performed; 2. that no mistake has occurred during the distribution of the control (dispensation of negative control instead of positive control. In this case, the negative control will have an OD450nm value > 0.050). 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the positive control has occurred.

If any of the above problems have occurred, report the problem to the supervisor for further actions.

P. CALCULATION OF THE CUT-OFF

The test results are calculated by means of a cut-off value determined on the mean OD450nm value of the negative control (NC) with the following formula:

NC + 0.050 = Cut-Off (Co)

The value found for the test is used for the interpretation of results as described in the next paragraph.

***Important note**: When the calculation of results is performed by the operating system of an ELISA automated work station, ensure that the proper formulation is used to calculate the cut-off value and generate the correct interpretation of results.*

Q. INTERPRETATION OF RESULTS

Test results are interpreted as a ratio of the sample OD450nm (S) and the Cut-Off value (Co), mathematically S/Co, according to the following table:

S/Co	Interpretation
< 0.9	Negative
0.9 – 1.1	Equivocal
> 1.1	Positive

A negative result indicates that the patient is not infected by HBV and that the blood unit may be transfused.
Any patient showing an equivocal result should be retested on a second sample taken 1-2 weeks after the initial sample; the blood unit should not be transfused.
A positive result is indicative of HBV infection and therefore the patient should be treated accordingly or the blood unit should be discarded.

Important notes:

1. *Interpretation of results should be done under the supervision of the laboratory supervisor to reduce the risk of judgment errors and misinterpretations.*
2. *Any positive result must be confirmed first by repeating the test on the sample, after having filtered it on 0.2-0.8 u filter to remove any microparticles interference. Then, if still positive, the sample has to be submitted to a confirmation test before a diagnosis of viral hepatitis is released.*
3. *When test results are transmitted from the laboratory to another department, attention must be paid to avoid erroneous data transfer.*
4. *Diagnosis of viral hepatitis infection has to be taken and released to the patient by a suitably qualified medical doctor.*

An example of calculation is reported below.

The following data must not be used instead or real figures obtained by the user.

Negative Control: 0.012 – 0.008 – 0.010 OD450nm
Mean Value: 0.010 OD450nm
Lower than 0.050 – Accepted
Positive Control: 2.489 OD450nm
Higher than 1.000 – Accepted
Cut-Off = 0.010+0.050 = 0.060
Calibrator: 0.350 - 0.370 OD450nm
Mean value: 0.360 OD450nm S/Co = 6.0
S/Co higher than 2.0 – Accepted
Sample 1: 0.028 OD450nm
Sample 2: 1.690 OD450nm
Sample 1 S/Co < 0.9 = negative
Sample 2 S/Co > 1.1 = positive

R. PERFORMANCE CHARACTERISTICS

Evaluation of Performances has been conducted in accordance to what reported in the Common Technical Specifications or CTS (art. 5, Chapter 3 of IVD Directive 98/79/EC). Version ULTRA proved to be at least equivalent to the original design in a study conducted for the validation of the new version.

1. Analytical Sensitivity

The limit of detection of the assay has been calculated on the 2nd WHO international standard, NIBSC code 00/588.
In the following table, results are given for three lots (P1, P2 and P3) of the version ULTRA in comparison with the reference device (Ref.):

WHO IU/ml	Lot # P1 S/Co	Lot # P2 S/Co	Lot # P3 S/Co	Ref. S/Co
0.4	**4.6**	**4.8**	**4.6**	**4.6**
0.2	**2.3**	**2.4**	**2.4**	**2.4**
0.1	**1.4**	**1.4**	**1.5**	**1.2**
0.05	0.8	0.8	***1.0***	0.7
0.025	0.6	0.6	0.6	0.4
FCS (NC)	0.3	0.2	0.3	0.1

The assay shows an Analytical Sensitivity better than 0.1 WHO IU/ml of HBsAg.

In addition two panels of sensitivity supplied by EFS, France, and by SFTS, France, were tested and gave in the best conditions the following results:

Panel EFS Ag HBs HB1-HB6 lot n° 04

Sample ID	Characteristics	ng/ml	S/Co
HB1	diluent	/	0,2
HB2	adw2+ayw3	0.05	0,6
HB3	adw2+ayw3	0.1	**1,0**
HB4	adw2+ayw3	0.2	**1,8**
HB5	adw2+ayw3	0.3	**2,4**
HB6	adw2+ayw3	0.5	**4,2**

Sensitivity panel SFTS, France, Ag HBs 2005

Sample ID	Characteristics	ng/ml	S/Co
171	Adw2 + ayw3	2.21 ± 0.15	**15,4**
172	Adw2 + ayw3	1.18 ± 0.10	**8,7**
173	Adw2 + ayw3	1.02 ± 0.05	**6,1**
174	Adw2 + ayw3	0.64 ± 0.04	**4,0**
175	Adw2 + ayw3	0.49 ± 0.03	**3,4**
176	Adw2 + ayw3	0.39 ± 0.02	**2,6**
177	Adw2 + ayw3	0.25 ± 0.02	**2,0**
178	Adw2 + ayw3	0.11 ± 0.02	**1,3**
179	Adw2 + ayw3	0.06 ± 0.01	***0,9***
180	Adw2 + ayw3	0.03 ± 0.01	0,8
181	Adw2	0.5 – 1.0	**4,7**
182	Adw4	0.5 – 1.0	**3,6**
183	Adr	0.5 – 1.0	**4,5**
184	Ayw1	0.5 – 1.0	**5,1**
185	Ayw2	0.5 – 1.0	**6,4**
186	Ayw3	0.5 – 1.0	**7,3**
187	Ayw3	0.5 – 1.0	**5,8**
188	Ayw4	0.5 – 1.0	**6,9**
189	Ayr	0.5 – 1.0	**6,1**
190	diluent	/	0,6

The panel # 808, supplied by Boston Biomedical Inc., USA, was also tested to define the limit of sensitivity.
Results in the best conditions are as follows :

BBI panel PHA 808

Sample ID	Characteristics	ng/ml	S/Co
01	ad	2,49	**10,2**
02	ad	1,17	**4,8**
03	ad	1,02	**4,3**
04	ad	0,96	**3,8**
05	ad	0,69	**2,9**
06	ad	0,50	**2,2**
07	ad	0,41	**1,5**
08	ad	0,37	**1,3**
09	ad	0,30	**1,2**
10	ad	0,23	***1,0***
11	ay	2,51	**11,2**
12	ay	1,26	**5,9**
13	ay	0,97	**4,1**
14	ay	0,77	**3,7**
15	ay	0,63	**2,0**
16	ay	0,48	**2,4**
17	ay	0,42	**2,0**
18	ay	0,33	**1,8**
19	ay	0,23	**1,6**
20	ay	0,13	**1,1**
21	negative	/	0,6

2. Diagnostic Sensitivity:
The diagnostic sensitivity was tested according to what required by Common Technical Specifications (CTS) of the directive 98/79/EC on IVD for HBsAg testing.
Positive samples, including HBsAg subtypes and a panel of "s" mutants from most frequent mutations, were collected from different HBV pathologies (acute, a-symptomatic and chronic hepatitis B) or produced synthetically, and were detected positive in the assay.
All the HBsAg known subtypes, "ay" and "ad", and isoforms "w" and "r", supplied by CNTS, France, were tested in the assay and determined positive by the kit as expected.
An overall value of 100% has been found in a study conducted on a total number of more than 400 samples positive with the original reference IVD code SAG1.CE, CE marked.
A total of 30 sero-conversions were studied, most of them produced by Boston Biomedica Inc., USA.

Results obtained by examining eight panels supplied by Boston Biomedica Inc., USA, are reported below for the version ULTRA in comparison with the reference device code SAG1.CE.

Panel ID	1st sample positive	HBsAg subtype	HBsAg ng/ml	Version ULTRA S/Co	Ref. device S/Co
PHM 906	02	ad	0.5	**3.7**	**1.4**
PHM 907 (M)	06	ay	1.0	**4.4**	**2.9**
PHM 909	04	ad	0.3	**1.2**	0.8
PHM 914	04	ad	0.5	**1.1**	**1.1**
PHM 918	02	ad	0.1	**1.8**	0.5
PHM 923	03	ay	< 0.2	**2.2**	**1.2**
PHM 925	03	Ind.	n.d.	**1.4**	*0.9*
PHM 934	01	ad	n.d.	**1.0**	0.8

3. Diagnostic Specificity:
It is defined as the probability of the assay of scoring negative in the absence of specific analyte. In addition to the first study, where more than 5000 negative samples from blood donors (two blood centers), classified negative with a CE marked device in use at the laboratory of collection were examined, the diagnostic specificity was recently assessed by testing a total of 2288 negative blood donors on seven different lots. A value of specificity of 100% was found.
Both plasma, derived with different standard techniques of preparation (citrate, EDTA and heparin), and sera have been used to determine the specificity.
No false reactivity due to the method of specimen preparation has been observed.
Frozen specimens have also been tested to check whether samples freezing interferes with the performance of the test. No interference was observed on clean and particle free samples.
Samples derived from patients with different viral (HCV, HAV) and non viral pathologies of the liver that may interfere with the test were examined. No cross reaction were observed.

4. Precision:
It has been calculated for the version ULTRA on two samples examined in 16 replicates in 3 different runs for three lots.
Results are reported in the following tables:

Average values Total n = 144	Negative Sample	Calibrator 0.5 IU/ml
OD450nm	0.026	0.332
Std.Deviation	0.004	0.027
CV %	16%	8%

The variability shown in the tables did not result in sample misclassification.

S. LIMITATIONS
Repeatable false positive results were assessed on freshly collected specimens in less than 0.1% of the normal population, mostly due to high titers Heterophilic Anti Mouse Antibodies (HAMA).
Interferences in fresh samples were also observed when they were not particles-free or were badly collected (see chapter G).
Old or frozen samples, presenting fibrin clots, crioglobulins, lipid-containing micelles or microparticles after storage or thawing, can generate false positive results.

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All the IVD Products manufactured by the company are under the control of a certified Quality Management System approved by an EC Notified Body. Each lot is submitted to a quality control and released into the market only if conforming with the EC technical specifications and acceptance criteria.

Manufacturer:
Dia.Pro Diagnostic Bioprobes S.r.l.
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CE
0318

HEV IgG

Third generation Enzyme Immunoassay for the determination of IgG antibodies to Hepatitis E Virus in human serum and plasma

- for “in vitro” diagnostic use only -

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REF EVG.CE
96 Tests

HEV IgG

A. INTENDED USE
Third generation Enzyme ImmunoAssay (ELISA) for the qualitative determination of IgG antibodies to Hepatitis E Virus in human plasma and sera.
The kit is intended for the follow-up of HEV-infected patients.
For "in vitro" diagnostic use only.

B. INTRODUCTION
Hepatitis E Virus or HEV is a recently discovered agent of enterically transmitted viral hepatitis. HEV is an un-enveloped single-strand RNA virus, after being provisionally assigned to the Caliciviridae family, HEV was re-classified as the sole member of the genus Hepevirus, family Hepeviridae, in 2004. HEV is found in the stool of infected patients and present in 4 strains (1, 2, 3 and 4) differently spread geographically and virulent.
HEV is a serious problem in many developing countries since its first outbreak was reported in 1955 in New Delhi, India.
A high case-fatality rate has been found among pregnant women and chronic hepatitis carriers.
The cloning and sequencing of HEV genome have led to the development of serological tests for the detection of anti HEV antibodies based on recombinant immunodominant antigens derived from conservative regions of the four virus strains.

C. PRINCIPLE OF THE TEST
Microplates are coated with HEV-specific recombinant antigens encoding for conservative and immunodominant determinants of all the 4 subtypes.
The solid phase is first treated with the diluted sample and anti HEV IgG are captured, if present, by the antigens.
After washing out all the other components of the sample, in the 2nd incubation bound anti-HEV IgG are detected by the addition of polyclonal specific anti hIgG antibodies, labelled with peroxidase (HRP).
The enzyme captured on the solid phase, acting on the substrate/chromogen mixture, generates an optical signal that is proportional to the amount of anti HEV IgG present in the sample. A cut-off value let optical densities be interpreted into anti-HEV IgG negative and positive results.

D. COMPONENTS
Code EVG.CE contains reagents for 96 tests.

1. Microplate MICROPLATE
n° 1 microplate. 12 strips of 8 microwells coated with HEV specific recombinant antigens. Plates are sealed into a bag with desiccant.

2. Negative Control CONTROL -
1x4.0ml/vial. Ready to use control. It contains 1% goat serum proteins, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.5% Tween 20, 0.09% Na-azide and 0.045% ProClin 300 as preservatives. The negative control is olive green colour coded.

3. Positive Control CONTROL +
1x4.0ml/vial. Ready to use control. It contains 1% goat serum proteins, human antibodies positive to HEV, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.5% Tween 20, 0.09% Na-azide and 0.045% ProClin 300 as preservatives.
The Positive Control is blue colour coded.

4. Calibrator CAL ….
n° 1 vial. Lyophilized calibrator. To be dissolved with the volume of EIA grade water reported on the label.
It contains foetal bovine serum proteins, human antibodies to HEV whose content is calibrated on 1st WHO reference reagent for HEV antibody, NIBSC code 95/584 at **1 IU/ml** +/-20%, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.3 mg/ml gentamicine sulphate and 0.045% ProClin 300 as preservatives.
Note: The volume necessary to dissolve the content of the vial may vary from lot to lot. Please use the right volume reported on the label.

5. Wash buffer concentrate WASHBUF 20X
1x60ml/bottle. 20x concentrated solution. Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2, 0.05% Tween 20 and 0.045% ProClin 300.

6. Enzyme Conjugate CONJ
1x16ml/vial. Ready to use and red colour coded reagent. It contains Horseradish Peroxidase conjugated goat polyclonal antibodies to human IgG, 5% BSA, 10 mM Tris buffer pH 6.8+/-0.1, 0.045% ProClin 300 and 0.02% gentamicine sulphate as preservatives.

7. Chromogen/Substrate SUBS TMB
1x16ml/vial. Ready-to-use component. It contains 50 mM citrate-phosphate buffer pH 3.5-3.8, 4% dimethylsulphoxide, 0.03% tetra-methyl-benzidine or TMB and 0.02% hydrogen peroxide or H2O2.
Note: To be stored protected from light as sensitive to strong illumination.

8. Assay Diluent DILAS
1x8ml/vial. 10 mM tris buffered solution pH 8.0 +/-0.1 containing 0.045% ProClin 300 for the pre-treatment of samples and controls in the plate, blocking interference.

9. Sulphuric Acid H_2SO_4 O.3 M
1x15ml/vial. It contains 0.3 M H_2SO_4 solution.
Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

10. Sample Diluent: DILSPE
1x50ml/vial. It contains 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.5% Tween 20, 0.09% Na-azide and 0.045% ProClin 300 as preservatives. To be used to dilute the sample.
Note: The diluent changes colour from olive green to dark bluish green in the presence of sample.

11. Plate sealing foils **n° 2**

12. Package insert **n° 1**

E. MATERIALS REQUIRED BUT NOT PROVIDED
1. Calibrated Micropipettes (200ul and 10ul) and disposable plastic tips.
2. EIA grade water (bidistilled or deionised, charcoal treated to remove oxidizing chemicals used as disinfectants).
3. Timer with 60 minute range or higher.
4. Absorbent paper tissues.
5. Calibrated ELISA microplate thermostatic incubator capable to provide a temperature of +37°C.
6. Calibrated ELISA microwell reader with 450nm (reading) and with 620-630nm (blanking) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.

F. WARNINGS AND PRECAUTIONS
1. The kit has to be used by skilled and properly trained technical personnel only, under the supervision of a medical doctor responsible of the laboratory.
2. When the kit is used for the screening of blood units and blood components, it has to be used in a laboratory certified and

qualified by the national authority in that field (Ministry of Health or similar entity) to carry out this type of analysis.
3. All the personnel involved in performing the assay have to wear protective laboratory clothes, talc-free gloves and glasses. The use of any sharp (needles) or cutting (blades) devices should be avoided. All the personnel involved should be trained in biosafety procedures, as recommended by the Center for Disease Control, Atlanta, U.S. and reported in the National Institute of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
4. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.
5. The laboratory environment should be controlled so as to avoid contaminants such as dust or air-born microbial agents, when opening kit vials and microplates and when performing the test. Protect the Chromogen/Substrate from strong light and avoid vibration of the bench surface where the test is undertaken.
6. Upon receipt, store the kit at +2..8°C into a temperature controlled refrigerator or cold room.
7. Do not interchange components between different lots of the kits. It is recommended that components between two kits of the same lot should not be interchanged.
8. Check that the reagents are clear and do not contain visible heavy particles or aggregates. If not, advise the laboratory supervisor to initiate the necessary procedures for kit replacement.
9. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each sample. Do not reuse disposable tips.
10. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one. Do not reuse disposable tips.
11. Do not use the kit after the expiration date stated on the external container and internal (vials) labels.
12. Treat all specimens as potentially infective. All human serum specimens should be handled at Biosafety Level 2, as recommended by the Center for Disease Control, Atlanta, U.S. in compliance with what reported in the Institutes of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
13. The use of disposable plastic-ware is recommended in the preparation of the liquid components or in transferring components into automated workstations, in order to avoid cross contamination.
14. Waste produced during the use of the kit has to be discarded in compliance with national directives and laws concerning laboratory waste of chemical and biological substances. In particular, liquid waste generated from the washing procedure, from residuals of controls and from samples has to be treated as potentially infective material and inactivated before waste. Suggested procedures of inactivation are treatment with a 10% final concentration of household bleach for 16-18 hrs or heat inactivation by autoclave at 121°C for 20 min..
15. Accidental spills from samples and operations have to be adsorbed with paper tissues soaked with household bleach and then with water. Tissues should then be discarded in proper containers designated for laboratory/hospital waste.
16. The Sulphuric Acid is an irritant. In case of spills, wash the surface with plenty of water
17. Other waste materials generated from the use of the kit (example: tips used for samples and controls, used microplates) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

G. SPECIMEN: PREPARATION AND RECOMMANDATIONS
1. Blood is drawn aseptically by venepuncture and plasma or serum is prepared using standard techniques of preparation of samples for clinical laboratory analysis. No influence has been observed in the preparation of the sample with citrate, EDTA and heparin.
2. Avoid any addition of preservatives to samples; especially sodium azide as this chemical would affect the enzymatic activity of the conjugate, generating false negative results.
3. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results. When the kit is used for the screening of blood units, bar code labeling and electronic reading is strongly recommended.
4. Haemolysed (red) and visibly hyperlipemic ("milky") samples have to be discarded as they could generate false results. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they could give rise to false results.
5. Sera and plasma can be stored at +2°…+8°C in primary collection tubes for up to five days after collection.
Do not freeze primary tubes of collection. For longer storage periods, sera and plasma samples, carefully removed from the primary collection tube, can be stored frozen at –20°C for at least 12 months. Any frozen samples should not be frozen/thawed more than once as this may generate particles that could affect the test result.
6. If particles are present, centrifuge at 2.000 rpm for 20 min or filter using 0.2-0.8u filters to clean up the sample for testing.

H. PREPARATION OF COMPONENTS AND WARNINGS
A study conducted on an opened kit has not pointed out any relevant loss of activity up to 6 re-use of the device and up to 6 months.

1. Microplates:
Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant is not turned to dark green, indicating a defect of storing.
In this case call Dia.Pro's customer service.
Unused strips have to be placed back into the aluminium pouch, in presence of desiccant supplied, firmly zipped and stored at +2°..8°C.
When opened the first time, residual strips are stable till the indicator of humidity inside the desiccant bag turns from yellow to green.

2. Negative Control:
Ready to use. Mix well on vortex before use.

3. Positive Control:
Ready to use. Mix well on vortex before use. Handle this component as potentially infective, even if HCV, eventually present in the control, has been chemically inactivated.

4. Calibrator:
Dissolve carefully the content of the lyophilized vial with the volume of EIA grade water reported on its label.
Mix well on vortex before use.
Handle this component as potentially infective, even if HEV, eventually present in the control, has been chemically inactivated.
***Note**: When dissolved the Calibrator is not stable. Store in aliquots at –20°C.*

5. Wash buffer concentrate:
The whole content of the 20x concentrated solution has to be diluted with bidistilled water up to 1200 ml and mixed gently end-over-end before use.
Once diluted, the wash solution is stable for 1 week at +2..8° C.
In the preparation avoid foaming as the presence of bubbles could give origin to a bad washing efficiency.
***Note:** Once diluted, the wash solution is stable for 1 week at +2..8° C.*

6. Enzyme conjugate:
Ready to use. Mix well on vortex before use.

Be careful not to contaminate the liquid with oxidizing chemicals, air-driven dust or microbes.
If this component has to be transferred use only plastic, possibly sterile disposable containers.

7. Chromogen/Substrate:
Ready to use. Mix well on vortex before use.
Be careful not to contaminate the liquid with oxidizing chemicals, air-driven dust or microbes.
Do not expose to strong illumination, oxidizing agents and metallic surfaces.
If this component has to be transferred use only plastic, possible sterile disposable container.

8. Assay Diluent:
Ready to use. Mix well on vortex before use.

9. Sulphuric Acid:
Ready to use. Mix well on vortex before use.
Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

Legenda:

Warning **H statements:**
H315 – Causes skin irritation.
H319 – Causes serious eye irritation.

Precautionary **P statements:**
P280 – Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352 – IF ON SKIN: Wash with plenty of soap and water.
P332 + P313 – If skin irritation occurs: Get medical advice/attention.
P305 + P351 + P338 – IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337 + P313 – If eye irritation persists: Get medical advice/attention.
P362 + P363 – Take off contaminated clothing and wash it before reuse.

10. Sample Diluent:
Ready to use. Mix well on vortex before use.

I. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

1. **Micropipettes** have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (household alcohol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample. They should also be regularly maintained in order to show a precision of 1% and a trueness of +/-2%. Decontamination of spills or residues of kit components should also be carried out regularly.
2. The **ELISA incubator** has to be set at +37°C (tolerance of +/-0.5°C) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water baths are suitable for the incubations, provided that the instrument is validated for the incubation of ELISA tests.
3. The **ELISA washer** is extremely important to the overall performances of the assay. The washer must be carefully validated in advance, checked for the delivery of the right dispensation volume and regularly submitted to maintenance according to the manufacturer's instructions for use. In particular the washer, at the end of the daily workload, has to be extensively cleaned out of salts with deionized water. Before use, the washer has to be extensively primed with the diluted Washing Solution.
   The instrument weekly has to be submitted to decontamination according to its manual (NaOH 0.1 M decontamination suggested).
   5 washing cycles (aspiration + dispensation of 350ul/well of washing solution + 20 sec soaking = 1 cycle) are sufficient to ensure the assay with the declared performances. If soaking is not possible add one more cycle of washing.
   An incorrect washing cycle or salt-blocked needles are the major cause of false positive reactions.
4. **Incubation times** have a tolerance of $\pm$5%.
5. The **ELISA reader** has to be equipped with a reading filter of 450nm and with a second filter (620-630nm, mandatory) for blanking purposes. Its standard performances should be (a) bandwidth $\leq$ 10 nm; (b) absorbance range from 0 to $\geq$ 2.0; (c) linearity to $\geq$ 2.0; repeatability $\geq$ 1%. Blanking is carried out on the well identified in the section "Assay Procedure". The optical system of the reader has to be calibrated regularly to ensure that the correct optical density is measured. It should be regularly maintained according to the manufacturer 's instructions.
6. When using an **ELISA automated work station**, all critical steps (dispensation, incubation, washing, reading, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the sections "Validation of Test" and "Assay Performances". The assay protocol has to be installed in the operating system of the unit and validated as for the washer and the reader. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set. Particular attention must be paid to avoid carry over by the needles used for dispensing and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells. The use of ELISA automated work stations is recommended for blood screening when the number of samples to be tested exceed 20-30 units per run.
7. Dia.Pro's customer service offers support to the user in the setting and checking of instruments used in combination with the kit, in order to assure compliance with the requirements described. Support is also provided for the installation of new instruments to be used with the kit.

L. PRE ASSAY CONTROLS AND OPERATIONS

1. Check the expiration date of the kit printed on the external label of the kit box. Do not use if expired.
2. Check that the liquid components are not contaminated by naked-eye visible particles or aggregates. Check that the Chromogen/Substrate is colorless or pale blue by aspirating a small volume of it with a sterile transparent plastic pipette. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box. Check that the aluminum pouch, containing the microplate, is not punctured or damaged.
3. Dilute all the content of the 20x concentrated Wash Solution as described above.
4. Dissolve the Calibrator as described above.
5. Allow all the other components to reach room temperature (about 1 hr) and then mix as described.
6. Set the ELISA incubator at +37°C and prepare the ELISA washer by priming with the diluted washing solution, according to the manufacturers instructions. Set the right number of washing cycles as reported in the specific section.
7. Check that the ELISA reader has been turned on at least 20 minutes before reading.
8. If using an automated workstation, turn it on, check settings and be sure to use the right assay protocol.
9. Check that the micropipettes are set to the required volume.
10. Check that all the other equipment is available and ready to use.
11. In case of problems, do not proceed further with the test and advise the supervisor.

M. ASSAY PROCEDURE

The assay has to be carried out according to what reported below, taking care to maintain the same incubation time for all the samples in testing.

Automated assay:

In case the test is carried out automatically with an ELISA workstation, we suggest to make the instrument aspirate 200 ul Sample Diluent and then 10 ul sample.

All the mixture is then carefully dispensed directly into the appropriate sample well of the microplate. Before the next sample is aspirated, needles have to be duly washed to avoid any cross-contamination among samples.

Do not dilute controls/calibrator as they are ready to use.

Dispense 200 ul controls/calibrator in the appropriate control/calibration wells.

Important Note: *Visually monitor that samples have been diluted and dispensed into appropriate wells. This is simply achieved by checking that the color of dispensed samples has turned to dark bluish-green while the color of the negative control has remained olive green.*

For the next operations follow the operative instructions reported below for the Manual Assay.

It is strongly recommended to check that the time lap between the dispensation of the first and the last sample will be calculated by the instrument and taken into consideration by delaying the first washing operation accordingly.

Manual assay:

1. Place the required number of Microwells in the microwell holder. Leave the 1st well empty for the operation of blanking.
2. Dispense 200 ul of Negative Control in triplicate, 200 ul Calibrator in duplicate and 200 ul Positive Control in single in proper wells. Do not dilute Controls and Calibrator as they are pre-diluted, ready to use !
3. Add 200 ul of Sample Diluent (DILSPE) to all the sample wells; then dispense 10 ul sample in each properly identified well. Mix gently the plate, avoiding overflowing and contaminating adjacent wells, in order to fully disperse the sample into its diluent.

Important note: *Check that the color of the Sample Diluent, upon addition of the sample, changes from light green to dark bluish green, monitoring that the sample has been really added.*

4. Dispense 50 ul Assay Diluent (DILAS) into all the controls/calibrator and sample wells. Check that the color of samples has turned to dark blue.
5. Incubate the microplate for **45 min at +37°C**.

Important note: *Strips have to be sealed with the adhesive sealing foil, supplied, only when the test is carried out manually. Do not cover strips when using ELISA automatic instruments.*

6. Wash the microplate with an automatic washer by delivering and aspirating 300ul/well of diluted washing solution as reported previously (section I.3).
7. Pipette 100µl Enzyme Conjugate into each well, except the 1st blanking well, and cover with the sealer. Check that this red colored component has been dispensed in all the wells, except A1.

Important note: *Be careful not to touch the plastic inner surface of the well with the tip filled with the Enzyme Conjugate. Contamination might occur.*

8. Incubate the microplate for **45 min at +37°C**.
9. Wash microwells as in step 6.
10. Pipette 100µl Chromogen/Substrate mixture into each well, the blank well included. Then incubate the microplate at **room temperature (18-24°C) for 15 minutes**.

Important note: *Do not expose to strong direct illumination. High background might be generated.*

11. Pipette 100µl Sulphuric Acid into all the wells using the same pipetting sequence as in step 10 to stop the enzymatic reaction. Addition of acid will turn the positive control and positive samples from blue to yellow.
12. Measure the color intensity of the solution in each well, as described in section I.5, at 450nm filter (reading) and at 620-630nm (background subtraction, mandatory), blanking the instrument on A1.

Important notes:

1. *Ensure that no finger prints are present on the bottom of the microwell before reading. Finger prints could generate false positive results on reading.*
2. *Reading has to be carried out just after the addition of the Stop Solution and anyway not any longer than 20 minutes after its addition. Some self oxidation of the chromogen can occur leading to high background.*

N. ASSAY SCHEME

Method	**Operations**
Controls & Calibrator(*) Samples Assay Diluent (DILAS)	200 ul 200ul dil.+10ul 50 ul
1st incubation	**45 min**
Temperature	+37°C
Wash step	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
Enzyme conjugate	100 ul
2nd incubation	**45 min**
Temperature	+37°C
Wash step	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
TMB/H2O2	100 ul
3rd incubation	**15 min**
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm/620-630nm

(*) Important Notes:

- *The Calibrator (CAL) does not affect the Cut Off calculation, therefore it does not affect the test's results calculation.*
- *The Calibrator (CAL) used only if a laboratory internal quality control is required by the Management.*

An example of dispensation scheme is reported below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S2										
B	NC	S3										
C	NC	S4										
D	NC	S5										
E	CAL(*)	S6										
F	CAL(*)	S7										
G	PC	S8										
H	S1	S9										

Legenda: BLK = Blank NC = Negative Control
CAL(*) = Calibrator - Not mandatory PC = Positive Control
S = Sample

O. INTERNAL QUALITY CONTROL
A check is carried out on the controls any time the kit is used in order to verify whether their OD450nm/620-630nm values are as expected and reported in the table below.

Check	Requirements
Blank well	< 0.100 OD450nm value
Negative Control (NC)	< 0.050 mean OD450nm value after blanking
Positive Control	> 1.000 OD450nm value

If the results of the test match the requirements stated above, proceed to the next section.
If they do not, do not proceed any further and operate as follows:

Problem	Check
Blank well > 0.100 OD450nm	1. that the Chromogen/Sustrate solution has not got contaminated during the assay
Negative Control (NC) > 0.050 OD450nm after blanking	1. that the washing procedure and the washer settings are as validated in the pre qualification study; 2. that the proper washing solution has been used and the washer has been primed with it before use; 3. that no mistake has been done in the assay procedure (dispensation of positive control instead of negative control; 4. that no contamination of the negative control or of their wells has occurred due to positive samples, to spills or to the enzyme conjugate; 5. that micropipettes haven't got contaminated with positive samples or with the enzyme conjugate 6. that the washer needles are not blocked or partially obstructed.
Positive Control < 1.000 OD450nm	1. that the procedure has been correctly executed; 2. that no mistake has been done in the distribution of controls (dispensation of negative control instead of positive control. In this case, the negative control will have an OD450nm value > 0.150, too. 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the positive control has occurred.

Should these problems happen, after checking, report any residual problem to the supervisor for further actions.

**** Note:**
If Calibrator has used, verify the following data:

Check	Requirements
Calibrator	S/Co > 1.1

If the results of the test doesn't match the requirements stated above, operate as follows:

Problem	Check
Calibrator S/Co < 1.1	1. that the procedure has been correctly executed; 2. that no mistake has been done in its distribution (e.g..: dispensation of negative control instead of Calibrator) 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the calibrator has occurred.

Anyway, if all other parameters (Blank, Negative Control, Positive Control), match the established requirements, the test may be considered valid.

Important note:
The analysis must be done proceeding as the reading step described in the section M, point 12.

P. CALCULATION OF THE CUT-OFF
The tests results are calculated by means of a cut-off value determined with the following formula:

Cut-Off = NC mean OD450nm/620-630nm + 0.350

The value found for the test is used for the interpretation of results as described in the next paragraph.
***Important note**: When the calculation of results is done by the operative system of an ELISA automated work station be sure that the proper formulation is used to calculate the cut-off value and generate the right interpretations of results.*

Q. INTERPRETATION OF RESULTS
Test results are interpreted as ratio of the sample OD450nm/620-630nm and the Cut-Off value (or S/Co) according to the following table:

S/Co	Interpretation
< 0.9	Negative
0.9 - 1.1	Equivocal
> 1.1	Positive

A negative result indicates that the patient has not been infected by HEV or that the blood unit may be transfused.
Any patient showing an equivocal result should be tested again on a second sample taken 1-2 weeks later from the patient and examined. The blood unit should not be transfused.
A positive result is indicative of HEV infection and therefore the patient should be treated accordingly or the blood unit should be discarded.

Important notes:
1. *Interpretation of results should be done under the supervision of the responsible of the laboratory to reduce the risk of judgment errors and misinterpretations.*
2. *By definition, any positive result should be confirmed by an alternative method before a diagnosis of viral hepatitis is formulated.*
3. *When test results are transmitted from the laboratory to an informatics center, attention has to be done to avoid erroneous data transfer.*
4. *Diagnosis of viral hepatitis infection has to be done and released to the patient only by a qualified medical doctor.*

An example of calculation is reported below (data obtained proceeding as the reading step described in the section M, point 12):

The following data must not be used instead or real figures obtained by the user.

Negative Control: 0.019 – 0.020 – 0.021 OD450nm
Mean Value: 0.020 OD450nm
Lower than 0.050 – Accepted
Positive Control: 2.189 OD450nm
Higher than 1.000 – Accepted
Cut-Off = 0.020+0.350 = 0.370
Calibrator: 0.600 - 0.640 OD450nm
Mean value: 0.620 OD450nm S/Co = 1.7
S/Co higher than 1.1 – Accepted

Sample 1: 0.070 OD450nm
Sample 2: 1.690 OD450nm
Sample 1 S/Co < 0.9 = negative
Sample 2 S/Co > 1.1 = positive

R. PERFORMANCES
Evaluation of Performances has been conducted on negative and positive samples in an external clinical center with reference to a FDA approved kit.

1. LIMIT OF DETECTION
The limit of detection of the assay has been calculated by means of 1st WHO reference reagent for HEV antibody, NIBSC code 95/584. The assay shows an analytical sensitivity of about 0.1 WHO IU/ml.

2. DIAGNOSTIC SPECIFICITY AND SENSITIVITY
They were checked with a sensitivity set at 0.25 WHO IU/ml on more than total 700 samples.

2.1 Diagnostic Specificity:
It is defined as the probability of the assay of scoring negative in the absence of specific analyte.
A total number of more than one hundred 1-5 years old children, by definition negative for HEV antibodies as they never chanced to eat uncooked swine meat and get therefore infected, were tested; a value of 100% specificity (negativity) was assured at a sensitivity set at a cut-off of 0.25 WHO IU/ml.

In addition, a total of more 500 unselected donors, including 1st time donors, and HEV negative hospitalized patients, coming from Italy, were examined maintaining the sensitivity of 0.25 WHO IU/ml. About 5% of such population turned out to be repeatedly positive; confirmation was not carried out in absence of a commercial Confirmation kit. However, these samples were detected positive with a commercial CE-marked ELISA. From this study a diagnostic specificity of 100% was observed.
Moreover, the Diagnostic Specificity was also assessed by testing more than 100 potentially interfering specimens (other infectious diseases, patients affected by non viral hepatic diseases, dialysis patients, pregnant women, haemolized, lipemic, etc.). A value of specificity of 100% was assessed.
No false reactivity due to the method of specimen preparation has been observed. Both plasma, derived with different standard techniques of preparation (citrate, EDTA and heparin), and sera have been used to determine the value of specificity.
Frozen specimens have been tested, as well, to check for interferences due to collection and storage.
No interference was observed.

2.2 Diagnostic Sensitivity
It is defined as the probability of the assay of scoring positive in the presence of specific analyte.
The diagnostic sensitivity has been assessed externally (Institute of Virology, University of Milan) and internally on a total number of 200 positive specimens (maintaining a sensitivity set at a cut-off of 0.25 WHO IU/ml); a diagnostic sensitivity (or correlation with a commercial reference kit manufactured in Europe and CE marked) of 100% was found.

3. PRECISION:
It has been calculated on two samples, one negative and one low positive, examined in 16 replicates in three separate runs.
CV values ranging between 5-10%, depending on OD450nm/620-630nm values, were found. The variability seen did not result in sample misclassification.

S. LIMITATIONS
Frozen samples containing fibrin particles or aggregates after thawing have been observed to generate some false results.

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All the IVD Products manufactured by the company are under the control of a certified Quality Management System in compliance with ISO 13485 rule. Each lot is submitted to a quality control and released into the market only if conforming with the EC technical specifications and acceptance criteria.

Manufacturer:
Dia.Pro Diagnostic Bioprobes Srl
Via G. Carducci n° 27 – Sesto San Giovanni (MI) – Italy

CE

HEV IgG

Ensayo Inmunoenzimático de tercera generación para la determinación de anticuerpos IgG al Virus de la Hepatitis E en plasma y suero humanos.

Uso exclusivo para diagnóstico “in vitro”

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REF EVG.CE
96 Pruebas

HEV IgG

A. OBJETIVO DEL ESTUCHE.
Ensayo inmunoenzimático (ELISA) de tercera generación para la determinación de anticuerpos IgG al virus de la Hepatitis E en plasma y suero humanos.
El equipo está diseñado para utilizarse en el seguimiento de pacientes infectados por VHE.
Uso exclusivo para diagnóstico "in vitro".

B. INTRODUCCIÓN
El virus de la hepatitis E o VHE es un agente descubierto recientemente de hepatitis viral transmitida por vía entérica. El VHE es un virus ARN monocatenario no encapsulado. Tras asignarse de forma provisional a la familia Caliciviridae, el VHE se reclasificó como el único miembro del género Hepevirus, familia Hepeviridae, en 2004. El VHE se encuentra en las heces de pacientes infectados y se presenta en 4 cepas (1, 2, 3 y 4) con distinta distribución geográfica y virulencia.
El VHE es un problema grave en muchos países en desarrollo desde que se registró el primer brote en 1955 en Nueva Delhi, India. Se ha encontrado una tasa alta de mortalidad en mujeres embarazadas y en portadores de hepatitis crónica.
La clonación y la secuenciación del genoma del VHE ha llevado al desarrollo de pruebas serológicas para la detección de anticuerpos anti-VHE basadas en antígenos inmunodominantes recombinantes derivados de regiones conservadoras de las cuatro cepas del virus.

C. PRINCIPIOS DEL ENSAYO
Las microplacas se recubren con antígenos codificados recombinantes específicos del VHE para identificar los determinantes inmunodominantes y conservados de todos los subtipos del virus (1, 2, 3 y 4).
La fase sólida es tratada primero con la muestra diluida y los anticuerpos anti-VHE IgG son capturados, si están presentes en la muestra, por los antígenos.
Después de lavar para eliminar otros componentes de la muestra, en la segunda incubación los anticuerpos anti-VHE son detectados mediante la adición de anticuerpos anti-IgG policlonales humanos específicos, marcados con peroxidasa (HRP).
La enzima capturada en la fase sólida, actúa sobre la mezcla substrato/cromógeno, generando una señal óptica que es proporcional a la cantidad de anticuerpos anti-VHE IgG presentes en la muestra. Un valor de corte, permite que las densidades ópticas sean interpretadas como resultados anti-VHE IgG negativos o positivos .

D. COMPONENTES
Código EVG.CE ontiene reactivos suficientes para realizar 96 pruebas.

1. Microplaca MICROPLATE
n° 1 microplaca.12 tiras de 8 micropocillos recubiertos con antígenos recombinantes específicos del VHE. Las placas están envasadas en bolsas selladas con desecante.

2. Control Negativo CONTROL -
1x4.0ml/vial. Listo para el uso. Contiene 1% de proteínas del suero de cabra, tampón Citrato sódico 10mM pH 6.0 +/-0.1, 0.5% de Tween 20, además de azida sódica 0.09% y ProClin 300 0.045% como preservativos. El control negativo está codificado con el color verde olivo.

3. Control Positivo CONTROL +
1x4.0ml/vial. Listo para el uso. Contiene 1% de proteínas del suero de cabra, anticuerpos humanos anti-HEV, tampón Citrato sódico10mM pH 6.0 +/-0.1, 0.5% de Tween 20, así como azida sódica 0.09% y ProClin 300 0.045% como preservativos.
El control positivo está codificado con el color azul.

4. Calibrador CAL ….
n° 1 vial. Calibrador Liofilizado. Para ser disuelto con el volumen de agua de grado EIA indicado en la etiqueta. Contiene proteínas de suero fetal bovino, anticuerpos humanos contra HEV, cuio contenido se calibra con el 1st OMS reactivo de referencia para anticuerpo VHE, NIBSC código 99/588-003-WI, at **1 IU/ml** (+/-20%), 10mM buffer Na Citrato sódico pH 6.0 +/-0.1, 0.3 mg/ml de gentamicina sulfato y ProClin 300 0.045% como conservantes.
Nota: El volumen necesario para disolver el contenido del frasco varía en cada lote. Se recomienda usar el volumen indicado en la etiqueta.

5. Tampón de Lavado Concentrado: WASHBUF 20X
1x60ml/botella. Solución concentrada 20x. Una vez diluida, la solución de lavado contiene tampón fosfato 10 mM a pH 7.0 +/-0.2, Tween 20 al 0.05% y ProClin 300 al 0.045%.

6.Conjugado encimático CONJ
1x16ml/vial.
Preparado para el uso y reactivo codificado con el color rojo. Contiene peroxidasa de rábano conjugada con anticuerpos policlonales de cabra para IgG humanos, 5% BSA, 10 mM tampón Tris pH 6.8+/-0.1, 0.045% ProClin 300 y 0.02% de sulfato de gentamicina como conservantes.

7. Cromógemo/Substrato SUBS TMB
1x16ml/vial. Listo para el uso.Contiene una solución tamponada citrato-fosfato 50mM pH 3.5-3.8, dimetilsulfóxido 4%, tetra-metil-benzidina (TMB) 0.03% y peróxido de hidrógeno (H_2O_2) 0.02%.
Nota: Evitar la exposición a la luz, la sustancia es fotosensible.

8. Diluente de ensayo DILAS
1x8ml/vial. Contiene una solución tamponada Tris 10 mM pH 8.0 +/- 0.1 y 0.045% de ProClin 300 para el pre-tratamiento de muestras y controles, bloquea posibles interferencias.

9. Ácido Sulfúrico H_2SO_4 O.3 M
1x15ml/vial. Contiene solución de H_2SO_4 0.3M
Atención: Irritante (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

10. Diluente de muestras: DILSPE
1x50ml/vial. Contiene una solución tamponada de citrato sódico 6.0 +/-0.1, 0.5% Tween 20, 0.09% de azida sódica y 0.045% de ProClin 300 como preservativos. Se usa para diluir las muestras.
Nota: El diluente cambia de color verde olivo a verde azul oscuro en presencia de la muestra.

11. Sellador adhesivo **n° 2**

12. Manual de instrucciones **n° 1**

E. MATERIALES NECESARIOS NO SUMINISTRADOS.
1. Micropipetas calibradas (200µl y 10µl) y puntas plásticas desechables.
2. Agua de calidad EIA (bidestilada o desionizada, tratada con carbón para remover químicos oxidantes usados como desinfectantes).
3. *Timer* con un rango de 60 minutos como mínimo.
4. Papel absorbente.
5. Incubador termostatizado de microplacas ELISA, calibrado (en seco o húmedo) fijo a 37ºC.
6. Lector calibrado de microplacas de ELISA con filtros de 450nm (lectura) y de 620-630 nm.
7. Lavador calibrado de microplacas ELISA.

8. Vórtex o similar.

F. ADVERTENCIAS Y PRECAUCIONES.

1. El estuche debe ser usado por personal técnico adecuadamente entrenado, bajo la supervisión de un doctor responsable del laboratorio.
2. Cuando el estuche es usado para pesquisaje en unidades de sangre, el laboratorio debe estar certificado y calificado para realizar este tipo de análisis (Ministerio de Salud o entidad similar).
3. Todas las personas encargadas de la realización de las pruebas deben llevar las ropas protectoras adecuadas de laboratorio, guantes y gafas. Evitar el uso de objetos cortantes (cuchillas) o punzantes (agujas). El personal debe ser adiestrado en procedimientos de bioseguridad, según ha sido recomendado por el Centro de Control de Enfermedades de Atlanta, Estados Unidos, y publicado por el Instituto Nacional de Salud: "Biosafety in Microbiological and Biomedical Laboratories", ed.1984.
4. Todo el personal involucrado en el manejo de muestras debe estar vacunado contra HBV y HAV, para lo cual existen vacunas disponibles, seguras y eficaces.
5. Se debe controlar el ambiente del laboratorio para evitar la contaminación de los componentes con polvo o agentes microbianos cuando se abran los estuches, así como durante la realización del ensayo. Evitar la exposición del substrato a la luz y las vibraciones de la mesa de trabajo durante el ensayo.
6. Conservar el estuche a temperaturas entre 2-8 ºC, en un refrigerador con temperatura regulada o en cámara fría.
7. No intercambiar reactivos de diferentes lotes ni tampoco de diferentes estuches.
8. Comprobar que los reactivos no contienen precipitados ni agregados en el momento del uso. De darse el caso, informar al supervisor para realizar el procedimiento pertinente y reemplazar el estuche.
9. Evitar contaminación cruzada entre muestras de suero/ plasma usando puntas desechables y cambiándolas luego de cada uso. No reutilizar puntas desechables
10. Evitar contaminación cruzada entre los reactivos del estuche usando puntas desechables y cambiándolas luego de cada uso. No reutilizar puntas desechables
11. No usar el producto después de la fecha de caducidad indicada en el estuche e internamente en los reactivos. Según estudios realizados, no se ha detectado pérdida relevante de actividad en estuches abiertos, en uso por un período de hasta 6 meses.
12. Tratar todas las muestras como potencialmente infecciosas. Las muestras de suero humano deben ser manipuladas al nivel 2 de bioseguridad, según ha sido recomendado por el Centro de Control de Enfermedades de Atlanta, Estados Unidos y publicado por el Instituto Nacional de Salud: "Biosafety in Microbiological and Biomedical Laboratories", ed.1984.
13. Se recomienda el uso de material plástico desechable para la preparación de las soluciones de lavado y para la transferencia de los reactivos a los diferentes equipos automatizados a fin de evitar contaminaciones.
14. Los desechos producidos durante el uso del estuche deben ser eliminados según lo establecido por las directivas nacionales y las leyes relacionadas con el tratamiento de los residuos químicos y biológicos de laboratorio. En particular, los desechos líquidos provenientes del proceso de lavado deben ser tratados como potencialmente infecciosos y deben ser inactivados. Se recomienda la inactivación con lejía al 10% de 16 a 18 horas o el uso de la autoclave a 121°C por 20 minutos.
15. En caso de derrame accidental de algún producto, se debe utilizar papel absorbente embebido en lejía y posteriormente en agua. El papel debe eliminarse en contenedores designados para este fin en hospitales y laboratorios.
16. El ácido sulfúrico es irritante. En caso de derrame, se debe lavar la superficie con abundante agua.
17. Otros materiales de desecho generados durante la utilización del estuche (por ejemplo: puntas usadas en la manipulación de las muestras y controles, microplacas usadas) deben ser manipuladas como fuentes potenciales de infección de acuerdo a las directivas nacionales y leyes para el tratamiento de residuos de laboratorio.

G. MUESTRA: PREPARACIÓN Y RECOMENDACIONES.

1. Extraer la sangre asépticamente por punción venosa y preparar el suero o plasma según las técnicas estándar de los laboratorios de análisis clínico. No se ha detectado que el tratamiento con citrato, EDTA o heparina afecte las muestras.
2. Evitar el uso de preservativos, en particular azida sódica, ya que pudiera afectar la actividad enzimática del conjugado, generando resultados falsos negativos.
3. Las muestras deben estar identificadas claramente mediante código de barras o nombres, a fin de evitar errores en los resultados. Cuando el estuche se emplea para el pesquisaje en unidades de sangre, se recomienda el uso del código de barras.
4. Las muestras hemolizadas (color rojo) o hiperlipémicas (aspecto lechoso) deben ser descartadas para evitar falsos resultados, al igual que aquellas donde se observe la presencia de precipitados, restos de fibrina o filamentos microbianos.
5. El suero y el plasma pueden conservarse a una temperatura entre +2° y +8°C en tubos de recolección principales hasta cinco días después de la extracción. No congelar tubos de recolección principales. Para periodos de almacenamiento más prolongados, las muestras de plasma o suero, retiradas cuidadosamente del tubo de extracción principal, pueden almacenarse congeladas a –20°C durante almenos 12 meses, evitando luego descongelar cada muestra más de una vez, ya que se pueden generar partículas que podrían afectar al resultado de la prueba.
6. Si hay presencia de agregados, la muestra se puede aclarar mediante centrifugación a 2000 rpm durante 20 minutos o por filtración con un filtro de 0,2-0,8 micras.

H. PREPARACIÓN DE LOS COMPONENTES Y PRECAUCIONES.

Según estudios realizados, no se ha detectado pérdida relevante de actividad en estuches abiertos, utilizados hasta 6 veces, en un período de hasta 6 meses.

1. Microplacas:

Dejar la microplaca a temperatura ambiente (aprox. 1 hora) antes de abrir el envase. Compruebe que el desecante no esté de un color verde oscuro, lo que indicaría un defecto de fabricación. De ser así, debe solicitar el servicio de Dia.Pro: atención al cliente.

Las tiras de pocillos no utilizadas, deben guardarse herméticamente cerradas en la bolsa de aluminio con el desecante a 2-8ºC.

Una vez abierto el envase, las tiras sobrantes, se mantienen estables hasta que el indicador de humedad dentro de la bolsa del desecante cambie de amarillo a verde.

2. Control Negativo:

Listo para el uso. Mezclar bien con la ayuda de un vórtex, antes de usar.

3. Control Positivo:

Listo para el uso. Mezclar bien con la ayuda de un vórtex, antes de usar. Manipule este reactivo como potencialmente infeccioso, aunque las partículas virales presentes en el control han sido inactivadas químicamente.

4. Calibrador:
Disolver cuidadosamente el contenido del vial en el volumen de agua de calidad EIA indicado en la etiqueta. Mezclar bien con el vórtex antes de usar.
Manipule este reactivo como potencialmente infeccioso, aunque las partículas virales presentes en el control han sido inactivadas químicamente.
Nota: *Una vez reconstituida, la solución no es estable. Se recomienda mantenerla congelada en alícuotas a –20ºC.*

5. Solución de Lavado Concentrada:
Todo el contenido de la solución concentrada 20x debe diluirse con agua bidestilada fino a 1200 ml y mezclarse suavemente antes de usarse.
Durante la preparación evitar la formación de espuma y burbujas, lo que podría influir en la eficiencia de los ciclos de lavado.
Nota: *Una vez diluida, la solución es estable por una semana a temperaturas entre +2 y 8ºC.*

6. Conjugado:
Listo para el uso. Mezclar bien con un vórtex antes de usar. Evitar posible contaminación del líquido con oxidantes químicos, polvo o microbios. En caso de que deba transferirse el reactivo, usar contenedores de plástico, estériles y desechables, siempre que sea posible.

7. Cromógeno/ Substrato:
Listo para el uso. Mezclar bien con un vórtex antes de usar. Evitar posible contaminación del líquido con oxidantes químicos, polvo o microbios. Evitar la exposición a la luz, agentes oxidantes y superficies metálicas. En caso de que deba transferirse el reactivo, usar contenedores de plástico, estériles y desechables, siempre que sea posible.

8. Diluente de ensayo:
Listo para el uso. Mezclar bien con un vórtex antes de usar.

9. Ácido Sulfúrico:
Listo para el uso. Mezclar bien con un vórtex antes de usar.
Atención: Irritante (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

Leyenda:

Indicación de peligro, **Frases H**
H315 – Provoca irritación cutánea.
H319 – Provoca irritación ocular grave.

Consejo de prudencia, **Frases P**
P280 – Llevar guantes/prendas/gafas/máscara de protección.
P302 + P352 – EN CASO DE CONTACTO CON LA PIEL: Lavar con agua y jabón abundantes.
P332 + P313 – En caso de irritación cutánea: Consultar a un médico.
P305 + P351 + P338 – EN CASO DE CONTACTO CON LOS OJOS: Aclarar cuidadosamente con agua durante varios minutos. Quitar las lentes de contacto, si lleva y resulta fácil. Seguir aclarando.
P337 + P313 – Si persiste la irritación ocular: Consultar a un médico.
P362 + P363 – Quitarse las prendas contaminadas y lavarlas antes de volver a usarlas.

10. Diluente de muestras :
Listo para el uso. Mezclar bien con un vórtex antes de usar.

I. INSTRUMENTOS Y EQUIPAMIENTO UTILIZADOS EN COMBINACIÓN CON EL ESTUCHE.

1. Las micropipetas deben ser calibradas para dispensar correctamente el volumen requerido en el ensayo y sometidas a una descontaminación periódica de las partes que pudieran entrar accidentalmente en contacto con la muestra o los reactivos (lejía 10%, de calidad de los desinfectantes hospitalarios). Deben además, ser regularmente revisadas para mantener una precisión del 1% y una confiabilidad de +/- 2%. Deben descontaminarse periódicamente los residuos de los componentes del estuche.
2. La incubadora de ELISA debe ser ajustada a 37ºC (+/- 0.5ºC) y controlada periódicamente para mantener la temperatura correcta. Pueden emplearse incubadoras secas o baños de agua siempre que estén validados para la incubación de pruebas de ELISA.
3. El **lavador ELISA** es extremadamente importante para el rendimiento global del ensayo. El lavador debe ser validado de forma minuciosa previamente, revisado para comprobar que suministra el volumen de dispensación correcto y enviado regularmente a mantenimiento de acuerdo con las instrucciones de uso del fabricante. En particular, deben lavarse minuciosamente las sales con agua desionizada del lavador al final de la carga de trabajo diaria. Antes del uso, debe suministrarse extensivamente solución de lavado diluida al lavador. Debe enviarse el instrumento semanalmente a descontaminación según se indica en su manual (se recomienda descontaminación con NaOH 0.1 M). Para asegurar que el ensayo se realiza conforme a los rendimientos declarados, basta con 5 ciclos de lavado (aspiración + dispensado de 350 µl/pocillo de solución de lavado + 20 segundos de remojo = 1 ciclo). Si no es posible remojar, añadir un ciclo de lavado adicional. Un ciclo de lavado incorrecto o agujas obstruidas con sal son las principales causas de falsas reacciones positivas.
4. Los tiempos de incubación deben tener un margen de $\pm$5%.
5. El lector de microplacas ELISA debe estar provisto de un filtro de lectura de 450nm y de un segundo filtro (620-630nm, obligatorio) para reducir interferencias en la lectura. El procedimiento estándar debe contemplar: a) Ancho de banda <= 10nm b) Rango de absorbancia de 0 a >=2.0, c) Linealidad >=2.0, reproducibilidad >=1%. El blanco se prueba en el pocillo indicado en la sección “Procedimiento del ensayo”. El sistema óptico del lector debe ser calibrado periódicamente para garantizar la correcta medición de la densidad óptica, según las normas del fabricante.
6. En caso de usar un sistema automatizado de ELISA, los pasos críticos (dispensado, incubación, lavado, lectura, agitación y procesamiento de datos) deben ser cuidadosamente fijados, calibrados, controlados y periódicamente ajustados, para garantizar los valores indicados en la seccione “Control interno de calidad”. El protocolo del ensayo debe ser instalado en el sistema operativo de la unidad y validado tanto para el lavador como para el lector. Por otro lado, la parte del sistema que maneja los líquidos (dispensado y lavado) debe ser validada y fijada correctamente. Debe prestarse particular atención a evitar el arrastre por las agujas de dispensación y de lavado, a fin de minimizar la posibilidad de ocurrencia de falsos positivos por contaminación de los pocillos adyacentes por muestras fuertemente reactivas. Se recomienda el uso de sistemas automatizados para el pesquisaje en unidades de sangre y cuando la cantidad de muestras supera las 20-30 unidades por ensayo.
7. El servicio de atención al cliente en Dia.Pro, ofrece apoyo al usuario para calibrar, ajustar e instalar los equipos a usar en combinación con el estuche, con el propósito de asegurar el cumplimiento de los requerimientos descritos.

L. OPERACIONES Y CONTROLES PREVIOS AL ENSAYO.

1. Compruebe la fecha de caducidad indicada en la parte externa del estuche (envase primario). No usar si ha caducado.
2. Compruebe que los componentes líquidos no están contaminados con partículas o agregados visibles. Asegúrese de que el Cromógeno/Sustrato es incoloro o azul pálido, aspirando un pequeño volumen de este con una

pipeta estéril de plástico. Compruebe que no han ocurrido rupturas ni derrames de líquido dentro de la caja (envase primario) durante el transporte. Asegurarse de que la bolsa de aluminio que contiene la microplaca no esté rota o dañada.
3. Diluir totalmente la solución de lavado concentrada 20X, como se ha descrito anteriormente.
4. Disolver el Calibrador como se ha descrito anteriormente.
5. Dejar los componentes restantes alcanzar la temperatura ambiente (aprox. 1 hora), luego mezclar como se ha descrito.
6. Ajustar la incubadora de ELISA a 37ºC y cebar el lavador de ELISA utilizando la solución de lavado, según las instrucciones del fabricante. Fijar el número de ciclos de lavado según se indica en la sección específica.
7. Comprobar que el lector de ELISA esté conectado al menos 20 minutos antes de realizar la lectura.
8. En caso de trabajar automáticamente, conectar el equipo y comprobar que los protocolos estén correctamente programados.
9. Comprobar que las micropipetas estén fijadas en el volumen requerido.
10. Asegurarse de que el equipamiento a usar esté en perfecto estado, disponible y listo para el uso.
11. En caso de surgir algún problema, se debe detener el ensayo y avisar al supervisor.

M. PROCEDIMIENTO DEL ENSAYO.

El ensayo debe realizarse según las instrucciones que siguen a continuación, es importante mantener en todas las muestras el mismo tiempo de incubación.

Ensayos Automatizados.

En el caso de que el ensayo se realice de manera automatizada con un sistema ELISA, se recomienda programar al equipo para aspirar 200µl de Diluente de Muestras, y posteriormente 10µl de muestra.

La mezcla debe ser dispensada cuidadosamente en los pocillos correspondientes a cada muestra. Antes de aspirar la muestra siguiente, las agujas deben lavarse debidamente para evitar cualquier contaminación cruzada entre las muestras.

No diluir el Calibrador ni los controles ya que están listos para el uso.

Dispensar 200ul de controles/Calibrador en los pocillos correspondientes.

Nota importante: *Controle a simple vista que las muestras han sido diluidas y dispensadas en los pocillos adecuados, para lo cual el color de las muestras dispensadas debe ser verde azul oscuro, mientras que el del control negativo debe permanecer verde olivo.*

Para las operaciones siguientes, consulte las instrucciones que aparecen debajo para el Ensayo Manual.

Es muy importante comprobar que el tiempo entre el dispensado de la primera y la última muestra sea calculado por el instrumento y considerado para los lavados.

Ensayo Manual.

1. Poner el número de tiras necesarias en el soporte de plástico. Dejar el primer pocillo vacío para el blanco.
2. Dispensar 200µl del Control Negativo, por triplicado, 200µl de Calibrador por duplicado y 200µl del Control Positivo. No diluir el Calibrador ni los controles ya que están listos para el uso!
3. Dispensar 200µl del Diluente de muestras (DILSPE) a todos los pocillos de muestras, luego dispensar 10 ul de cada muestra en su pocillo correspondiente. Resuspender suavemente evitando la formación de espuma y la contaminación de los pocillos adyacentes.

Nota importante*: Comprobar que el color del Diluente de muestras cambia de verde a verde azul oscuro, una vez adicionada la muestra..*

4. Dispensar 50 ul de Diluente de ensayo (DILAS) en los pocillos de los controles/Calibrador y muestras. Compruebe que el color de las muestras sea azul oscuro.
5. Incubar la microplaca **45 min a +37°C**.

Nota importante*: Las tiras se deben sellar con el adhesivo suministrado solo cuando se hace el test manualmente. No sellar cuando se emplean equipos automatizados de ELISA.*

6. Lavar la microplaca con el lavador automático dispensando y aspirando 300 ul/pocillo de solución de lavado diluída, segun según se indica (sección I.3).
7. Dispensar 100uL del Conjugado en todos los pocillos, excepto en el A1 y cubrir con el sellador. Compruebe que este reactivo de color rojo ha sido añadido en todos los pocillos excepto el A1.

Nota importante*: Tener cuidado de no tocar la pared interna del pocillo con la punta de la pipeta al dispensar el conjugado. Podría producirse contaminación.*

8. Incubar la microplaca **45 min a +37°C**.
9. Lavar la microplaca, de igual forma que en el paso 6.
10. Dispensar 100µl del Cromógeno/Substrato en todos los pocillos, incluido el A1. Incubar la microplaca a **temperatura ambiente (18-24°C) durante 15 minutos**.

Nota importante*: No exponer directamente a fuerte iluminación, de lo contrario se generan interferencias.*

11. Dispensar 100µl de ácido sulfúrico en todos los pocillos para detener la reacción enzimática, usar la misma secuencia que en el paso 10. La adición de la solución de parada cambia el color del Control Positivo y las muestras positivas de azul a amarillo.
12. Medir la intensidad del color de la solución en cada pocillo, según se indica en la sección I.5, con un filtro de 450 nm (lectura) y otro de 620-630 nm (substracción del fondo, obligatorio), calibrando el instrumento con el pocillo A1 (blanco).

Notas importantes:

1. *Asegurarse de que no hay impresiones digitales ni polvo en el fondo de los pocillos antes de leer. Podrían generarse falsos positivos en la lectura.*
2. *La lectura debe hacerse inmediatamente después de añadir la solución de parada y, en cualquier caso, nunca transcurridos 20 minutos después de su adición. Se podría producir auto oxidación del cromógeno causando un elevado fondo.*

N. ESQUEMA DEL ENSAYO.

Método	**Operaciones**
Controles & Calibrador(*) Muestras Diluente de ensayo (DILAS)	200 ul 200ul dil.+10ul 50 ul
1ra incubación	**45 min**
Temperatura	+37°C
Lavado	5 ciclos con 20''de remojo o 6 ciclos sin remojo
Conjugado	100 ul
2da incubación	**45 min**
Temperatura	+37°C
Lavado	5 ciclos con 20''de remojo o 6 ciclos sin remojo

TMB/H2O2	100 ul
3r[a] incubación	**15 min**
Temperatura	t.a
Acido Sulfúrico	100 ul
Lectura D.O.	450nm/620-630nm

(*) Notas importantes:

- *El calibrador (CAL) no afecta al cálculo del valor de corte y, por lo tanto, no afecta al cálculo de los resultados de la prueba.*
- *El calibrador (CAL) se usa solo si la gestión requiere un control interno de calidad del laboratorio.*

A continuación se describe un ejemplo del esquema de dispensado.

Microplaca

	1	2	3	4	5	6	7	8	9	10	11	12
A	BL	M2										
B	CN	M3										
C	CN	M4										
D	CN	M5										
E	CAL(*)	M6										
F	CAL(*)	M7										
G	CP	M8										
H	M 1	M9										

Leyenda: BL = Blanco CN = Control Negativo
CAL = Calibrador(*) - No obligatorio CP = Control Positivo
M = Muestra

O. CONTROL DE CALIDAD INTERNO.

Se realiza un grupo de pruebas con los controles cada vez que se usa el estuche para verificar si los valores DO450nm/620-630nm son los esperados.
Asegurar el cumplimiento de los siguientes parámetros:

Parámetro	Exigencia
Pocillo Blanco	Valor < 0.100 DO450nm
Control Negativo (CN)	Valor medio < 0.050 DO450nm después de leer el blanco
Control Positivo	Valor > 1.000 DO450nm

Si los resultados del ensayo coinciden con lo establecido anteriormente, pase a la siguiente sección.

En caso contrario, detenga el ensayo y compruebe:

Problema	Compruebe que
Pocillo blanco > 0.100DO450nm	la solución cromógeno/substrato no se ha contaminado durante el ensayo.
Control Negativo (CN) > 0.050 DO450nm después de leer el blanco	1. el proceso de lavado y los parámetros del lavador estén validados según los estudios previos de calificación. 2. se ha usado la solución de lavado apropiada y que el lavador ha sido cebado con la misma antes del uso. 3. no se han cometido errores en el procedimiento (dispensar el control positivo en lugar del negativo). 4. no ha existido contaminación del control negativo o de sus pocillos debido a muestras positivas derramadas, o al conjugado. 5. las micropipetas no se han contaminado con muestras positivas o con el conjugado. 6. las agujas del lavador no estén parcial o totalmente obstruidas.
Control Positivo < 1.000 DO450nm	1. el procedimiento ha sido realizado correctamente. 2. no se han cometido errores en el procedimiento (dispensar el control negativo en lugar del positivo). En este caso el control negativo debe tener un valor de DO450nm > 0.150. 3. el proceso de lavado y los parámetros del lavador estén validados según los estudios previos de calificación. 4. no ha ocurrido contaminación externa del control positivo.

Si ocurre alguno de los problemas anteriores, después de comprobar, informe al supervisor para tomar las medidas pertinentes.

**** Nota:**

Si se ha usado el Calibrador, comprobar los siguientes datos:

Parámetro	Exigencia
Calibrador	M/Co > 1.1

Si los resultados de la prueba no se corresponden con los requisitos indicados anteriormente, proceder del siguiente modo

Problema	Compruebe que
Calibrador M/Co < 1.1	1. el procedimiento ha sido realizado correctamente. 2. no ha habido errores durante su distribución (dispensar el control negativo en lugar del calibrador). 3. el proceso de lavado y los parámetros del lavador estén validados según los estudios previos de calificación. 4. no ha ocurrido contaminación externa del calibrador.

En cualquier caso, si todos los demás parámetros (blanco, control negativo, control positivo) se corresponden con los requisitos establecidos, la prueba puede considerarse válida.

Nota importante:
El análisis debe seguir el paso de lectura descrito en la sección M, punto 12.

P. CÁLCULO DEL VALOR DE CORTE.

Los resultados se calculan por medio de un valor de corte (cut-off) hallado con la siguiente fórmula:

Valor de corte = CN medio DO450nm/620-630nm **+ 0.350**

El valor encontrado para el ensayo se usa para la interpretación de los resultados, según se describe a continuación:

***Nota Importante**: Cuando el cálculo de los resultados se halla mediante el sistema operativo de un equipo de ELISA automático, asegurarse de que la formulación usada para el cálculo del valor de corte, y para la interpretación de los resultados sea correcta.*

Q. INTERPRETACIÓN DE LOS RESULTADOS.

Los resultados de la prueba son interpretados mediante la relación entre las DO a 450nm/620-630nm de las muestras y el Valor de corte (M/Co), de acuerdo a la la siguiente tabla.

(M/Co)	Interpretación
< 0.9	Negativo
0.9 – 1.1	Equívoco
> 1.1	**Positivo**

Un resultado negativo indica que el paciente no ha sido infectado por VHE o que la unidad de sangre se puede transfundir.
Cualquier paciente que muestre un resultado equívoco debe someterse a una nueva prueba con una segunda muestra de sangre tomada 1 ó 2 semanas después de la inicial. En este caso la unidad de sangre no debe ser transfundida.
Un resultado positivo es indicativo de infección por VHE, por consiguiente el paciente debe ser tratado adecuadamente o la unidad de sangre debe ser descartada.

Notas importantes:

1. *La interpretación de los resultados debe hacerse bajo la vigilancia del supervisor del laboratorio para reducir el riesgo de errores de juicio y de interpretación.*
2. *Por definición, antes de formular un diagnóstico de hepatitis viral, los resultados positivos deben comprobarse a través de un método alternativo*
3. *Cuando se transmiten los resultados de la prueba, del laboratorio a otras instalaciones, debe ponerse mucha atención para evitar el traslado de datos erróneos.*
4. *El diagnóstico de infección con un virus de la hepatitis debe ser evaluado y comunicado al paciente por un médico calificado.*

A continuación se muestra un ejemplo de los cálculos a realizar (datos obtenidos siguiendo el paso de lectura descrito en la sección M, punto 12):

Los siguientes datos no deben usarse en lugar de los valores reales obtenidos en el laboratorio.

Control Negativo: 0.019 – 0.020 – 0.021 DO450nm
Valor medio: 0.020 DO450nm
Menor de 0.050 – Válido

Control Positivo: 2.189 DO450nm
Mayor de 1.000 – Válido
Valor de corte = 0.020+0.350 = 0.370

Calibrador: 0.600 – 0.640 DO450nm
Valor medio: 0.620 DO450nm M/Co = 1.7
M/Co Mayor de 1.1 – Válido

Muestra 1: 0.070 DO450nm
Muestra 2: 1.690 DO450nm
Muestra 1 M/Co < 0.9 = negativa
Muestra 2 M/Co > 1.1 = positiva

R. FUNCIONAMIENTO
La Evaluación del funcionamiento ha sido realizada en muestras positivas y negativas en un centro clínico externo utilizando el kit aprobado por la FDA de los Estados Unidos.

1. LIMITE DE DETECCIÓN
El límite de detección del ensayo ha sido calculado por medio del estándar de referencia 1st WHO anti- HEV NIBSC código 95/584.
Los ensayos muestran una sensibilidad de alrededor de 0.1 WHO UI/ml.

2. ESPECIFICIDAD Y SENSIBILIDAD DIAGNÓSTICA
Se comprobaron en un total de más de 700 muestras con una sensibilidad de 0.25 WHO UI/ml (UI definida por la OMS).

2.1 Especificidad Diagnóstica:
Se define como la probabilidad del ensayo de detectar negativos en ausencia del analito específico.
Se examinaron más de 100 niños de 1 a 5 años de edad, negativos por definición a los anticuerpos HEV porque nunca habían comido carne de cerdo cruda y por tanto no podían haberse infectado, asegurando un especificidad (negatividad) del 100% y una sensibilidad de 0.25 WHO IU/ml.
Asimismo, siempre en la sensibilidad de 0.25 WHO IU/ml, se examinaron más de 500 muestras provenientes de donantes incluyendo aquellos que lo hacían por primera vez, así como de pacientes HEV negativos hospitalizados provenientes de Italia. Alrededor del 5% de esta población resultó ser repetidamente positiva. No se llevó a cabo el test de confirmación dada la ausencia de un kit comercial de confirmación; de todos modos la positividad de estas muestras se detectó mediante un kit comercial ELISA con marca CE.
La especificidad obtenida en este estudio fue del 100%.
No se ha observado falsa reactividad debida a los métodos de tratamiento de muestras. Se emplearon además, plasma sometido a métodos de tratamiento estándar (citrato, EDTA y heparina) y suero humanos.
Por último, se analizaron muestras congeladas, para determinar posibles interferencias debidas a la toma de muestra y al almacenamiento.
No se observaron interferencias.

2.2 Sensibilidad Diagnóstica
Se define como la probabilidad que tiene el ensayo de detectar positivos en presencia del analito específico.
La sensibilidad diagnóstica (manteniendo una sensibilidad fijado en un valor de corte de 0,25 WHO UI/ml) ha sido estimada de forma externa (Instituto de Virología de Milan) e interna en un total de 200 muestras positivas; el valor de sensibilidad diagnóstica (o correlación con el kit de referencia comercial con marca CE) encontrado fue del 100%.

3. PRECISÓN:
Ha sido calculada en dos muestras, una negativa y una débil positiva, examinadas en 16 réplicas en tres corridas separadas.
Los valores de CV varían entre 5-10%, dependiendo de los valores de DO450nm/620-630nm encontrados. La variabilidad observada no dio como resultado una clasificación errónea de las muestras.

S. LIMITACIONES.
Las muestras que después de ser descongeladas presentan partículas de fibrina o partículas agregadas, generan algunos resultados falsos positivos.

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Todos los productos de diagnóstico in vitro fabricados por la empresa son controlados por un sistema certificado de control de calidad conforme a la norma ISO 13485 .Cada lote se somete a un control de calidad y se libera al mercado únicamente si se ajusta a las especificaciones técnicas y criterios de aceptación de la CE.

Fabricante:
Dia.Pro Diagnostic Bioprobes S.r.l
Via G. Carducci n° 27 – Sesto San Giovanni (MI) – Italia

CE

Dia.Pro
Diagnostic
BioProbes

EC DECLARATION OF CONFORMITY

MANUFACTURER	DIA.PRO DIAGNOSTIC BIOPROBES S.R.L. VIA G. CARDUCCI N° 27 – 20099 SESTO SAN GIOVANNI (MILANO) – ITALY
PRODUCT	**HEV IgG** CODE: **EVG.CE** (96 tests)
CLASSIFICATION	GENERAL IVD
CONFORMITY ASSESSMENT ROUTE	SELF CERTIFICATION

WE HEREBY DECLARE THAT THE ABOVE MENTIONED PRODUCT MEETS THE PROVISIONS OF THE COUNCIL DIRECTIVE 98/79/EC FOR IN VITRO DIAGNOSTIC DEVICES.

ISO CERTIFICATE	UNE EN ISO 13485 N° 2013 11 0039 EN, RELEASED BY AEMPS (AGENCIA ESPAÑOLA DE MEDICAMENTOS Y PRODUCTOS SANITARIOS)

PLACE & DATE OF FIRST ISSUE	MILANO – JULY 2004
PLACE & DATE OF CURRENT ISSUE	SESTO SAN GIOVANNI (MI) – MARCH 2019
SIGNATURE **Legal Representative** **Dr.ssa Fiorenza Scozzesi**	

Rev:	05/2018

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EC DECLARATION OF CONFORMITY

MANUFACTURER	DIA.PRO DIAGNOSTIC BIOPROBES S.R.L. VIA G. CARDUCCI N° 27 – 20099 SESTO SAN GIOVANNI (MILANO) – ITALY
PRODUCT	**HEV IgM** CODE: **EVM.CE** (96 tests)
CLASSIFICATION	GENERAL IVD
CONFORMITY ASSESSMENT ROUTE	SELF CERTIFICATION

WE HEREBY DECLARE THAT THE ABOVE MENTIONED PRODUCT MEETS THE PROVISIONS OF THE COUNCIL DIRECTIVE 98/79/EC FOR IN VITRO DIAGNOSTIC DEVICES.

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Rev:	05/2018

HEV IgM

Enzyme Immunoassay (ELISA) for the determination of IgM antibodies to Hepatitis E Virus in human serum and plasma

- for “in vitro” diagnostic use only -

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e-mail: info@diapro.it

REF EVM.CE
96 Tests

HEV IgM

A. INTENDED USE

Enzyme ImmunoAssay (ELISA) for the determination of IgM antibodies to Hepatitis E Virus in human plasma and sera.
The kit may be used for the determination of the acute phase of infection where IgM antibodies are generated before the other immunoglobulins and for the follow-up of HEV-infected patients.
For "in vitro" diagnostic use only.

B. INTRODUCTION

Hepatitis E Virus or HEV is a recently discovered agent of enterically transmitted viral hepatitis. HEV is an un-enveloped single-strand RNA virus, after being provisionally assigned to the Caliciviridae family, HEV was re-classified as the sole member of the genus Hepevirus, family Hepeviridae, in 2004. HEV is found in the stool of infected patients and present in 4 strains (1, 2, 3 and 4) differently spread geographically and virulent.
HEV is a serious problem in many developing countries since its first outbreak was reported in 1955 in New Delhi, India.
A high case-fatality rate has been found among pregnant women and chronic hepatitis carriers.
The cloning and sequencing of HEV genome have led to the development of serological tests for the detection of anti HEV antibodies based on recombinant immunodominant antigens derived from conservative regions of the four virus strains.

C. PRINCIPLE OF THE TEST

Microplates are coated with HEV-specific recombinant antigens encoding for conservative and immunodominant determinants of all the 4 subtypes.
The solid phase is first treated with the diluted sample and anti HEV IgM are captured, if present, by the antigens.
After washing out all the other components of the sample, in the 2nd incubation bound anti-HEV IgM are detected by the addition of polyclonal specific anti hIgM antibodies, labelled with peroxidase (HRP).
The enzyme captured on the solid phase, acting on the substrate/chromogen mixture, generates an optical signal that is proportional to the amount of anti HEV IgM present in the sample. A cut-off value let optical densities be interpreted into anti-HEV IgM negative and positive results.
Neutralization of IgG anti-HEV and Rheumatoid Factor, carried out directly in the well, is performed in the assay in order to block such kind of interferences.

D. COMPONENTS

Code EVM.CE contains reagents for 96 tests.

1. Microplate MICROPLATE
n° 1 microplate. 12 strips of 8 microwells coated with HEV specific recombinant antigens. Plates are sealed into a bag with desiccant.

2. Negative Control CONTROL -
1x4.0ml/vial. Ready to use control. It contains 1% goat serum proteins, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.5% Tween 20, 0.09% Na-azide and 0.045% ProClin 300 as preservatives. The negative control is yellow colour coded.

3. Positive Control CONTROL +
1x4.0ml/vial. Ready to use control. It contains 1% goat serum proteins, human anti HEV IgM, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.5% Tween 20, 0.09% Na-azide and 0.045% ProClin 300 as preservatives.
The Positive Control is dark green colour coded.

4. Wash buffer concentrate WASHBUF 20X
1x60ml/bottle. 20x concentrated solution. Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2, 0.05% Tween 20 and 0.045% ProClin 300.

5. Enzyme Conjugate CONJ
1x16ml/vial. Ready to use and red colour coded reagent. It contains Horseradish Peroxidase conjugated goat polyclonal antibodies to human IgM, 5% BSA, 10 mM Tris buffer pH 6.8+/-0.1, 0.045% ProClin 300 and 0.02% gentamicine sulphate as preservatives.

6. Chromogen/Substrate SUBS TMB
1x16ml/vial. Ready-to-use component. It contains 50 mM citrate-phosphate buffer pH 3.5-3.8, 4% dimethylsulphoxide, 0.03% tetra-methyl-benzidine or TMB and 0.02% hydrogen peroxide or H2O2.
Note: To be stored protected from light as sensitive to strong illumination.

7. Sulphuric Acid H_2SO_4 O.3 M
1x15ml/vial. It contains 0.3 M H_2SO_4 solution.
Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

8. Specimen Diluent: DILSPE
2x60ml/vial. It contains 2% casein, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.1% Tween 20, 0.09% Na-azide and 0.045% ProClin 300 as preservatives. To be used to dilute the sample.

9. Neutralizing Reagent: SOLN NTR
1x8ml/vial. Ready-to-use Reagent. It contains goat anti hIgG, 2% casein, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.1% Tween 20, 0.09% Na-azide and 0.045% ProClin 300 as preservatives.

10. Plate sealing foils **n° 2**

11. Package insert **n° 1**

E. MATERIALS REQUIRED BUT NOT PROVIDED

1. Calibrated Micropipettes (100ul and 10ul) and disposable plastic tips.
2. EIA grade water (bidistilled or deionised, charcoal treated to remove oxidizing chemicals used as disinfectants).
3. Timer with 60 minute range or higher.
4. Absorbent paper tissues.
5. Calibrated ELISA microplate thermostatic incubator capable to provide a temperature of +37°C.
6. Calibrated ELISA microwell reader with 450nm (reading) and with 620-630nm (blanking) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.

F. WARNINGS AND PRECAUTIONS

1. The kit has to be used by skilled and properly trained technical personnel only, under the supervision of a medical doctor responsible of the laboratory.
2. All the personnel involved in performing the assay have to wear protective laboratory clothes, talc-free gloves and glasses. The use of any sharp (needles) or cutting (blades) devices should be avoided. All the personnel involved should be trained in biosafety procedures, as recommended by the Center for Disease Control, Atlanta, U.S. and reported in the National Institute of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
3. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.
4. The laboratory environment should be controlled so as to avoid contaminants such as dust or air-born microbial agents, when opening kit vials and microplates and when performing the

test. Protect the Chromogen/Substrate from strong light and avoid vibration of the bench surface where the test is undertaken.
5. Upon receipt, store the kit at 2..8°C into a temperature controlled refrigerator or cold room.
6. Do not interchange components between different lots of the kits. It is recommended that components between two kits of the same lot should not be interchanged.
7. Check that the reagents are clear and do not contain visible heavy particles or aggregates. If not, advise the laboratory supervisor to initiate the necessary procedures for kit replacement.
8. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each sample. Do not reuse disposable tips.
9. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one. Do not reuse disposable tips.
10. Do not use the kit after the expiration date stated on the external container and internal (vials) labels.
11. Treat all specimens as potentially infective. All human serum specimens should be handled at Biosafety Level 2, as recommended by the Center for Disease Control, Atlanta, U.S. in compliance with what reported in the Institutes of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
12. The use of disposable plastic-ware is recommended in the preparation of the liquid components or in transferring components into automated workstations, in order to avoid cross contamination.
13. Waste produced during the use of the kit has to be discarded in compliance with national directives and laws concerning laboratory waste of chemical and biological substances. In particular, liquid waste generated from the washing procedure, from residuals of controls and from samples has to be treated as potentially infective material and inactivated before waste. Suggested procedures of inactivation are treatment with a 10% final concentration of household bleach for 16-18 hrs or heat inactivation by autoclave at 121°C for 20 min..
14. Accidental spills from samples and operations have to be adsorbed with paper tissues soaked with household bleach and then with water. Tissues should then be discarded in proper containers designated for laboratory/hospital waste.
15. The Sulphuric Acid is an irritant. In case of spills, wash the surface with plenty of water
16. Other waste materials generated from the use of the kit (example: tips used for samples and controls, used microplates) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

G. SPECIMEN: PREPARATION AND RECOMMANDATIONS
1.Blood is drawn aseptically by venepuncture and plasma or serum is prepared using standard techniques of preparation of samples for clinical laboratory analysis. No influence has been observed in the preparation of the sample with citrate, EDTA and heparin.
2. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results. When the kit is used for the screening of blood units, bar code labeling and electronic reading is strongly recommended.
3. Haemolysed (red) and visibly hyperlipemic ("milky") samples have to be discarded as they could generate false results. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they could give rise to false results.
4.Sera and plasma can be stored at +2°…+8°C in primary collection tubes for up to five days after collection.
Do not freeze primary tubes of collection. For longer storage periods, sera and plasma samples, carefully removed from the primary collection tube, can be stored frozen at –20°C for at least 12 months. Any frozen samples should not be frozen/thawed more than once as this may generate particles that could affect the test result.
5. If particles are present, centrifuge at 2.000 rpm for 20 min or filter using 0.2-0.8u filters to clean up the sample for testing.

H. PREPARATION OF COMPONENTS AND WARNINGS
A study conducted on an opened kit has not pointed out any relevant loss of activity up to 6 re-use of the device and up to 6 months.

Microplates:
Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant is not turned to dark green, indicating a defect of conservation.
In this case call Dia.Pro's customer service.
Unused strips have to be placed back into the aluminium pouch, in presence of desiccant supplied, firmly zipped and stored at +2°..8°C.
When opened the first time, residual strips are stable till the indicator of humidity inside the desiccant bag turns from yellow to green.

Negative Control:
Ready to use. Mix well on vortex before use.

Positive Control:
Ready to use. Mix well on vortex before use. Handle this component as potentially infective, even if HCV, eventually present in the control, has been chemically inactivated.

Wash buffer concentrate:
The whole content of the 20x concentrated solution has to be diluted with bidistilled water up to 1200 ml and mixed gently end-over-end before use.
Once diluted, the wash solution is stable for 1 week at +2..8° C.
In the preparation avoid foaming as the presence of bubbles could give origin to a bad washing efficiency.
Note: *Once diluted, the wash solution is stable for 1 week at +2..8° C.*

Enzyme conjugate:
Ready to use. Mix well on vortex before use.
Be careful not to contaminate the liquid with oxidizing chemicals, air-driven dust or microbes.
If this component has to be transferred use only plastic, possibly sterile disposable containers.

Chromogen/Substrate:
Ready to use. Mix well on vortex before use.
Be careful not to contaminate the liquid with oxidizing chemicals, air-driven dust or microbes.
Do not expose to strong illumination, oxidizing agents and metallic surfaces.
If this component has to be transferred use only plastic, possible sterile disposable container.

Neutralizing Reagent
Ready to use component. Mix carefully on vortex before use.

Sample Diluent:
Ready to use. Mix well on vortex before use.

Sulphuric Acid:
Ready to use. Mix well on vortex before use.
Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

Legenda:

Warning **H statements:**
H315 – Causes skin irritation.
H319 – Causes serious eye irritation.

Precautionary **P statements:**
P280 – Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352 – IF ON SKIN: Wash with plenty of soap and water.
P332 + P313 – If skin irritation occurs: Get medical advice/attention.
P305 + P351 + P338 – IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337 + P313 – If eye irritation persists: Get medical advice/attention.
P362 + P363 – Take off contaminated clothing and wash it before reuse.

I. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

1. **Micropipettes** have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (household alcohol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample. They should also be regularly maintained in order to show a precision of 1% and a trueness of +/-2%. Decontamination of spills or residues of kit components should also be carried out regularly.
2. The **ELISA incubator** has to be set at +37°C (tolerance of +/-0.5°C) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water baths are suitable for the incubations, provided that the instrument is validated for the incubation of ELISA tests.
3. The **ELISA washer** is extremely important to the overall performances of the assay. The washer must be carefully validated in advance, checked for the delivery of the right dispensation volume and regularly submitted to maintenance according to the manufacturer's instructions for use. In particular the washer, at the end of the daily workload, has to be extensively cleaned out of salts with deionized water. Before use, the washer has to be extensively primed with the diluted Washing Solution.
   The instrument weekly has to be submitted to decontamination according to its manual (NaOH 0.1 M decontamination suggested).
   5 washing cycles (aspiration + dispensation of 350ul/well of washing solution + 20 sec soaking = 1 cycle) are sufficient to ensure the assay with the declared performances. If soaking is not possible add one more cycle of washing.
   An incorrect washing cycle or salt-blocked needles are the major cause of false positive reactions.
4. Incubation times have a tolerance of $\pm$5%.
5. The **ELISA reader** has to be equipped with a reading filter of 450nm and with a second filter of 620-630nm, mandatory for blanking purposes. Its standard performances should be (a) bandwidth $\leq$ 10 nm; (b) absorbance range from 0 to $\geq$ 2.0; (c) linearity to $\geq$ 2.0; repeatability $\geq$ 1%. Blanking is carried out on the well identified in the section "Assay Procedure". The optical system of the reader has to be calibrated regularly to ensure that the correct optical density is measured. It should be regularly maintained according to the manufacturer 's instructions.
6. When using an **ELISA automated work station**, all critical steps (dispensation, incubation, washing, reading, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the sections "Validation of Test" and "Assay Performances". The assay protocol has to be installed in the operating system of the unit and validated as for the washer and the reader. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set. Particular attention must be paid to avoid carry over by the needles used for dispensing and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells. The use of ELISA automated work stations is recommended when the number of samples to be tested exceed 20-30 units per run.
7. Dia.Pro's customer service offers support to the user in the setting and checking of instruments used in combination with the kit, in order to assure compliance with the requirements described. Support is also provided for the installation of new instruments to be used with the kit.

L. PRE ASSAY CONTROLS AND OPERATIONS

1. Check the expiration date of the kit printed on the external label (primary container). Do not use if expired.
2. Check that the liquid components are not contaminated by visible particles or aggregates.
3. Check that the Chromogen (TMB) is colourless or pale blue by aspirating a small volume of it with a sterile plastic pipette.
4. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box (primary container). Check that the aluminium pouch, containing the microplate, is not punctured or damaged.
5. Dissolve the content of the Control Serum as reported.
6. Dilute all the content of the 20x concentrated Wash Solution as described above.
7. Allow all the other components to reach room temperature (about 1 hr) and then mix gently on vortex all liquid reagents.
8. Set the ELISA incubator at +37°C and prepare the ELISA washer by priming with the diluted washing solution, according to the manufacturers instructions. Set the right number of washing cycles as as reported in the specific section.
9. Check that the ELISA reader is turned on or ensure it will be turned on at least 20 minutes before reading.
10. If using an automated work station, turn on, check settings and be sure to use the right assay protocol.
11. Check that the micropipettes are set to the required volume.
12. Check that all the other equipment is available and ready to use.
13. In case of problems, do not proceed further with the test and advise the supervisor.

M. ASSAY PROCEDURE

The assay has to be carried out according to what reported below, taking care to maintain the same incubation time for all the samples in testing.

1. Dilute samples 1:101 into a properly defined dilution tube (example: 1000 µl Sample Diluent + 10 µl sample). Do not dilute the negative Control and the Positive Control as they are ready to use. Mix carefully all the liquid components on vortex and then proceed as described below.
2. Place the required number of Microwells in the microwell holder. Leave A1 well empty for the operation of blanking.
3. Dispense 50 µl of the Neutralizing Reagent (SOLN NTR) in all the wells of the samples. Do not add it in the wells used for Controls and in A1!

Important note: *The Neutralizing Reagent is able to block false positive reactions due to RF. Positive samples in internal QC panels might be detected negative if such samples were tested positive with an IVD that does not carry out any RF blocking reaction.*

4. Dispense 100 µl of Negative Control in triplicate and 100 µl of Positive control in single. Then dispense 100 µl of diluted samples in each properly identified well.
5. Incubate the microplate for **60 min at +37°C**.

Important note: *Strips have to be sealed with the adhesive sealing foil, supplied, only when the test is carried out manually. Do not cover strips when using ELISA automatic instruments.*

6. Wash the microplate with an automatic washer by delivering and aspirating 300 µl/well of diluted washing solution as reported previously (section I.3).
7. Pipette 100 µl Enzyme Conjugate into each well, except the A1 well, and cover with the sealer. Check that this red coloured component has been dispensed in all the wells, except A1.

Important note: *Be careful not to touch the plastic inner surface of the well with the tip filled with the Enzyme Conjugate. Contamination might occur.*

8. Incubate the microplate for **60 min at +37°C**.
9. Wash microwells as in step 6.
10. Pipette 100 µl Chromogen/Substrate mixture into each well, the blank well included. Then incubate the microplate at **room temperature (18-24°C) for 20 minutes**.

Important note: *Do not expose to strong direct illumination. High background might be generated.*

11. Pipette 100 µl Sulphuric Acid into all the wells using the same pipetting sequence as in step 10. Addition of acid will turn the positive calibrators, the control serum and the positive samples from blue to yellow.
12. Measure the colour intensity of the solution in each well, as described in section I.5, at 450nm filter (reading) and at 620-630nm (background subtraction, mandatory), blanking the instrument on A1.

General Important notes:

1. *Ensure that no finger prints are present on the bottom of the microwell before reading. Finger prints could generate false positive results on reading.*
2. *Reading has to be carried out just after the addition of the Stop Solution and anyway not any longer than 20 minutes after its addition. Some self oxidation of the chromogen can occur leading to high background.*

N. ASSAY SCHEME

Method	Operations
Neutralizing Reagent (in sample wells only !) Negative and Positive Controls Samples diluted 1:101	50 µl 100 µl 100 µl
1st incubation	**60 min**
Temperature	+37°C
Wash step	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
Enzyme conjugate	100 µl
2nd incubation	**60 min**
Temperature	+37°C
Wash step	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
TMB/H2O2	100 µl
3rd incubation	**20 min**
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm/620-630nm

An example of dispensation scheme is reported below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S4										
B	NC	S5										
C	NC	S6										
D	NC	S7										
E	PC	S8										
F	S1	S9										
G	S2	S10										
H	S3	S11										

Legenda: BLK = Blank NC = Negative Control
PC = Positive Control S = Sample

O. INTERNAL QUALITY CONTROL

A check is carried out on the controls any time the kit is used in order to verify whether their OD450nm/620-630nm values are as expected and reported in the table below.

Check	Requirements
Blank well	< 0.100 OD450nm value
Negative Control (NC)	< 0.100 mean OD450nm value after blanking
Positive Control	> 0.500 OD450nm value

If the results of the test match the requirements stated above, proceed to the next section.
If they do not, do not proceed any further and operate as follows:

Problem	Check
Blank well > 0.100 OD450nm	1. that the Chromogen/Sustrate solution has not got contaminated during the assay
Negative Control (NC) > 0.100 OD450nm after blanking	1. that the washing procedure and the washer settings are as validated in the pre qualification study; 2. that the proper washing solution has been used and the washer has been primed with it before use; 3. that no mistake has been done in the assay procedure (dispensation of positive control instead of negative control; 4. that no contamination of the negative control or of their wells has occurred due to positive samples, to spills or to the enzyme conjugate; 5. that micropipettes haven't got contaminated with positive samples or with the enzyme conjugate 6. that the washer needles are not blocked or partially obstructed.
Positive Control < 0.500 OD450nm	1. that the procedure has been correctly executed; 2. that no mistake has been done in the distribution of controls (dispensation of negative control instead of positive control. In this case, the negative control will have an OD450nm value > 0.150, too. 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the positive control has occurred.

Should these problems happen, after checking, report any residual problem to the supervisor for further actions.

Important note:
The analysis must be done proceeding as the reading step described in the section M, point 12.

P. CALCULATION OF THE CUT-OFF
The tests results are calculated by means of a cut-off value determined with the following formula:

Cut-Off = NC mean OD450nm/620-630nm + 0.250

The value found for the test is used for the interpretation of results as described in the next paragraph.

***Important note**: When the calculation of results is done by the operative system of an ELISA automated work station be sure that the proper formulation is used to calculate the cut-off value and generate the right interpretations of results.*

Q. INTERPRETATION OF RESULTS
Test results are interpreted as ratio of the sample OD450nm/620-630nm and the Cut-Off value (or S/Co) according to the following table:

S/Co	Interpretation
< 1.0	Negative
1.0 – 1.2	Equivocal
> 1.2	Positive

A negative result indicates that the patient has no detectable anti HEV IgM reactivity.
Any patient showing an equivocal result should be tested again on a second sample taken 1-2 weeks later from the patient and examined.
A positive result is indicative of HEV infection and therefore the patient should be treated accordingly.

Important notes:
1. *Interpretation of results should be done under the supervision of the responsible of the laboratory to reduce the risk of judgment errors and misinterpretations.*
2. *Any positive result should be confirmed by an alternative method before a diagnosis of viral hepatitis is formulated.*
3. *When test results are transmitted from the laboratory to an informatics center, attention has to be done to avoid erroneous data transfer.*
4. *Diagnosis of viral hepatitis E infection has to be done and released to the patient only by a qualified medical doctor.*

An example of calculation is reported below (data obtained proceeding as the the reading step described in the section M, point 12):

The following data must not be used instead or real figures obtained by the user.

Negative Control: 0.060 – 0.080 – 0.070 DO 450nm
Mean Value: 0.070 OD450nm
Lower than 0.100 – Accepted
Positive Control: 1.589 OD450nm
Higher than 0.500 – Accepted
Cut-Off = 0.070+0.250 = 0.320

Sample 1: 0.070 OD450nm
Sample 2: 1.690 OD450nm
Sample 1 S/Co < 1.0 = negative
Sample 2 S/Co > 1.2 = positive

R. PERFORMANCES
Evaluation of Performances has been conducted on negative and positive samples in an external clinical center with reference to a FDA approved kit.

1. LIMIT OF DETECTION
The limit of detection of the product has been checked on the international reference reagent for HEV antibody supplied by NIBSC/WHO with code n° 95/584. This material was assessed to be positive also for anti HEV IgM, low titer. The observed value is about 1 IU/ml.

2. DIAGNOSTIC SPECIFICITY AND SENSITIVITY
They were checked on about 700 sample derived from acute infections, HEV Ab positive patients, random individuals under diagnostic examination and healthy individuals with a sensitivity set at about **5 IU/ml** in order to assure the highest sensitivity and be able to detect primary infection at the earliest phase.

2.1 Diagnostic specificity:
It is defined as the probability of the assay of scoring negative in the absence of specific analyte.
A total of more 500 unselected donors and HEV negative hospitalized patients, including 1st time donors, were examined.
The diagnostic specificity was assessed against a kit US FDA approved. A diagnostic specificity of $\geq$ 95% was observed.
Moreover, diagnostic specificity was assessed by testing more than 100 potentially interfering specimens (other infectious diseases, patients affected by non viral hepatic diseases, dialysis patients, pregnant women, haemolized, lipemic, etc.).
A value of specificity of 100% was assessed.
No false reactivity due to the method of specimen preparation has been observed. Both plasma, derived with different standard techniques of preparation (citrate, EDTA and heparin), and sera have been used to determine the value of specificity.
Frozen specimens have been tested, as well, to check for interferences due to collection and storage.
No interference was observed.
High reactive RF positive samples were observed to give origin to false positive results in not more than 5% of HEV Ab negative individuals.

2.2 Diagnostic Sensitivity
It defined as the probability of the assay of scoring positive in the presence of specific analyte.
The diagnostic sensitivity has been assessed externally and internally on a total number of 100 positive specimens coming from Germany, Mexico and from Burma.
A diagnostic sensitivity of 100% was found.

3. PRECISION:
It has been calculated on two samples, one negative and one positive, examined in 16 replicates in three separate runs.
CV values ranging between 5-15%, depending on OD450nm values, were found. The variability seen did not result in sample misclassification.

S. LIMITATIONS
Repeatable false positive results were assessed for high titer RF positive samples, escaping the effect of the Neutralizing Reagent.
Frozen samples containing fibrin particles or aggregates after thawing have been observed to generate some false results.

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All the IVD Products manufactured by the company are under the control of a certified Quality Management System in compliance with ISO 13485 rule. Each lot is submitted to a quality control and released into the market only if conforming with the EC technical specifications and acceptance criteria.

Manufacturer:
Dia.Pro. Diagnostic Bioprobes Srl.
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CE

HEV IgM

Ensayo Inmunoenzimático (ELISA) para la determinación de anticuerpos IgM frente al virus de la Hepatitis E en suero y plasma humano

- Uso exclusivo para diagnóstico *in vitro* -

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REF EVM.CE
96 pruebas

HEV IgM

A. OBJETIVO DEL ESTUCHE
Ensayo Inmunoenzimático (ELISA) para la detección de anticuerpos IgM frente al virus de la Hepatitis E en plasma y suero humano.
El equipo puede utilizarse tanto en la determinación de la fase aguda de la infección, en la que se generan anticuerpos IgM antes que otras inmunoglobulinas, como en el seguimiento de pacientes infectados por VHE.
Uso exclusivo de diagnóstico *in vitro*.

B. INTRODUCCIÓN
El virus de la hepatitis E o VHE es un agente descubierto recientemente de hepatitis viral transmitida por vía entérica. El VHE es un virus ARN monocatenario no encapsulado. Tras asignarse de forma provisional a la familia Caliciviridae, el VHE se reclasificó como el único miembro del género Hepevirus, familia Hepeviridae, en 2004. El VHE se encuentra en las heces de pacientes infectados y se presenta en 4 cepas (1, 2, 3 y 4) con distinta distribución geográfica y virulencia.
El VHE es un problema grave en muchos países en desarrollo desde que se registró el primer brote en 1955 en Nueva Delhi, India. Se ha encontrado una tasa alta de mortalidad en mujeres embarazadas y en portadores de hepatitis crónica.
La clonación y la secuenciación del genoma del VHE ha llevado al desarrollo de pruebas serológicas para la detección de anticuerpos anti-VHE basadas en antígenos inmunodominantes recombinantes derivados de regiones conservadoras de las cuatro cepas del virus.

C. PRINCIPIOS DEL ENSAYO
Las microplacas se recubren con antígenos codificados recombinantes específicos del VHE para identificar los determinantes inmunodominantes y conservados de todos los 4 subtipos del virus
La muestra diluida se añade a la microplacas y los anticuerpos anti-HEV IgM, presentes en la muestra, son secuestrados por los antígenos de la fase sólida unida a la microplaca.
Después de los lavados, el resto de componentes presentes en la muestra que no se hayan unido a la fase sólida, son eliminados mediante los lavados. En la segunda incubación, los anticuerpos anti-HEV IgM presentes en la muestra y unidos a la fase sólida, son detectados gracias a la adición de anticuerpos policlonales específicos anti-IgM, marcados con la enzima peroxidasa (HRP).
La enzima peroxidasa unida ahora a la fase sólida de la placa, reacciona con el cromógeno/substrato, generando una señal óptica que es proporcional a la cantidad de anticuerpo anti-HEV presente en la muestra.
Posteriormente, mediante un valor de corte calculado, las densidades ópticas pueden interpretarse como resultados negativos o positivos a la presencia de anticuerpos para HEV.
Es necesario bloquear la presencia de anti-HEV IgG y de Factor Reumatoide, para eliminar interferencias.

D. COMPONENTES
Cada estuche con Codigo EVM.CE contiene reactivos para 96 pruebas.

1. Microplaca MICROPLATE
n° 1 microplaca.
12 tiras de 8 micropocillos recubiertos con antígenos recombinantes específicos del VHE.Las placas están envasadas en bolsas selladas con desecante.

2. Control Negativo CONTROL -
1x4.0ml/vial.
Listo para el uso. Contiene: 1% de proteínas de suero de cabra, 10mM de tampón Citrato Sódico pH 6.0 +/-0.1, 0.5% Tween 20, 0.09% Azida sódica y 0.045% ProClin 300, como conservantes.
El control negativo está codificado con el color amarillo.

3. Control Positivo CONTROL +
1x4.0ml/vial.
Listo para el uso. Contiene: 1% de proteínas de suero de cabra, anticuerpos humanos anti-HEV IgM, 10mM de tampón Citrato Sódico pH 6.0 +/-0.1, 0.5% Tween 20, 0.09% Azida sódica y 0.045% ProClin 300, como conservantes.
El control positivo está codificado en el color verde oscuro.

4. Tampón de Lavado Concentrado WASHBUF 20X
1x60ml/botella. Solución concentrada 20x.
Una vez diluida, la solución de lavado contiene 10mM de tampon fosfato pH 7.0+/-0.2, 0.05% Tween 20 y 0.045% ProClin 300.

5. Enzima Conjugada CONJ
1x16ml/vial.
Listo para el uso y codificada con el color rojo. Contiene: Peroxidasa de rabano unida a anticuerpos policlonales de cabra que reconocen la IgM humana, 5% BSA, 10 mM de tampón Tris pH 6.8+/-0.1, 0.045% ProClin 300 y 0.02% de sulfato de gentamicina como conservantes.

6. Cromogeno/Substrato SUBS TMB
1x16ml/vial.
Listo para el uso. Contiene: 50 mM de tempón citrato-fosfato pH 3.5-3.8, 4% dimetilsulfóxido, 0.03% tetra-metil-benzidina (TMB) y 0.02% de peróxido de hidrógeno (H2O2).
Nota: Evitar la exposición a la luz, es fotosensible.

7. Ácido Sulfúrico H_2SO_4 O.3 M
1x15ml/vial.
Contiene: solución 0.3 M de H_2SO_4.
Atención: Irritante (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

8. Diluyente de muestras DILSPE
2x60ml/vial.
Contiene: 2% caseina, 10 mM de tampon Citrato-Sódico pH 6.0 +/-0.1, 0.1% Tween 20, 0.09% Azida sódica y 0.045% ProClin 300 como conservantes. Usar para diluir las muestras.

9. Reactivo Neutralizante SOLN NTR
1x8ml/vial.
Listo para el uso. Contiene: anticuerpos de cabra anti-IgG humana, 2% caseina, 10 mM de tampón Citrato sódico pH 6.0 +/-0.1, 0.1% Tween 20, 0.09% Azida sódica y 0.045% ProClin 300 como conservantes.

10. Sellador adhesivo n° 2

11. Libro de Instrucciones n° 1

E. MATERIALES NECESARIOS PERO NO SUMINISTRADOS
1. Micropipetas calibradas (100ul and 10ul) y puntas de plástico desechables.
2. Agua de calidad EIA (bidestilada o desionizada, tratada con carbón para eliminar agentes químicos oxidantes usados como desinfectantes).
3. Reloj con un rango de 60 minutos o más.
4. Papel absorbente.
5. Incubador termostático de microplacas ELISA capaz de alcanzar una temperatura de 37ºC.
6. Lector calibrado de microplacas de ELISA con filtros de 450nm (lectura) y de filtros de 620-630 nm.
7. Lavador calibrado de microplacas ELISA.
8. Agitador o similar.

F. ADVERTENCIAS Y PRECAUCIONES

1. El estuche debe ser usado por personal técnico adecuadamente entrenado, bajo la supervisión de un doctor responsable del laboratorio.
2. Todas las personas encargadas de la realización de las pruebas deben llevar las ropas protectoras adecuadas de laboratorio, guantes y gafas. Evitar el uso de objetos cortantes (cuchillas) o punzantes (agujas). El personal debe ser entrenadon en procedimientos de bioseguridad, según ha sido recomendado por el Centro de Control de Enfermedades de Atlanta, Estados Unidos, y publicado por el Instituto Nacional de Salud: "Biosafety in Microbiological and Biomedical Laboratories", ed.1984.
3. Todo el personal involucrado en el manejo de muestras debe estar vacunado contra HBV y HAV, para lo cual existen vacunas disponibles, seguras y eficaces.
4. Se debe controlar el ambiente del laboratorio para evitar la contaminación de los componentes con polvo o agentes microbianos cuando se abran los estuches, así como durante la realización del ensayo. Evitar la exposición del cromógeno/ substrato a la luz y también las vibraciones de la mesa de trabajo durante el ensayo.
5. Conservar el estuche a temperaturas entre 2-8 ºC, en un refrigerador con temperatura regulada o en cámara fría.
6. No intercambiar reactivos de diferentes lotes ni tampoco de diferentes estuches.
7. Comprobar que los reactivos son transparentes y no contienen precipitados ni agregados en el momento del uso. En caso contrario, informar al supervisor del laboratorio para realizar el procedimiento pertinente y reemplazar el estuche.
8. Evitar contaminación cruzada entre muestras de suero/ plasma usando puntas desechables y cambiándolas después de cada uso. No reutilizar puntas desechables.
9. Evitar contaminación cruzada entre los reactivos del estuche usando puntas desechables y cambiándolas después de cada uso. No reutilizar puntas desechables.
10. No usar el producto después de la fecha de caducidad indicada en el estuche e internamente en los reactivos.
11. Tratar todas las muestras como potencialmente infecciosas. Las muestras de suero humano deben ser manipuladas al nivel 2 de bioseguridad, según ha sido recomendado por el Centro de Control de Enfermedades de Atlanta, Estados Unidos y publicado por el Instituto Nacional de Salud: "Biosafety in Microbiological and Biomedical Laboratories", ed.1984.
12. Se recomienda el uso de material plástico desechable para la preparación de las soluciones de lavado y para la transferencia de los reactivos a los diferentes equipos automatizados a fin de evitar contaminaciones.
13. Los desechos producidos durante el uso del estuche deben ser eliminados según lo establecido por las directivas nacionales y las leyes relacionadas con el tratamiento de los residuos químicos y biológicos de laboratorio. En particular, los desechos líquidos provenientes del proceso de lavado deben ser tratados como potencialmente infecciosos y deben ser inactivados antes de tirar. Se recomienda la inactivación con lejía al 10% de 16 a 18 horas o el uso de la autoclave a 121°C por 20 minutos.
14. En caso de derrame accidental de algún producto, se debe utilizar papel absorbente embebido en lejía y posteriormente en agua. El papel debe eliminarse en contenedores designados para este fin en hospitales y laboratorios.
15. El ácido sulfúrico es irritante. En caso de derrame, se debe lavar la superficie con abundante agua.
16. Otros materiales de desecho generados durante la utilización del estuche (por ejemplo: puntas usadas en la manipulación de las muestras y controles, microplacas usadas) deben ser manipuladas como fuentes potenciales de infección de acuerdo a las directivas nacionales y leyes para el tratamiento de residuos de laboratorio.

G. MUESTRA: PREPARACIÓN Y RECOMENDACIONES

1. Extraer la sangre asépticamente por punción venosa y preparar el suero o plasma según las técnicas estándar de los laboratorios de análisis clínico. No se ha detectado que el tratamiento con citrato, EDTA o heparina afecte las muestras.
2. Las muestras deben estar identificadas claramente mediante código de barras o nombres, a fin de evitar errores en los resultados. Cuando el estuche se emplea para el pesquisaje en unidades de sangre, se recomienda el uso del código de barras.
3. Las muestras hemolizadas (color rojo) o hiperlipémicas (aspecto lechoso) deben ser descartadas para evitar falsos resultados, al igual que aquellas donde se observe la presencia de precipitados, restos de fibrina o filamentos microbianos.
4. El suero y el plasma pueden conservarse a una temperatura entre +2° y +8°C en tubos de recolección principales hasta cinco días después de la extracción. No congelar tubos de recolección principales. Para periodos de almacenamiento más prolongados, las muestras de plasma o suero, retiradas cuidadosamente del tubo de extracción principal, pueden almacenarse congeladas a –20°C durante almenos 12 meses, evitando luego descongelar cada muestra más de una vez, ya que se pueden generar partículas que podrían afectar al resultado de la prueba.
5. Si hay presencia de agregados, la muestra se puede aclarar mediante centrifugación a 2000 rpm durante 20 minutos o por filtración con un filtro de 0,2-0,8 micras.

H. PREPARACIÓN DE COMPONENTES Y PRECAUCIONES

Según estudios realizados, no se ha detectado pérdida relevante de actividad en estuches abiertos, utilizados hasta 6 veces, en un período de hasta 6 meses.

Microplacas:
Dejar la microplaca a temperatura ambiente (aprox. 1 hora) antes de abrir el envase. Compruebe que el desecante no esté de un color verde oscuro, lo que indicaría un defecto de fabricación. De ser así, debe solicitar el servicio de Dia.Pro: atención al cliente.
Las tiras de pocillos no utilizadas, deben guardarse herméticamente cerradas en la bolsa de aluminio con el desecante a 2-8ºC. Una vez abierto el envase, las tiras sobrantes, se mantienen estables hasta que el indicador de humedad dentro de la bolsa del desecante cambie de amarillo a verde.

Control Negativo:
Listo para el uso. Mezclar bien con la ayuda de un vórtex, antes de usar.

Control Positivo:
Listo para el uso. Mezclar bien con la ayuda de un vórtex, antes de usar. Manipule este reactivo como potencialmente infeccioso, aunque las partículas virales presentes en el control han sido inactivadas quimicamente.

Tampón de lavado concentrado:
Todo el contenido del tampón concentrado 20x debe diluirse con agua bidestilada a 1200 ml y mezclarse suavemente antes de usarse.
Una vez diluido, el tampón de lavado es estable durante una semana conservándolo a una temperatura de 2-8ºC
Durante la preparación evitar la formación de espuma y burbujas, lo que podría influir en la eficiencia de los ciclos de lavado.
Nota: *Una vez diluida, la solución es estable por una semana a temperaturas entre +2 y 8ºC.*

Enzima conjugada:
Listo para el uso. Mezclar bien con un vórtex antes de usar. Evitar posible contaminación del líquido con oxidantes químicos, polvo o microbios.

En caso de que deba transferirse el reactivo, usar contenedores de plástico, estériles y desechables, siempre que sea posible.

Cromogeno/Substrato:
Listo para el uso. Mezclar bien con un vórtex antes de usar. Evitar posible contaminación del líquido con oxidantes químicos, polvo o microbios.
Evitar la exposición a la luz, agentes oxidantes y superficies metálicas.
En caso de que deba transferirse el reactivo, usar contenedores de plástico, estériles y desechables, siempre que sea posible.

Reactivo Neutralizante:
Listo para el uso. Mezclar bien con un vórtex antes de usar.

Diluyente de Muestras:
Listo para el uso. Mezclar bien con un vórtex antes de usar.

Ácido Sulfúrico:
Listo para el uso. Mezclar bien con un vórtex antes de usar.
Atención: Irritante (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

Leyenda:

Indicación de peligro, **Frases H**
H315 – Provoca irritación cutánea.
H319 – Provoca irritación ocular grave.

Consejo de prudencia, **Frases P**
P280 – Llevar guantes/prendas/gafas/máscara de protección.
P302 + P352 – EN CASO DE CONTACTO CON LA PIEL: Lavar con agua y jabón abundantes.
P332 + P313 – En caso de irritación cutánea: Consultar a un médico.
P305 + P351 + P338 – EN CASO DE CONTACTO CON LOS OJOS: Aclarar cuidadosamente con agua durante varios minutos. Quitar las lentes de contacto, si lleva y resulta fácil. Seguir aclarando.
P337 + P313 – Si persiste la irritación ocular: Consultar a un médico.
P362 + P363 – Quitarse las prendas contaminadas y lavarlas antes de volver a usarlas.

I. INSTRUMENTOS Y EQUIPAMIENTO UTILIZADOS EN COMBINACIÓN CON EL ESTUCHE.

1. Las micropipetas deben ser calibradas para dispensar correctamente el volumen requerido en el ensayo y sometidas a una descontaminación periódica de las partes que pudieran entrar accidentalmente en contacto con la muestra o los reactivos (alcohol, lejía 10%, desinfectantes hospitalarios). Deben además, ser regularmente revisadas para mantener una precisión del 1% y una confiabilidad de +/- 2%. Deben descontaminarse periódicamente los residuos de los componentes del estuche.
2. El incubador de ELISA debe ser ajustada a 37ºC (+/- 0.5ºC) y controlada periódicamente para mantener la temperatura correcta. Pueden emplearse incubadoras secas o baños de agua siempre que estén validados para la incubación de pruebas de ELISA.
3. El **lavador ELISA** es extremadamente importante para el rendimiento global del ensayo. El lavador debe ser validado de forma minuciosa previamente, revisado para comprobar que suministra el volumen de dispensación correcto y enviado regularmente a mantenimiento de acuerdo con las instrucciones de uso del fabricante. En particular, deben lavarse minuciosamente las sales con agua desionizada del lavador al final de la carga de trabajo diaria. Antes del uso, debe suministrarse extensivamente solución de lavado diluida al lavador. Debe enviarse el instrumento semanalmente a descontaminación según se indica en su manual (se recomienda descontaminación con NaOH 0.1 M). Para asegurar que el ensayo se realiza conforme a los rendimientos declarados, basta con 5 ciclos de lavado (aspiración + dispensado de 350 µl/pocillo de solución de lavado + 20 segundos de remojo = 1 ciclo). Si no es posible remojar, añadir un ciclo de lavado adicional. Un ciclo de lavado incorrecto o agujas obstruidas con sal son las principales causas de falsas reacciones positivas.
4. Los tiempos de incubación deben tener un margen de ±5%.
5. El lector de microplacas ELISA debe estar provisto de un filtro de lectura de 450nm y de un segundo filtro de 620-630nm, obligatorio para reducir interferencias en la lectura. El procedimiento estándar debe contemplar: a) Ancho de banda <= 10 b) Rango de absorbancia de 0 a >=2.0, c) Linealidad >=2.0, reproducibilidad >=1%. El blanco se prueba en el pocillo indicado en la sección “Procedimiento del ensayo”. El sistema óptico del lector debe ser calibrado periódicamente para garantizar la correcta medición de la densidad óptica, según las normas del fabricante.
6. En caso de usar un sistema automatizado de ELISA, los pasos críticos (dispensado, incubación, lavado, lectura, agitación y procesamiento de datos) deben ser cuidadosamente fijados, calibrados, controlados y periódicamente ajustados, para garantizar los valores indicados en la seccione “Control interno de calidad” y “Procedimiento del ensayo”. El protocolo del ensayo debe ser instalado en el sistema operativo de la unidad y validado tanto para el lavador como para el lector. Por otro lado, la parte del sistema que maneja los líquidos (dispensado y lavado) debe ser validada y fijada correctamente. Debe prestarse particular atención a evitar el arrastre por las agujas de dispensación y de lavado, a fin de minimizar la posibilidad de ocurrencia de falsos positivos por contaminación de los pocillos adyacentes por muestras fuertemente reactivas. Se recomienda el uso de sistemas automatizados de ELISA cuando el numero de muestras para analizar supera las 20-30 unidades por ensayo.
7. El servicio de atención al cliente en Dia.Pro, ofrece apoyo al usuario para calibrar, ajustar e instalar los equipos a usar en combinación con el estuche, con el propósito de asegurar el cumplimiento de los requerimientos descritos.

L. OPERACIONES Y CONTROLES PREVIOS AL ENSAYO

1. Compruebe la fecha de caducidad indicada en la parte externa del estuche (envase primario). No usar si ha caducado.
2. Compruebe que los componentes líquidos no están contaminados con partículas o agregados visibles.
3. Asegúrese de que el cromógeno (TMB) es incoloro o azul pálido, aspirando un pequeño volumen de este con una pipeta estéril de plástico.
4. Compruebe que no han ocurrido rupturas ni derrames de líquido dentro de la caja (envase primario) durante el transporte. Asegurarse de que la bolsa de aluminio que contiene la microplaca no esté rota o dañada.
5. Disolver totalmente el contenido del Suero Control, como se ha descrito anteriormente.
6. Diluir totalmente el tampón de lavado concentrada 20X, como se ha descrito anteriormente.
7. Dejar los componentes restantes alcanzar la temperatura ambiente (aprox. 1 hora), mezclar luego suavemente en el vórtex todos los reactivos líquidos.
8. Ajustar la incubadora de ELISA a 37ºC y cebar el lavador de ELISA utilizando la solución de lavado, según las instrucciones del fabricante. Fijar el número de ciclos de lavado según se indica en la sección específica.
9. Comprobar que el lector de ELISA esté conectado al menos 20 minutos antes de realizar la lectura.
10. En caso de trabajar automáticamente, conectar el equipo y comprobar que los protocolos estén correctamente programados.

11. Comprobar que las micropipetas estén fijadas en el volumen requerido.
12. Asegurarse de que el equipamiento a usar esté en perfecto estado, disponible y listo para el uso.
13. En caso de surgir algún problema, se debe detener el ensayo y avisar al supervisor.

M. PROCEDIMIENTO DEL ENSAYO

El ensayo debe realizarse según las instrucciones que siguen a continuación, es importante mantener en todas las muestras el mismo tiempo de incubación.

1. Diluir las muestras 1:101 dentro de un apropiado tubo (ejemplo: 1000 µl Diluyente de muestras + 10 µl muestra). No diluir el Control Negativo y Positivo ya que están listos para usarse. Mezclar todos los reactivos líquidos en un agitador y continua como se describe a continuación.
2. Poner el número de tiras necesarias en el soporte de plástico. Dejar el primer pocillo (A1) vacío para el blanco.
3. Dispensar 50 µl del Reactivo Neutralizante (SOLN NTR) en todos los pocillos de las muestras. ¡No añadirlo dentro de los pocillos usados para los controles y en el primer pocillo A1!

***Nota importante**: El reactivo neutralizante puede bloquear falsas reacciones positivas debido a RF. Las muestras positivas en paneles de control de calidad internos podrían ser detectadas como negativas si estas muestras se analizaron como positivas con un IVD que no realiza ninguna reacción de bloqueo de RF.*

4. Dispensar 100 µl de Control Negativo por triplicado y 100 µl de Control Positivo. Una vez hecho esto, añadir 100 µl de las muestras diluidas en cada uno de los pocillos marcados específicamente.
5. Incubar la microplaca durante **60 min a +37°C**.

***Nota importante:** Las tiras se deben sellar con el adhesivo suministrado solo cuando se hace el test manualmente. No sellar cuando se emplean equipos automatizados de ELISA.*

6. Lavar la microplaca con el lavador automático dispensando y aspirando 300 ul/pocillo de solución de lavado diluída, segun según se indica (section I.3).
7. Dispensar 100uL de la Enzima Conjugada en todos los pocillos, excepto en el A1 y cubrir con el sellador. Compruebe que este reactivo de color rojo ha sido añadido en todos los pocillos excepto el A1.

***Nota importante**: Tener cuidado de no tocar la pared interna del pocillo con la punta de la pipeta al dispensar el conjugado. Podría producirse contaminación.*

8. Incubar la microplaca 60 **min a +37°C**.
9. Lavar los pocillos como en el paso 6.
10. Dispensar 100µl del Cromógeno/Substrato en todos los pocillos, incluido el A1. Incubar la microplaca a **temperatura ambiente (18-24°C) durante 20 minutos**.

***Nota importante**: No exponer directamente a fuerte iluminación, de lo contrario se generan interferencias.*

11. Dispensar 100µl de ácido sulfúrico en todos los pocillos para detener la reacción enzimática, usar la misma secuencia que en el paso 10. La adición de la solución de parada cambia el color del Control Positivo y las muestras positivas de azul a amarillo.
12. Medir la intensidad del color de la solución en cada pocillo, según se indica en la sección I.5, con un filtro de 450 nm (lectura) y otro de 620-630 nm (substracción del fondo, obligatorio), calibrando el instrumento con el pocillo A1 (blanco).

Notas importantes:

1. *Asegurarse de que no hay impresiones digitales ni polvo en el fondo de los pocillos antes de leer. Podrían generarse falsos positivos en la lectura.*
2. *La lectura debe hacerse inmediatamente después de añadir la solución de parada y, en cualquier caso, nunca transcurridos 20 minutos después de su adición. Se podría producir auto oxidación del cromógeno causando un elevado fondo.*

N. ESQUEMA DEL ENSAYO

Metodo	**Operaciones**
Reactivo Neutralizande (¡añadir solo en los pocillos donde se añada la muestra!)	50 µl
Control Negativo y Positivo	100 µl
Muestras diluidas 1:101	100 µl
1ª incubación	**60 min**
Temperatura	+37°C
Lavado	5 ciclos con 20''de remojo o 6 ciclos sin remojo
Enzime conjugada	100 µl
2ª incubación	**60 min**
Temperatura	+37°C
Lavado	5 ciclos con 20''de remojo o 6 ciclos sin remojo
TMB/H2O2	100 µl
3ª incubación	**20 min**
Temperatura	18-24°C
Ácido sulfúrico	100 ul
Lectura DO	450nm/620-630nm

A continuación se describe un ejemplo del esquema de dispensado:

Microplace

	1	2	3	4	5	6	7	8	9	10	11	12
A	BL	M4										
B	CN	M5										
C	CN	M6										
D	CN	M7										
E	CP	M8										
F	M1	M9										
G	M2	M10										
H	M3	M11										

Leyenda: BL = Blanco CN = Control Negativo
CP = Control Positivo M = Muestra

O. CONTROL DE CALIDAD INTERNO

Se realiza un grupo de pruebas con los controles cada vez que se usa el estuche para verificar si los valores DO 450nm/620-630nm son los esperados.
Asegurar el cumplimiento de los siguientes parámetros:

Parámetro	**Exigencia**
Pocillo blanco	Valor < 0.100 DO 450nm
Control Negativo (CN)	Valor Medio < 0.100 DO 450nm despés de leer el blanco
Control Positivo	Valor > 0.500 DO 450nm

Si los resultados del ensayo coinciden con lo establecido anteriormente, pase a la siguiente sección.

En caso contrario, detenga el ensayo y compruebe:

Problema	Comprueba que
Pocillo blanco > 0.100 DO 450nm	1. La solución Cromogeno/Substrato no se ha contaminado durante el ensayo
Control Negativo (CN) > 0.100 DO 450nm después de leer el blanco	1. el proceso de lavado y los parámetros del lavador estén validados según los estudios previos de calificación. 2. se ha usado la solución de lavado apropiada y que el lavador ha sido cebado con la misma antes del uso. 3. no se han cometido errores en el procedimiento (dispensar el control positivo en lugar del negativo). 4. no ha existido contaminación del control negativo o de sus pocillos debido a muestras positivas derramadas, o al conjugado. 5. las micropipetas no se han contaminado con muestras positivas o con el conjugado. 6. las agujas del lavador no estén parcial o totalmente obstruidas.
Control Positivo < 0.500 DO 450nm	1. el procedimiento ha sido realizado correctamente. 2. no se han cometido errores en el procedimiento (dispensar el control negativo en lugar del positivo). En este caso el control negativo debe tener un valor de DO450nm > 0.150. 3. el proceso de lavado y los parámetros del lavador estén validados según los estudios previos de calificación. 4. no ha ocurrido contaminación externa del control positivo.

Si ocurre alguno de los problemas anteriores, luego de comprobar, informe al supervisor para tomar las medidas pertinentes.

Nota importante:
El análisis debe seguir el paso de lectura descrito en la sección M, punto 12.

P. CÁLCULO DEL VALOR DE CORTE
Los resultados se calculan por medio de un valor de corte (cut-off) hallado con la siguiente fórmula:

Valor de corte = Media CN DO450nm/620-630nm + 0.250

El valor encontrado para el ensayo se usa para la interpretación de los resultados, según se describe a continuación:

Nota Importante*: Cuando el cálculo de los resultados se halla mediante el sistema operativo de un equipo de ELISA automático, asegurarse de que la formulación usada para el cálculo del valor de corte, y para la interpretación de los resultados sea correcta.*

Q. INTERPRETACIÓN DE LOS RESULTADOS
La interpretación de los resultados se realiza mediante la razón entre las DO a 450nm/620-630nm de las muestras y el Valor de corte (M/Co).
Los resultados se interpretan según la siguiente tabla:

M/Co	Interpretación
< 1.0	Negativo
1.0 – 1.2	Equivoco
> 1.2	Positivo

Un resultado negativo indica que el paciente no posee anti-HEV IgM detectables
Cualquier paciente, cuya muestra resulte equívoca debe someterse a una nueva prueba con una segunda muestra de sangre colectada 1 ó 2 semanas después de la inicial.
Un resultado positivo es indicativo de infección por HEV y por consiguiente el paciente debe ser tratado adecuadamente.

Notas importantes:
5. *La interpretación de los resultados debe hacerse bajo la vigilancia del supervisor del laboratorio para reducir el riesgo de errores de juicio y de interpretación.*
6. *Antes de formular un diagnóstico de hepatitis viral, los resultados positivos deben comprobarse a través de un método alternativo.*
7. *Cuando se transmiten los resultados de la prueba, del laboratorio a otras instalaciones, debe ponerse mucha atención para evitar el traslado de datos erróneos.*
8. *El diagnóstico de infección con virus de la hepatitis E debe ser evaluado y comunicado al paciente por un médico calificado.*

A continuación, un ejemplo de los cálculos a realizar (datos obtenidos siguiendo el paso de lectura descrito en la sección M, punto 12).

Los siguientes datos no deben usarse en lugar de los valores reales obtenidos en el laboratorio

Control Negativo: 0.060 – 0.080 – 0.070 DO 450nm
Media del valor: 0.070 DO 450nm
Menor de 0.100 –Válido
Control Positivo: 1.589 DO 450nm
Mayor que 0.500 – Válido

Valor de corte = 0.070+0.250 = 0.320

Muestra 1: 0.070 DO 450nm
Muestra 2: 1.690 DO 450nm
Muestra 1 M/Co < 1.0 = negativo
Muestra 2 M/Co > 1.2 = positivo

R. REALIZACIONES
La evaluación de las realizaciones ha sido realizada sobre muestras negativas y positivas en un centro clínico con referencias a un FDA de aprobación del estuche.

1. LIMITE DE DETECCIÓN

El límite de detección del producto ha sido calculado sobre el reactivo de referencia internacional para anticuerpos anti-HEV aportados por NIBSC/WHO con código nº 95/584. Este material ha mostrado resultados positivos incluso para bajos niveles de anti-HEV IgM. El valor observado es aproximadamente 1 IU/ml.

2. ESPECIFICIDAD Y SENSIBILIDAD DIAGNÓSTICAS
Estos parámetros furon testados sobre 700 muestras proecedentes de diferentes donantes sanos y enfermos con una sensibilidad de 5 UI/ml aproximadamente para garantizar la sensibilidad máxima y poder detectar la infección primaria en la fase muy inicial.

2.1 Especificidad Diagnóstica:
Se define como la probabilidad del ensayo de detectar negativos en ausencia del analito específico.
Un total de mas de 500 muestras provenientes de donantes y pacientes HEV negativos, incluyendo aquellos que lo hacían por vez primera, fueron examinados.
Se estableció la especificidad diagnóstica mediante la referencia de un estuche US FDA. Se obtuvo una especificidad diangnóstica ≥ 95%.
También se analizaron 100 muestras que pudieran provocar interferencia (por ejemplo: otras enfermedades infecciosas, pacientes afectados por hepatitis no virales, pacientes sometidos a diálisis, mujeres embarazadas, hemofílicos, lipémicos, etc.). La especificidad obtenida fue del 100%.

Se emplearon además, plasma sometido a métodos de tratamiento estándar (citrato, EDTA y heparina) y suero humanos.
No se ha observado falsa reactividad debida a los métodos de tratamiento de muestras.
Por último se analizaron muestras congeladas, para determinar posibles interferencias debidas a la toma de muestra y al almacenamiento. No se observaron interferencias.
Muestras positivas altamente reactivas RF dieron origen a falsos positivos.

2.3 Sensibilidad Diagnóstica
Se define como la probabilidad del ensayo de detectar positivos en presencia del analito específico.
La sensibilidad diagnóstica ha sido estimada de forma externa e interna en un total de 100 muestras positivas, procedentes de Alemania, Mexico y Burma. Obteniéndose una valor de Sensibilidad diagnóstica 100%.

3. PRECISIÓN:
Ha sido calculada utilizando dos muestras, una negativa y una débil positiva, examinadas en 16 réplicas en tres filas separadas.
La variabilidad mostrada en las tablas no dió como resultado una clasificación errónea de las muestras.

S. LIMITACIONES
Los falsos positivos repetibles fueron ensayados para altos RF muestras positivas, escapando al efecto del Reactivo Neutralizante.
Las muestras que después de ser descongeladas presentan partículas de fibrina o partículas agregadas, generan algunos resultados falsos positivos.

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Todos los productos de diagnóstico in vitro fabricados por la empresa son controlados por un sistema certificado de control de calidad conforme a la norma ISO 13485. Cada lote se somete a un control de calidad y se libera al mercado únicamente si se ajusta a las especificaciones técnicas y criterios de aceptación de la CE.

Fabricante
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CE

HDV Ab

Competitive Enzyme Immunoassay for the qualitative determination of antibodies to Hepatitis Delta Virus in human serum and plasma

- for “in vitro” diagnostic use only -

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REF DAB.CE
96 Tests

HDV Ab

A. INTENDED USE

Competitive Enzyme ImmunoAssay (ELISA) for the qualitative determination of antibodies to Hepatitis Delta Virus or HDV in human plasma and sera with a "two-steps" methodology.
The kit is used for the follow-up of patients infected by HDV.
For "in vitro" diagnostic use only.

B. INTRODUCTION

The Hepatitis Delta Virus or HDV is a RNA defective virus composed of a core presenting the delta-specific antigen, encapsulated by HBsAg, that requires the helper function of HBV to support its replication.
Infection by HDV occurs in the presence of acute or chronic HBV infection. When acute delta and acute HBV simultaneously occur, the illness becomes severe and clinical and biochemical features may be indistinguishable from those of HBV infection alone. In contrast, a patient with chronic HBV infection can support HDV replication indefinitely, usually with a less severe illness appearing as a clinical exacerbation.
The determination of HDV specific serological markers (HDV Ag, HDV Ab, HDV IgM and HDV IgG) represents in these cases an important tool to the clinician for the classification of the etiological agent, for the follow up of infected patients and their treatment. The detection of HDV total antibodies allows the classification of the illness and the monitoring of the seroconversion event.

C. PRINCIPLE OF THE TEST

Anti-HDV antibodies, if present in the sample, compete with a virus-specific polyclonal IgG, labeled with peroxidase (HRP), for a fixed amount of rec-HDV coated on the microplate. The test is carried out with a two steps incubation competitive system. First the sample is added to the plate and specific anti HDV antibodies bind to the adsorbed antigen. After washing, an enzyme conjugated antibody to HDV is added and binds to the free portion of the antigen coated. After washing a chromogen/substrate mixture is dispensed. The concentration of the bound enzyme on the solid phase becomes inversely proportional to the amount of anti-HDV antibodies in the sample and its activity is detected by the added chromogen/substrate. The concentration of HDV-specific antibodies in the sample is determined by means of a cut-off value that allows for the semi quantitative detection of anti-HDV antibodies.

D. COMPONENTS

Each kit contains sufficient reagents to perform 96 tests.

1. Microplate: MICROPLATE
8x12 microwell strips coated with recombinant HDV-specific antigen and sealed into a bag with desiccant. Allow the microplate to reach room temperature before opening; reseal unused strips in the bag with desiccant and store at 4°C.

2. Negative Control: CONTROL -
1x2.0ml/vial. Ready to use. Contains goat serum proteins, 100 mM Tris-HCl buffer pH 7.4 +/-0.1, 0.09% Sodium Azide and 0.045% ProClin 300 as preservatives. The negative control is colour coded pale yellow.

3. Positive Control: CONTROL +
1x2.0ml/vial. Ready to use. Contains goat serum proteins, high titer anti HDV antibodies, 100 mM Tris-HCl buffer pH 7.4 +/-0.1, 0.09% Sodium Azide and 0.045% ProClin 300 as preservatives.
The positive control is colour coded green.

4. Calibrator: CAL ….
n° 1 vial. Lyophilised. To be dissolved with EIA grade water as reported in the label. Contains bovine serum proteins, low titer human antibodies to HDV, 0.2 mg/ml gentamicine sulphate and 0.045% ProClin 300 as preservatives.
Note: The volume necessary to dissolve the content of the vial may vary from lot to lot. Please use the right volume reported on the label.

5. Wash buffer concentrate: WASHBUF 20X
1x60ml/bottle. 20x concentrated solution.
Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2, 0.05% Tween 20 and 0.045% ProClin 300.

6. Enzyme conjugate: CONJ
1x16ml/vial. Ready-to-use solution. Contains 5% bovine serum albumine, 10 mM tris buffer pH 6.8 +/-0.1, Horseradish peroxidase conjugated antibody to HDV in presence of 0.2 mg/ml gentamicine sulphate and 0.045% ProClin 300 as preservatives. The component is colour coded red.

7. Chromogen/Substrate: SUBS TMB
1x16ml/vial. Contains a 50 mM citrate-phosphate buffered solution at pH 3.5-3.8, 4% DMSO, 0.03% tetra-methyl-benzidine or TMB and 0.02% hydrogen peroxide of H_2O_2.
Note: To be stored protected from light as sensitive to strong illumination.

8. Sulphuric Acid: H_2SO_4 O.3 M
1x15ml/vial. Contains 0.3 M H_2SO_4 solution.
Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

Plate sealers n° 2

Instructions for Use n° 1

E. MATERIALS REQUIRED BUT NOT PROVIDED

1. Calibrated Micropipettes in the range 10-1000 ul and disposable plastic tips.
2. EIA grade water (double distilled or deionized, charcoal treated to remove oxidizing chemicals used as disinfectants).
3. Timer with 60 minute range or higher.
4. Absorbent paper tissues.
5. Calibrated ELISA microplate thermostatic incubator (dry or wet) set at +37°C.
6. Calibrated ELISA microwell reader with 450nm (reading) and with 620-630nm (blanking) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.

F. WARNINGS AND PRECAUTIONS

1. The kit has to be used by skilled and properly trained technical personnel only, under the supervision of a medical doctor responsible of the laboratory.
2. All the personnel involved in performing the assay have to wear protective laboratory clothes, talc-free gloves and glasses. The use of any sharp (needles) or cutting (blades) devices should be avoided. All the personnel involved should be trained in biosafety procedures, as recommended by the Center for Disease Control, Atlanta, U.S. and reported in the National Institute of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
3. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.

4. The laboratory environment should be controlled so as to avoid contaminants such as dust or air-born microbial agents, when opening kit vials and microplates and when performing the test. Protect the Chromogen/Substrate (TMB/H_2O_2) from strong light and avoid vibration of the bench surface where the test is undertaken.
5. Upon receipt, store the kit at +2..8°C into a temperature controlled refrigerator or cold room.
6. Do not interchange components between different lots of the kits. It is recommended that components between two kits of the same lot should not be interchanged.
7. Check that the reagents are clear and do not contain visible heavy particles or aggregates. If not, advise the laboratory supervisor to initiate the necessary procedures.
8. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each sample. Do not reuse disposable tips.
9. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one. Do not reuse disposable tips.
10. Do not use the kit after the expiration date stated on external (primary container) and internal (vials) labels.
11. Treat all specimens as potentially infective. All human serum specimens should be handled at Biosafety Level 2, as recommended by the Center for Disease Control, Atlanta, U.S. in compliance with what reported in the Institutes of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
12. The use of disposable plastic labware is recommended in the preparation of the washing solution or in transferring components into other containers of automated workstations, in order to avoid contamination.
13. Waste produced during the use of the kit has to be discarded in compliance with national directives and laws concerning laboratory waste of chemical and biological substances. In particular, liquid waste generated from the washing procedure, from residuals of controls and from samples has to be treated as potentially infective material and inactivated. Suggested procedures of inactivation are treatment with a 10% final concentration of household bleach for 16-18 hrs or heat inactivation by autoclave at 121°C for 20 min..
14. Accidental spills have to be adsorbed with paper tissues soaked with household bleach and then with water. Tissues should then be discarded in proper containers designated for laboratory/hospital waste.
15. The Sulphuric Acid is an irritant. In case of spills, wash the surface with plenty of water.
16. Other waste materials generated from the use of the kit (example: tips used for samples and controls, used microplates) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

G. SPECIMEN: PREPARATION AND RECOMMANDATIONS

1. Blood is drawn aseptically by venepuncture and plasma or serum is prepared using standard techniques of preparation of samples for clinical laboratory analysis. No influence has been observed in the preparation of the sample with citrate, EDTA and heparin.
2. Avoid any addition of preservatives to samples; especially sodium azide as this chemical would affect the enzymatic activity of the conjugate.
3. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results. When the kit is used for the screening of blood units, bar code labeling and electronic reading is strongly recommended.
4. Haemolysed (red) and visibly hyperlipemic ("milky") samples have to be discarded as they could generate false results. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they could give rise to false results.
5. Sera and plasma can be stored at +2°…+8°C in primary collection tubes for up to five days after collection.
   Do not freeze primary tubes of collection. For longer storage periods, sera and plasma samples, carefully removed from the primary collection tube, can be stored frozen at –20°C for at least 12 months. Any frozen samples should not be frozen/thawed more than once as this may generate particles that could affect the test result.
6. If particles are present, centrifuge at 2.000 rpm for 20 min or filter using 0.2-0.8u filters to clean up the sample for testing.

H. PREPARATION OF COMPONENTS AND WARNINGS

A study conducted on an opened kit has not pointed out any relevant loss of activity up to 6 re-uses of the device and up to 3 months.

1. Antigen coated microwells:
Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant has not turned dark green, indicating a defect in manufacturing.
In this case, call Dia.Pro's customer service.
Unused strips have to be placed back into the aluminium pouch, with the desiccant supplied, firmly zipped and stored at +2°-8°C.
When opened the first time, unused strips are stable until the humidity indicator inside the desiccant bag turns from yellow to green.

2. Negative Control:
Ready to use. Mix well on vortex before use.

3. Positive Control:
Ready to use. Mix well on vortex before use.

4. Calibrator:
Low positive control. Add precisely the volume of EIA grade water, reported on its label, to the lyophilized powder; let fully dissolve and then gently mix on vortex.
Note: *The dissolved calibrator is not stable. Store it frozen in aliquots at –20°C. When thawed do not freeze again; discard it.*

5. Wash buffer concentrate:
The whole content of the 20x concentrated solution has to be diluted with EIA grade water up to 1200 ml and mixed gently end-over-end before use. During preparation avoid foaming as the presence of bubbles could impact on the efficiency of the washing cycles.
Note: *Once diluted, the wash solution is stable for 1 week at +2..8° C.*

6. Enzyme conjugate:
Ready to use. Mix well on vortex before use.
Avoid contamination of the liquid with oxidizing chemicals, dust or microbes. If this component has to be transferred, use only plastic, and if possible, sterile disposable containers.

7. Chromogen/Substrate:
Ready to use. Mix well on vortex before use.
Avoid contamination of the liquid with oxidizing chemicals, air-driven dust or microbes. Do not expose to strong light, oxidizing agents and metallic surfaces.
If this component has to be transferred use only plastic, and if possible, sterile disposable container

8. Sulphuric Acid:
Ready to use. Mix well on vortex before use.
Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

Legenda:
Warning **H statements:**
H315 – Causes skin irritation.
H319 – Causes serious eye irritation.

Precautionary **P statements:**
P280 – Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352 – IF ON SKIN: Wash with plenty of soap and water.
P332 + P313 – If skin irritation occurs: Get medical advice/attention.
P305 + P351 + P338 – IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337 + P313 – If eye irritation persists: Get medical advice/attention.
P362 + P363 – Take off contaminated clothing and wash it before reuse.

I. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

1. Micropipettes have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (70% ethanol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample or the components of the kit. They should also be regularly maintained in order to show a precision of 1% and a trueness of ±2%.
2. The ELISA incubator has to be set at +37°C (tolerance of ±0.5°C) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water baths are suitable for the incubations, provided that the instrument is validated for the incubation of ELISA tests.
3. The **ELISA washer** is extremely important to the overall performances of the assay. The washer must be carefully validated in advance, checked for the delivery of the right dispensation volume and regularly submitted to maintenance according to the manufacturer's instructions for use. In particular the washer, at the end of the daily workload, has to be extensively cleaned out of salts with deionized water. Before use, the washer has to be extensively primed with the diluted Washing Solution.
   The instrument weekly has to be submitted to decontamination according to its manual (NaOH 0.1 M decontamination suggested).
   5 washing cycles (aspiration + dispensation of 350ul/well of washing solution + 20 sec soaking = 1 cycle) are sufficient to ensure the assay with the declared performances. If soaking is not possible add one more cycle of washing.
   An incorrect washing cycle or salt-blocked needles are the major cause of false positive reactions.
4. Incubation times have a tolerance of ±5%.
5. The ELISA microplate reader has to be equipped with a reading filter of 450nm and with a second filter of 620-630nm, mandatory for blanking purposes. Its standard performances should be (a) bandwidth ≤ 10 nm; (b) absorbance range from 0 to 4; (c) linearity to 4; repeatability ≥ 1%. Blanking is carried out on the well identified in the section "Assay Procedure". The optical system of the reader has to be calibrated regularly to ensure that the correct optical density is measured. It should be regularly maintained according to the manufacturer 's instructions.
6. When using an ELISA automated work station, all critical steps (dispensation, incubation, washing, reading, shaking, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the sections "Validation of Test" and "Assay Performances". The assay protocol has to be installed in the operating system of the unit and validated as for the washer and the reader. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set. Particular attention must be paid to avoid carry over by the needles used for dispensing samples and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells due to strongly reactive samples, leading to false positive results. The use of ELISA automated work stations is recommended for blood screening and when the number of samples to be tested exceed 20-30 units per run.
7. Dia.Pro's customer service offers support to the user in the setting and checking of instruments used in combination with the kit, in order to assure full compliance with the requirements described. Support is also provided for the installation of new instruments to be used with the kit.

L. PRE ASSAY CONTROLS AND OPERATIONS

1. Check the expiration date of the kit printed on the external label (primary container). Do not use if expired.
2. Check that the liquid components are not contaminated by visible particles or aggregates. Check that the Chromogen/Substrate is colorless or pale blue by aspirating a small volume of it with a sterile plastic pipette. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box (primary container). Check that the aluminum pouch, containing the microplate, is not punctured or damaged.
3. Dilute all the content of the 20x concentrated Wash Solution as described above.
4. Dissolve the Calibrator as described above and gently mix.
5. Allow all the other components to reach room temperature (about 1 hr) and then mix gently on vortex all liquid reagents.
6. Set the ELISA incubator at +37°C and prepare the ELISA washer by priming with the diluted washing solution, according to the manufacturers instructions. Set the right number of washing cycles as reported in the specific section.
7. Check that the ELISA reader is turned on or ensure it will be turned on at least 20 minutes before reading.
8. If using an automated work station, turn on, check settings and be sure to use the right assay protocol.
9. Check that the micropipettes are set to the required volume.
10. Check that all the other equipment is available and ready to use.
11. In case of problems, do not proceed further with the test and advise the supervisor.

M. ASSAY PROCEDURE

The assay has to be carried out according to what reported below, taking care to maintain the same incubation time for all the samples in testing.

1. Place the required number of strips in the microplate holder. Leave A1 well empty for the operation of blanking.
Store the other strips into the bag in presence of the desiccant at +2..8°C, sealed.

2. Pipette 100 µl of Negative Control in triplicate, 100 µl Positive Control in single and then 100 µl of samples. Check that controls and samples have been correctly added.
Then incubate the microplate at **+37°C for 60 min**.

3. Wash the microplate as reported in section I.3.

4. In all the wells except A1, pipette 100 µl Enzyme Conjugate. Check that the reagent has been correctly added.
Then incubate the microplate at **+37°C for 60 min.**

Important note: *Be careful not to touch the inner surface of the well with the pipette tip when dispensing the Enzyme Conjugate. Contamination might occur.*

5. Wash the microplate as described.

6. Pipette 100 µl TMB/H_2O_2 mixture in each well, the blank wells included. Check that the reagent has been correctly added. Then incubate the microplate at **room temperature for 20 min**.

Important note: *Do not expose to strong direct light as a high background might be generated.*

7. Pipette 100 µl Sulphuric Acid into all the wells using the same pipetting sequence as in step n° 6 to stop the enzymatic reaction. Addition of the stop solution will turn the negative control and negative samples from blue to yellow.

8. Measure the colour intensity of the solution in each well, as described in section I.5 using a 450nm filter (reading) and a 620-630nm filter (background subtraction, mandatory), blanking the instrument on A1.

Important notes:

1. *Ensure that no finger prints are present on the bottom of the microwell before reading. Finger prints could generate false positive results on reading.*
2. *Reading has should ideally be performed immediately after the addition of the Stop Solution but definitely no longer than 20 minutes afterwards. Some self oxidation of the chromogen can occur leading to a higher background.*
3. *The use of the Calibrator, a low positive control, is not mandatory for the assay as the CAL does not enter into the cut-off calculation. The CAL may be used as a low titer positive control when a laboratory internal quality verification is required by the management. When used for such purpose, dispense 100 ul of it, possibly in duplicate.*

N. ASSAY SCHEME

Controls/Calibrator Samples	100 ul 100 ul
1st incubation	**60 min**
Temperature	+37°C
Washing step	n° 5 cycles with 20" of soaking OR n° 6 cycles without soaking
Enzyme Conjugate	100 ul
2nd incubation	**60 min**
Temperature	+37°C
Washing step	n° 5 cycles with 20" of soaking OR n° 6 cycles without soaking
TMB/H2O2 mix	100 ul
3rd incubation	**20 min**
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm / 620-630nm

An example of dispensation scheme (including CAL) is reported in the table below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S2										
B	NC	S3										
C	NC	S4										
D	NC	S5										
E	CAL	S6										
F	CAL	S7										
G	PC	S8										
H	S1	S9										

Legenda: BLK = Blank NC = Negative Control
CAL = Calibrator PC = Positive Control S = Sample

O. INTERNAL QUALITY CONTROL

A check is performed on the negative and positive controls any time, and on the Calibrator in addition when the kit is used for the first time, in order to verify whether the expected OD450nm / 620-630nm or Co/S values have been matched in the analysis. Ensure that the following parameters are met:

Parameter	Requirements
Blank well	< 0.100 OD450nm value
Negative Control (NC)	> 1.000 OD450nm after blanking If lower carefully control the washing procedure and decrease the number of cycles or the soaking time coefficient of variation < 30%
Positive Control (PC)	OD450 nm < NC/10
Calibrator (CAL)	PC $\leq$ OD450nm < (NC+PC)/5

If the results of the test match the requirements stated above, proceed to the next section.
If they don't, do not proceed any further and perform the following checks:

Problem	Check
Blank well > 0.100 OD450nm	that the Chromogen/Substrate solution has not become contaminated during the assay
Negative Control (NC) < 1.000 OD450nm after blanking coefficient of variation > 30%	1. that the washing procedure and the washer settings are as validated in the pre qualification study; 2. that the proper washing solution has been used and the washer has been primed with it before use; 3. that no mistake has been done in the assay procedure (dispensation of positive control instead of negative control); 4. that no contamination of the negative control or of the wells where the control was dispensed has occurred due to positive samples, to spills or to the enzyme conjugate; 5. that micropipettes have not become contaminated with positive samples or with the enzyme conjugate; 6. that the washer needles are not blocked or partially obstructed.

Calibrator OD450nm Outside the range	1. that the procedure has been correctly performed; 2. that no mistake has occurred during its distribution (ex.: dispensation of negative control instead of Calibrator); 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the calibrator has occurred.
Positive Control OD450nm > NC/10	1. that the procedure has been correctly performed; 2. that no mistake has occurred during the distribution of the control (dispensation of negative control instead of positive control). 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the positive control has occurred.

If any of the above problems have occurred, report the problem to the supervisor for further actions.

Important note:
The analysis must be done proceeding as the reading step described in the section M, point 8.

P. RESULTS
The results are calculated by means of a cut-off value determined with the following formula:

Cut-Off = (NC + PC) / 5

***Important note**: When the calculation of results is performed by the operating system of an ELISA automated work station, ensure that the proper formulation is used to calculate the cut-off value and generate the correct interpretation of results.*

Q. INTERPRETATION OF RESULTS
Results are interpreted as ratio between the cut-off value and the sample OD450nm / 620-630nm or Co/S. Results are interpreted according to the following table:

Co/S	Interpretation
< 0.9	Negative
0.9 – 1.1	Equivocal
> 1.1	Positive

A negative result indicates that the patient has not been infected by HDV.
Any patient showing an equivocal result should be re-tested on a second sample taken 1-2 weeks after the initial sample.
A positive result is indicative of HDV infection and therefore the patient should be treated accordingly.

Important notes:
1. *Interpretation of results should be done under the supervision of the laboratory supervisor to reduce the risk of judgement errors and misinterpretations.*
2. *When test results are transmitted from the laboratory to another facility, attention must be paid to avoid erroneous data transfer.*
3. *Diagnosis of viral hepatitis infection has to be taken by and released to the patient by a suitably qualified medical doctor.*

An example of calculation is reported below (data obtained proceeding as the the reading step described in the section M, point 8).

The following data must not be used instead of real figures obtained by the user.

Negative Control: 2.100 – 2.200 – 2.000 OD450nm
Mean Value: 2.100 OD450nm
Higher than 1.000 – Accepted

Positive Control: 0.100 OD450nm
Lower than NC/10 – Accepted

Cut-Off = (2.100 + 0.100) / 5 = 0.440

Calibrator: 0.300-0.260 OD450nm
Mean value: 0.280 OD450nm
Within the range PC ≤ OD450nm < (NC+PC)/5 – *Accepted*

Sample 1: 0.020 OD450nm
Sample 2: 1.900 OD450nm
Sample 1 Co/S > 1.1 positive
Sample 2 Co/S < 0.9 negative

R. PERFORMANCES
Evaluation of Performances has been conducted in accordance to what reported in the Common Technical Specifications or CTS (art. 5, Chapter 3 of IVD Directive 98/79/EC)

1. LIMIT OF DETECTION:
In absence of an international standard, the sensitivity of the assay has been calculated by means of the product named Accurun n° 127 supplied by Boston Biomedica Inc. – USA .
The table below reports the OD450nm shown by this preparation when diluted in Fetal Calf Serum to prepare a limiting dilution curve, in three different lots.

Co/S values

	DAB.CE	**Lot # 1102**	**DAB.CE**	**Lot # 0103**	**DAB.CE**	**Lot # 0403**
Accurun # 127	**OD450 nm**	**Co/S value**	**OD450 nm**	**Co/S value**	**OD450 nm**	**Co/S value**
1x	0.171	**3.0**	0.163	**2.9**	0.156	**2.8**
2x	0.187	**2.7**	0.176	**2.6**	0.179	**2.5**
4x	0.230	**2.2**	0.220	**2.1**	0.202	**2.2**
8x	0.298	**1.7**	0.285	**1.6**	0.271	**1.6**
16x	0.417	**1.2**	0.405	**1.1**	0.402	**1.1**
32x	0.514	**1.0**	0.490	***0.9***	0.482	***0.9***
64x	0.717	0.7	0.700	0.7	0.705	0.6
128x	1.063	0.5	1.006	0.5	1.015	0.4
CTRL (-)	2.484	//////////	2.261	//////////	2.114	//////////

2. DIAGNOSTIC SPECIFICITY AND SENSITIVITY
The diagnostic performances were evaluated in a clinical trial conducted by the Department of Gastro-Hepatology, Prof. M.Rizzetto, S.Giovanni Battista hospital, Torino, Italy, on more than 400 samples against a reference kit.
Negative, positive and potentially interfering samples were examined in the trial.
Both plasma, derived with different standard techniques of preparation (citrate, EDTA and heparin), and sera have been used to determine the specificity. No false reactivity due to the method of specimen preparation has been observed.
Results are briefly reported in the tables below:

Sensitivity	> 98 %
Specificity	> 98 %

3. PRECISION

The mean values obtained from a study conducted on two samples of different anti-HDV antibody reactivity, examined in 16 replicates in three separate runs for three lots of product, is reported below:

DAB.CE: lot #1102

Negative Control (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	2.342	2.428	2.433	2.401
Std.Deviation	0.113	0.106	0.122	0.114
CV %	4.8	4.4	5.0	4.7

Calibrator (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.298	0.289	0.286	0.291
Std.Deviation	0.023	0.027	0.026	0.025
CV %	7.7	9.3	9.1	8.7
Co/S	1.6	1.7	1.7	1.7

DAB.CE: lot #0103

Negative Control (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	2.208	2.237	2.246	2.230
Std.Deviation	0.105	0.108	0.108	0.107
CV %	4.7	4.8	4.8	4.8

Calibrator (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.269	0.277	0.266	0.271
Std.Deviation	0.026	0.024	0.025	0.025
CV %	9.8	8.5	9.5	9.3
Co/ S	1.7	1.7	1.7	1.7

DAB.CE: lot # 0403

Negative Control (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	2.246	2.221	2.182	2.216
Std.Deviation	0.097	0.103	0.118	0.106
CV %	4.3	4.6	5.4	4.8

Calibrator (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.286	0.273	0.280	0.280
Std.Deviation	0.027	0.023	0.026	0.025
CV %	9.3	8.5	9.1	9.0
Co/S	1.6	1.7	1.6	1.6

The variability shown in the tables did not result in sample misclassification.

Important note:
The performance data have been obtained proceeding as the reading step described in the section M, point 8.

S. LIMITATIONS

Bacterial contamination or heat inactivation of the specimen may affect the absorbance values of the samples with consequent alteration of the level of the analyte.
This test is suitable only for testing single samples and not pooled ones.
Diagnosis of an infectious disease should not be established on the basis of a single test result. The patient's clinical history, symptomatology, as well as other diagnostic data should be considered.

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All the IVD Products manufactured by the company are under the control of a certified Quality Management System approved by an EC Notified Body. Each lot is submitted to a quality control and released into the market only if conforming with the EC technical specifications and acceptance criteria.

Manufacturer:
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CE
0318

HDV Ab

Ensayo inmunoenzimático competitivo para la determinación cualitativa de anticuerpos frente al Virus de la Hepatitis Delta en plasma y suero humanos

Uso exclusivo para diagnóstico “in vitro”

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REF DAB.CE
96 pruebas

HDV Ab

A. OBJETIVO DEL EQUIPO.
Ensayo inmunoenzimático competitivo (ELISA) para la determinación cualitativa de anticuerpos frente al Virus de la Hepatitis Delta (HDV) en plasma y suero humanos con una metodología de "dos pasos".
El equipo ha sido desarrollado para el seguimiento de pacientes infectados con HDV.
Uso exclusivo para diagnóstico "in vitro".

B. INTRODUCCIÓN.
El Virus de la Hepatitis Delta es un virus ARN defectivo. Se compone de un núcleo con los antígenos delta específicos, y está encapsulado por el HBsAg. Para su replicación necesita ayuda funcional de HBV.
La infección por HDV ocurre en presencia de una infección aguda o crónica por HBV. Cuando se presenta simultáneamente la infección aguda por los dos virus, la enfermedad es grave y el cuadro clínico, así como las características bioquímicas son practicamente indistinguibles de una infección por HBV. Sin embargo, una persona infectada por HBV de forma crónica puede soportar indefinidamente la replicación por HDV, normalmente la enfermedad es menos severa y aparece como exacerbación clínica.
La determinación de los marcadores serológicos específicos de HDV (HDV Ag, HDV Ab, HDV IgM y HDV IgG) representa una herramienta importante para los clínicos en la clasificación del agente etiológico, en el seguimiento de los pacientes así como en el tratamiento.
La detección de anticuerpos totales permite la clasificación de la enfermedad y el seguimiento de la seroconversión.

C. PRINCIPIOS DEL ENSAYO.
El ensayo es de tipo competitivo, donde los anticuerpos anti-HDV de la muestra compiten con un anticuerpo policlonal (IgG) específico para el virus y conjugado con peroxidasa (HRP), por el antígeno recombinate-HDV de la fase sólida.
El ensayo se realiza mediante un sistema de dos pasos con incubación competitiva. La muestra se añade a la placa y los anticuerpos específicos anti-HDV se combinan con el antígeno de la fase sólida. Después del lavado, se añade un anticuerpo conjugado con peroxidasa (HRP) que se une al antígeno libre en la placa. Previo lavado, se añade el substrato cromogénico.
La concentración de la enzima conjugada, unida a la fase sólida es inversamente proporcional a la cantidad de anticuerpos al HDV presentes en la muestra y su actividad se detecta por la adición del substrato cromogénico.
La concentración de anticuerpos específicos al HDV en la muestra se determina de manera semicuantitativa a través del cálculo de un valor de corte.

D. COMPONENTES.
Cada equipo contiene reactivos suficientes para realizar 96 pruebas.

1. Microplaca: MICROPLATE
12 tiras de 8 pocillos recubiertos con antígeno recombinante específico de HDV, en bolsas selladas con desecante. Se deben poner las placas a temperatura ambiente antes de abrirlas, sellar las tiras sobrantes en la bolsa con el desecante y almacenar a 4ºC.

2. Control Negativo: CONTROL -
1x2.0ml/vial. Listo para el uso. Contiene proteinas del suero de cabra, tampón Tris-HCl 100 mM pH 7.4 +/-0.1, además de azida sódica 0.09% y ProClin 300 0.045% como conservantes. El control negativo está codificado con el color amarillo pálido.

3. Control Positivo: CONTROL +
1x2.0ml/vial. Listo para el uso. Contiene proteinas del suero de cabra, alto título de anticuerpos anti-HDV, tampón Tris-HCl 100 mM pH 7.4 +/-0.1, además de azida sódica 0.09% y ProClin 300 0.045% como conservantes. El control positivo está codificado con el color verde.

4. Calibrador: CAL ….
n° 1 vial. Liofilizado. Para disolver en agua calidad EIA como se indica en la etiqueta. Contiene suero bovino fetal, bajo título de anticuerpos humanos al HDV, además de sulfato de gentamicina 0.02 mg/ml y ProClin 300 0.045% como conservantes.
Nota: El volumen necesario para disolver el contenido del frasco, varía en cada lote. Se recomienda usar el volumen indicado en la etiqueta.

5. Tampón de Lavado Concentrado: WASHBUF 20X
1x60ml/botella. Solución concentrada 20x.
Una vez diluida, la solución de lavado contiene tampón fosfato 10 mM a pH 7.0 +/- 0.2, Tween 20 al 0.05% y ProClin 300 al 0.045%

6. Conjugado CONJ
1x16ml/vial. Solución lista para el uso. Contiene 5% de albúmina de suero bovino, tampón Tris 10mM a pH 6.8 +/- 0.1, anticuerpo anti-HDV conjugado con peroxidasa (HPR) en presencia de 0.2 mg/ml de sulfato de gentamicina y ProClin 300 0.045% como conservante. El conjugado está codificado con el color rojo.

7. Cromógeno/Substrato SUBS TMB
1x16ml/vial. Contiene una solución tamponada citrato-fosfato 50mM pH 3.5-3.8, tetra-metil-benzidina (TMB) 0.03% y peróxido de hidrógeno (H_2O_2) 0.02% así como dimetilsulfóxido 4%.
Nota: Evitar la exposición a la luz, ya que la sustancia es fotosensible.

8. Ácido Sulfúrico: H_2SO_4 O.3 M
1x15ml/vial. Contiene solución de H_2SO_4 0.3M
Atención: Irritante (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

Sellador adhesivo, n° 2

Manual de instrucciones, n° 1

E. MATERIALES NECESARIOS NO SUMINISTRADOS.
1. Micropipetas calibradas (10-1000 µl) y puntas plásticas desechables.
2. Agua de calidad EIA (Bidestilada o desionizada, tratada con carbón para remover químicos oxidantes usados como desinfectantes).
3. *Timer* con un rango de 60 minutos como mínimo.
4. Papel absorbente.
5. Incubador termostático de microplacas ELISA, calibrado (en seco o húmedo) fijo a 37ºC.
6. Lector calibrado de microplacas de ELISA con filtros de 450nm (lectura) y de 620-630 nm.
7. Lavador calibrado de microplacas ELISA.
8. Vórtex o similar.

F. ADVERTENCIAS Y PRECAUCIONES.
1. El equipo debe ser usado por personal técnico adecuadamente entrenado, bajo la supervisión de un doctor responsable del laboratorio.
2. Todas las personas encargadas de la realización de las pruebas deben llevar las ropas protectoras adecuadas de laboratorio, guantes y gafas. Evitar el uso de objetos cortantes (cuchillas) o punzantes (agujas). El personal

debe ser adiestrado en procedimientos de bioseguridad, según ha sido recomendado por el Centro de Control de Enfermedades de Atlanta, Estados Unidos, y publicado por el Instituto Nacional de Salud: "Biosafety in Microbiological and Biomedical Laboratories", ed.1984.

3. Todo el personal involucrado en el manejo de muestras debe estar vacunado contra HBV y HAV, para lo cual existen vacunas disponibles, seguras y eficaces.
4. Se debe controlar el ambiente del laboratorio para evitar la contaminación de los componentes con polvo o agentes microbianos cuando se abran los equipos, así como durante la realización del ensayo. Evitar la exposición del substrato (TMB/H_2O_2) a la luz y las vibraciones de la mesa de trabajo durante el ensayo.
5. Conservar el equipo a temperaturas entre 2-8 ºC, en un refrigerador con temperatura regulada o en cámara fría.
6. No intercambiar reactivos de diferentes lotes ni tampoco de diferentes equipos.
7. Comprobar que los reactivos no contienen precipitados ni agregados en el momento del uso. De darse el caso, informar al responsable para realizar el procedimiento pertinente.
8. Evitar contaminación cruzada entre muestras de suero/ plasma usando puntas desechables y cambiándolas después de cada uso. No reutilizar puntas desechables.
9. Evitar contaminación cruzada entre los reactivos del equipo usando puntas desechables y cambiándolas después de cada uso. No reutilizar puntas desechables.
10. No usar el producto después de la fecha de caducidad indicada en el equipo e internamente en los reactivos.
11. Tratar todas las muestras como potencialmente infecciosas. Las muestras de suero humano deben ser manipuladas al nivel 2 de bioseguridad, según ha sido recomendado por el Centro de Control de Enfermedades de Atlanta, Estados Unidos y publicado por el Instituto Nacional de Salud: "Biosafety in Microbiological and Biomedical Laboratories", ed.1984.
12. Se recomienda el uso de material plástico desechable para la preparación de las soluciones de lavado y para la transferencia de los reactivos a los diferentes equipos automatizados a fin de evitar contaminaciones.
13. Los desechos producidos durante el uso del equipo deben de ser eliminados según lo establecido por las directivas nacionales y las leyes relacionadas con el tratamiento de los residuos químicos y biológicos de laboratorio. En particular, los desechos líquidos provenientes del proceso de lavado deben ser tratados como potencialmente infecciosos y deben ser inactivados. Se recomienda la inactivación con lejía al 10% de 16 a 18 horas o el uso de la autoclave a 121°C por 20 minutos.
14. En caso de derrame accidental de algún producto, se debe utilizar papel absorbente embebido en lejía y posteriormente en agua. El papel debe eliminarse en contenedores designados para este fin en hospitales y laboratorios.
15. El ácido sulfúrico es irritante. En caso de derrame, se debe lavar la superficie con abundante agua.
16. Otros materiales de desecho generados durante la utilización del equipo (por ejemplo: puntas usadas en la manipulación de las muestras y controles, microplacas usadas) deben ser manipuladas como fuentes potenciales de infección de acuerdo a las directivas nacionales y leyes para el tratamiento de residuos de laboratorio.

G. MUESTRA: PREPARACIÓN Y RECOMENDACIONES.

1. Extraer la sangre asépticamente por punción venosa y preparar el suero o plasma según las técnicas estándar de los laboratorios de análisis clínico. No se ha detectado que el tratamiento con citrato, EDTA o heparina afecte las muestras.
2. Evitar el uso de conservantes, en particular azida sódica, ya que pudiera afectar la actividad enzimática del conjugado.
3. Las muestras deben estar identificadas claramente mediante código de barras o nombres, a fin de evitar errores en los resultados. Cuando el equipo se emplea para el pesquisaje en unidades de sangre, se recomienda el uso del código de barras.
4. Las muestras hemolizadas (color rojo) o hiperlipémicas (aspecto lechoso) deben ser descartadas para evitar falsos resultados, al igual que aquellas donde se observe la presencia de precipitados, restos de fibrina o filamentos microbianos.
5. El suero y el plasma pueden conservarse a una temperatura entre +2° y +8°C en tubos de recolección principales hasta cinco días después de la extracción. No congelar tubos de recolección principales. Para periodos de almacenamiento más prolongados, las muestras de plasma o suero, retiradas cuidadosamente del tubo de extracción principal, pueden almacenarse congeladas a –20°C durante al menos 12 meses. Evitar congelar/descongelar cada muestra más de una vez, ya que pueden generarse partículas que podrían afectar al resultado de la prueba.
6. Si hay presencia de agregados, la muestra se puede aclarar mediante centrifugación a 2000 rpm durante 20 minutos o por filtración con un filtro de 0,2-0,8 micras.

H. PREPARACIÓN DE LOS COMPONENTES Y PRECAUCIONES.

Según estudios realizados, no se ha detectado pérdida relevante de actividad en equipos abiertos, en uso por un período de hasta 3 meses.

1. Microplacas:
Dejar la microplaca a temperatura ambiente (aprox. 1 hora) antes de abrir el envase. Compruebe que el desecante no esté de un color verde oscuro, lo que indicaría un defecto de fabricación. De ser así, debe solicitar el servicio de Dia.Pro: atención al cliente.
Las tiras de pocillos no utilizadas, deben guardarse herméticamente cerradas en la bolsa de aluminio con el desecante a 2-8ºC. Una vez abierto el envase, las tiras sobrantes, se mantienen estables hasta que el indicador de humedad dentro de la bolsa del desecante cambie de amarillo a verde.

2. Control Negativo:
Listo para el uso. Mezclar bien con la ayuda de un vórtex, antes de usar.

3. Control Positivo:
Listo para el uso. Mezclar bien con la ayuda de un vórtex, antes de usar.

4. Calibrador:
Control positivo bajo. Añadir de manera precisa al polvo liofilizado el volumen de agua de calidad EIA indicado en la etiqueta. Dejar disolver totalmente y mezclar suavemente en el vórtex.
Note: *Una vez reconstituida, la solución no es estable. Se recomienda mantenerla congelada en alícuotas a –20ºC. Cuando se descongele, descartar el agua en lugar de congelarla nuevamente.*

5. Solución de Lavado Concentrada:
Todo el contenido de la solución concentrada 20x debe diluirse con agua bidestilada hasta 1200ml y mezclarse suavemente antes de usarse. Durante la preparación evitar la formación de espuma y burbujas, lo que podría influir en la eficiencia de los ciclos de lavado.
Nota: *Una vez diluida, la solución es estable por una semana a temperaturas entre +2 y 8ºC.*

6. Conjugado:
Listo para el uso. Mezclar bien con un vórtex antes de usar. Evitar posible contaminación del líquido con oxidantes químicos, polvo o microbios. En caso de que deba transferirse el reactivo, usar contenedores de plástico, estériles y desechables, siempre que sea posible.

7. Cromógeno/ Substrato:
Listo para el uso. Mezclar bien con un vórtex antes de usar. Evitar posible contaminación del líquido con oxidantes químicos, polvo o microbios. Evitar la exposición a la luz, agentes oxidantes y superficies metálicas. En caso de que deba transferirse el reactivo, usar contenedores de plástico, estériles y desechables, siempre que sea posible.

8. Ácido Sulfúrico:
Listo para el uso. Mezclar bien con un vórtex antes de usar.
Atención: Irritante (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

Leyenda:

Indicación de peligro, **Frases H**
H315 – Provoca irritación cutánea.
H319 – Provoca irritación ocular grave.

Consejo de prudencia, **Frases P**
P280 – Llevar guantes/prendas/gafas/máscara de protección.
P302 + P352 – EN CASO DE CONTACTO CON LA PIEL: Lavar con agua y jabón abundantes.
P332 + P313 – En caso de irritación cutánea: Consultar a un médico.
P305 + P351 + P338 – EN CASO DE CONTACTO CON LOS OJOS: Aclarar cuidadosamente con agua durante varios minutos. Quitar las lentes de contacto, si lleva y resulta fácil. Seguir aclarando.
P337 + P313 – Si persiste la irritación ocular: Consultar a un médico.
P362 + P363 – Quitarse las prendas contaminadas y lavarlas antes de volver a usarlas.

I. INSTRUMENTOS Y EQUIPAMIENTO UTILIZADOS EN COMBINACIÓN CON EL EQUIPO.

1. Las micropipetas deben ser calibradas para dispensar correctamente el volumen requerido en el ensayo y sometidas a una descontaminación periódica de las partes que pudieran entrar accidentalmente en contacto con la muestra o los reactivos (etanol 70%, lejía 10%, de calidad de los desinfectantes hospitalarios). Deben además, ser regularmente revisadas para mantener una precisión del 1% y una confiabilidad de +/- 2%.
2. La incubadora de ELISA debe ser ajustada a 37ºC (+/- 0.5ºC) y controlada periódicamente para mantener la temperatura correcta. Pueden emplearse incubadoras secas o baños de agua siempre que estén validados para la incubación de pruebas de ELISA.
3. El **lavador ELISA** es extremadamente importante para el rendimiento global del ensayo. El lavador debe ser validado de forma minuciosa previamente, revisado para comprobar que suministra el volumen de dispensación correcto y enviado regularmente a mantenimiento de acuerdo con las instrucciones de uso del fabricante. En particular, deben lavarse minuciosamente las sales con agua desionizada del lavador al final de la carga de trabajo diaria. Antes del uso, debe suministrarse extensivamente solución de lavado diluida al lavador. Debe enviarse el instrumento semanalmente a descontaminación según se indica en su manual (se recomienda descontaminación con NaOH 0.1 M). Para asegurar que el ensayo se realiza conforme a los rendimientos declarados, basta con 5 ciclos de lavado (aspiración + dispensado de 350 µl/pocillo de solución de lavado + 20 segundos de remojo = 1 ciclo). Si no es posible remojar, añadir un ciclo de lavado adicional. Un ciclo de lavado incorrecto o agujas obstruidas con sal son las principales causas de falsas reacciones positivas.
4. Los tiempos de incubación deben tener un margen de $\pm$5%.
5. El lector de microplaca ELISA debe estar provisto de un filtro de lectura de 450nm y de un segundo filtro de 620-630 nm, obligatorio para reducir interferencias en la lectura. El procedimiento estándar debe contemplar: a) Ancho de banda $\leq$10nm b) Rango de absorbancia de 0 a 4, c) Linealidad a 4, reproducibilidad $\geq$1%. El blanco se prueba en el pocillo indicado en la sección “Procedimiento del ensayo”. El sistema óptico del lector debe ser calibrado periódicamente para garantizar que se mide la densidad óptica correcta. Periódicamente debe procederse al mantenimiento según las instrucciones del fabricante.
6. En caso de usar un sistema automatizado de ELISA, los pasos críticos (dispensado, incubación, lavado, lectura, agitación y procesamiento de datos) deben ser cuidadosamente fijados, calibrados, controlados y periódicamente ajustados, para garantizar los valores indicados en las secciones “Control interno de calidad” y “Procedimiento del ensayo”. El protocolo del ensayo debe ser instalado en el sistema operativo de la unidad y validado tanto para el lavador como para el lector. Por otro lado, la parte del sistema que maneja los líquidos (dispensado y lavado) debe ser validada y fijada correctamente. Debe prestarse particular atención a evitar el arrastre por las agujas de dispensación y las de lavado, a fin de minimizar la posibilidad de ocurrencia de falsos positivos por contaminación de los pocillos adyacentes por muestras fuertemente reactivas. Se recomienda el uso de sistemas automatizados para el pesquisaje en unidades de sangre y cuando la cantidad de muestras supera las 20-30 unidades por ensayo.
7. El servicio de atención al cliente en Dia.Pro, ofrece apoyo al usuario para calibrar, ajustar e instalar los equipos a usar en combinación con el equipo, con el propósito de asegurar el cumplimiento de los requerimientos descritos.

L. OPERACIONES Y CONTROLES PREVIOS AL ENSAYO.

1. Compruebe la fecha de caducidad indicada en la parte externa del equipo (envase primario). No usar si ha caducado.
2. Compruebe que los componentes líquidos no están contaminados con partículas o agregados visibles. Asegúrese de que el cromógeno (TMB) es incoloro o azul pálido, aspirando un pequeño volumen de este con una pipeta estéril de plástico. Compruebe que no han ocurrido rupturas ni derrames de líquido dentro de la caja (envase primario) durante el transporte. Asegurarse de que la bolsa de aluminio que contiene la microplaca no esté rota o dañada.
3. Diluir totalmente la solución de lavado 20x concentrada, como se ha descrito anteriormente.
4. Disolver el Calibrador como se ha descrito anteriormente y mezclar suavemente usando un vórtex.
5. Dejar los componentes restantes alcanzar la temperatura ambiente (aprox. 1 hora), mezclar luego suavemente en el vórtex todos los reactivos líquidos.
6. Ajustar la incubadora de ELISA a 37ºC y cebar el lavador de ELISA utilizando la solución de lavado, según las instrucciones del fabricante. Fijar el número de ciclos de lavado según se indica en la sección específica.
7. Comprobar que el lector de ELISA esté conectado al menos 20 minutos antes de realizar la lectura.
8. En caso de trabajar automáticamente, conectar el equipo y comprobar que los protocolos estén correctamente programados.
9. Comprobar que las micropipetas estén fijadas en el volumen requerido.
10. Asegurarse de que el equipamiento a usar esté en perfecto estado, disponible y listo para el uso.

11. En caso de surgir algún problema, se debe detener el ensayo y avisar al responsable.

M. PROCEDIMIENTO DEL ENSAYO.

El ensayo debe realizarse según las instrucciones que siguen a continuación, es importante mantener en todas las muestras el mismo tiempo de incubación.

1. Poner el número necesario de tiras en el soporte plástico. Dejar el pocillo A1 vacío para el blanco.
Almacenar las tiras restantes en la bolsa con el desecante a temperaturas entre 2 y 8°C.

2. Dispensar 100µl del Control Negativo, por triplicado, 100µl del Control Positivo una vez y, posteriormente, añadir 100µl de muestras. Comprobar que los controles y muestras se han añadido correctamente.
Después incubar la microplaca durante **60 minutos a +37ºC.**

3. Lavar la microplaca según lo descrito previamente (sección I.3).

4. Dispensar 100µl de Conjugado en todos los pocillos, excepto A1; comprobar que los reactivos se han añadido correctamente. Incubar la microplaca durante **60 minutos a +37°C.**

***Nota importante**: Tener cuidado de no tocar la pared interna del pocillo con la punta de la pipeta al dispensar el conjugado. Podría producirse contaminación.*

5. Lavar la microplaca según lo descrito previamente (sección I.3).

6. Dispensar 100µl del Cromógeno/Substrato en todos los pocillos, incluido el A1.
Incubar la microplaca protegida de la luz a **temperatura ambiente (18-24°C) durante 20 minutos**.

***Nota importante**: No exponer directamente a fuerte iluminación, de lo contrario se generan interferencias.*

7. Dispensar 100µl de ácido sulfúrico en todos los pocillos para detener la reacción enzimática, usar la misma secuencia que en el paso 6. La adición de la solución de parada cambia el color del Control Negativo y las muestras negativas de azul a amarillo.

8. Medir la intensidad del color de la solución en cada pocillo, según se indica en la sección I.5, con un filtro de 450 nm (lectura) y otro de 620-630 nm (substracción del fondo, obligatorio), calibrando el instrumento con el pocillo A1 (blanco).

Notas importantes:

1. *Asegurarse de que no hay impresiones digitales en el fondo de los pocillos antes de leer. Podrían generarse falsos positivos en la lectura.*
2. *La lectura debe hacerse inmediatamente después de añadir la solución de parada y, en cualquier caso, nunca transcurridos 20 minutos después de su adición. Se podría producir auto oxidación del cromógeno causando un elevado fondo.*
3. *El uso del calibrador (CAL), un control negativo bajo, no es obligatorio para el ensayo ya que el calibrador (CAL) no afecta al cálculo del valor de corte. El calibrador (CAL) puede usarse como un control negativo bajo si la gestión requiere un control interno de calidad del laboratorio. Dispensar 100µl del calibrador (CAL), posiblemente por duplicado, cuando se utilice para este propósito.*

N. ESQUEMA DEL ENSAYO.

Controles/Calibrador Muestras	100 µl 100 µl
1ra incubación	**60 min**
Temperatura	+37°C
Lavado	5 ciclos con 20''de remojo o 6 ciclos sin remojo
Conjugado	100 µl
2da incubación	**60 min**
Temperatura	+37°C
Lavado	5 ciclos con 20''de remojo o 6 ciclos sin remojo
Mezcla TMB/H2O2	100 µl
3ra incubación	**20 min**
Temperatura	t.a.*
Ácido Sulfúrico	100 µl
Lectura D.O.	450nm / 620-630nm

t.a. temperatura ambiente*

A continuación se describe un ejemplo del esquema de dispensado (incluido el calibrador (CAL):

Microplaca

	1	2	3	4	5	6	7	8	9	10	11	12
A	BL	M2										
B	CN	M3										
C	CN	M4										
D	CN	M5										
E	CAL	M6										
F	CAL	M7										
G	CP	M8										
H	M1	M9										

Leyenda: BL = Blanco CN = Control Negativo
CAL = Calibrador CP = Control Positivo M = Muestra

O. CONTROL DE CALIDAD INTERNO.

Se realiza un grupo de pruebas con los controles negativo y positivo cada vez que se usa el equipo, y con el calibrador la primera vez que se usa el equipo, para verificar si los valores DO450nm o Co/M son los esperados.

Asegurar el cumplimiento de los siguientes parámetros:

Parámetro	Exigencia
Pocillo Blanco	valor < 0.100 DO450nm
Control Negativo (CN)	> 1.000 DO450nm después de leer el blanco Si es menor, controle cuidadosamente el proceso de lavado y disminuya los ciclos o el tiempo entre los mismos. Coeficiente de variación < 30%
Control Positivo (CP)	DO450 nm < CN/10
Calibrador (CAL)	CP ≤ DO450nm < (CN+CP)/5

Si los resultados del ensayo coinciden con lo establecido anteriormente, pase a la siguiente sección.

En caso contrario, no siga adelante y compruebe:

Problema	Compruebe que
Pocillo blanco > 0.100DO450nm	la solución cromógeno/substrato no se ha contaminado durante el ensayo.
Control	1. el proceso de lavado y los parámetros

Negativo (CN) < 1.000DO450nm después de leer el blanco Coeficiente de variación > 20%	del lavador estén validados según los estudios previos de calificación. 2. se ha usado la solución de lavado apropiada y que el lavador ha sido cebado con la misma antes del uso. 3. no se han cometido errores en el procedimiento (dispensar el control positivo en lugar del negativo). 4. no ha existido contaminación del control negativo o de sus pocillos debido a muestras positivas derramadas, o al conjugado. 5. las micropipetas no se han contaminado con muestras positivas o con el conjugado. 6. las agujas del lavador no estén parcial o totalmente obstruidas.
Calibrador DO450nm Fuera de rango	1. el procedimiento ha sido realizado correctamente. 2. no ha habido errores durante su distribución (dispensar el control negativo en lugar del calibrador). 3. el proceso de lavado y los parámetros del lavador estén validados según los estudios previos de calificación. 4. no ha ocurrido contaminación externa del calibrador.
Control Positivo DO450nm > CN/10	1. el procedimiento ha sido realizado correctamente. 2. no se han cometido errores en el procedimiento (dispensar el control negativo en lugar del positivo). 3. el proceso de lavado y los parámetros del lavador estén validados según los estudios previos de calificación. 4. no ha ocurrido contaminación externa del control positivo.

Si ocurre alguno de los problemas anteriores, informe al responsable para tomar las medidas pertinentes.

Nota importante:
El análisis debe seguir el paso de lectura descrito en la sección M, punto 8.

P. RESULTADOS.
Los resultados se calculan por medio de un valor de corte (cut-off) hallado con la siguiente fórmula:

Valor de corte = (CN + CP) / 5

Nota Importante*: Cuando el cálculo de los resultados se halla mediante el sistema operativo de un equipo de ELISA automático, asegurarse de que la formulación usada para el cálculo del valor de corte, y para la interpretación de los resultados sea correcta.*

Q. INTERPRETACIÓN DE LOS RESULTADOS.
La interpretación de los resultados se realiza mediante la razón entre las DO a 450nm / 620-630nm de las muestras y el Valor de corte Co/M.
Los resultados se interpretan según la siguiente tabla:

Co/M	Interpretación
< 0.9	Negativo
0.9 - 1.1	Equívoco
> 1.1	Positivo

Un resultado negativo indica que el paciente no está infectado por HDV.
Cualquier paciente, cuya muestra resulte equívoca debe someterse a una nueva prueba con una segunda muestra de sangre colectada 1 ó 2 semanas después de la inicial.
Un resultado positivo es indicativo de infección por HDV y por consiguiente el paciente debe ser tratado adecuadamente.

Notas importantes:
1. *La interpretación de los resultados debe hacerse bajo la vigilancia del responsable del laboratorio para reducir el riesgo de errores de juicio y de interpretación.*
2. *Cuando se transmiten los resultados de la prueba, del laboratorio a otras instalaciones, debe ponerse mucha atención para evitar el traslado de datos erróneos.*
3. *El diagnóstico de infección con un virus de la hepatitis debe ser evaluado y comunicado al paciente por un médico calificado.*

A continuación se incluye un ejemplo de los cálculos (datos obtenidos siguiendo el paso de lectura descrito en la sección M, punto 8).

Los siguientes datos no deben usarse en lugar de los valores reales obtenidos en el laboratorio.

Control Negativo: 2.100 – 2.200 – 2.000 DO450nm
Valor medio: 2.100 DO450nm
Mayor de 1.000 – Válido

Control Positivo: 0.100 DO450nm
Menor de CN/10 – Válido

Valor de corte = (2.100 + 0.100) / 5 = 0.440

Calibrador: 0.300-0.260 DO450nm
Valor medio: 0.280 DO450nm
Dentro del rango CP ≤ DO450nm < (CN+CP)/5 – Válido

Muestra 1: 0.020 DO450nm
Muestra 2: 1.900 DO450nm
Muestra 1 Co/M > 1.1 positiva
Muestra 2 Co/M < 0.9 negativa

R. FUNCIONAMIENTO.
La evaluación del funcionamiento ha sido realizada según lo reportado en las Especificaciones Técnicas Comunes (ETC) (art. 5, Capítulo 3 de las Directivas IVD 98/79/EC).

1. LÍMITE DE DETECCIÓN.
En ausencia de un estándar internacional, la sensibilidad del ensayo ha sido calculada por medio de un producto denominado Accurun n° 127 suministrado por Boston Biomedical Inc., Estados Unidos.
La siguiente tabla muestra los valores de DO450nm para esta preparación, diluido en suero bovino fetal (SFB), para construir la curva de dilución límite en tres lotes diferentes:

Valores Co/M

	DAB.CE	Lote # 1102	DAB.CE	Lote # 0103	DAB.CE	Lote # 0403
Accurun # 127	**DO450 nm**	**Co/M valor**	**DO450 nm**	**Co/M valor**	**DO450 nm**	**Co/M valor**
1x	0.171	**3.0**	0.163	**2.9**	0.156	**2.8**
2x	0.187	**2.7**	0.176	**2.6**	0.179	**2.5**
4x	0.230	**2.2**	0.220	**2.1**	0.202	**2.2**
8x	0.298	**1.7**	0.285	**1.6**	0.271	**1.6**
16x	0.417	**1.2**	0.405	**1.1**	0.402	**1.1**
32x	0.514	**1.0**	0.490	***0.9***	0.482	***0.9***
64x	0.717	0.7	0.700	0.7	0.705	0.6
128x	1.063	0.5	1.006	0.5	1.015	0.4
CTRL (-)	2.484	//////////	2.261	//////////	2.114	//////////

2. ESPECIFICIDAD Y SENSIBILIDAD DIAGNÓSTICA.

La evaluación del procedimiento diagnóstica se realizó mediante un ensayo con más de 400 muestras frente a un equipo de referencia. Este ensayo clínico fue conducido por el Prof. M. Rizzetto, Departamento de Gastro-Hepatología del hospital S. Giovanni Battista de Turín, Italia.
Se examinaron muestras negativas, positivas y otras que pudieran provocar interferencia.
Se emplearon además plasma sometido a métodos de tratamiento estándar (citrato, EDTA y heparina) y suero humanos. No se ha observado falsa reactividad debida a los métodos de tratamiento de muestras.
A continuación se muestran brevemente los resultados obtenidos:

Sensibilidad	> 98 %
Especificidad	> 98 %

3. PRECISIÓN.

Se realizó un estudio con 3 lotes y dos muestras de diferente reactividad anti-HDV, examinadas en 16 réplicas, en tres tandas separadas. Los valores medios obtenidos se reportan a continuación:

DAB.CE: lote #1102

Control Negativo (N = 16)

Valores medios	1ra tanda	2da tanda	3ra tanda	Valor Promedio
DO 450nm	2.342	2.428	2.433	2.401
Desviación estándar	0.113	0.106	0.122	0.114
CV %	4.8	4.4	5.0	4.7

Calibrador (N = 16)

Valores medios	1ra tanda	2da tanda	3ra tanda	Valor Promedio
DO 450nm	0.298	0.289	0.286	0.291
Desviación estándar	0.023	0.027	0.026	0.025
CV %	7.7	9.3	9.1	8.7
Co/M	1.6	1.7	1.7	1.7

DAB.CE: lote #0103
Control Negativo (N = 16)

Valores medios	1ra tanda	2da tanda	3ra tanda	Valor Promedio
DO 450nm	2.208	2.237	2.246	2.230
Desviación estándar	0.105	0.108	0.108	0.107
CV %	4.7	4.8	4.8	4.8

Calibrador (N = 16)

Valores medios	1ra tanda	2da tanda	3ra tanda	Valor Promedio
DO 450nm	0.269	0.277	0.266	0.271
Desviación estándar	0.026	0.024	0.025	0.025
CV %	9.8	8.5	9.5	9.3
Co/M	1.7	1.7	1.7	1.7

DAB.CE: lote # 0403

Control Negativo (N = 16)

Valores medios	1ra tanda	2da tanda	3ra tanda	Valor Promedio
DO 450nm	2.246	2.221	2.182	2.216
Desviación estándar	0.097	0.103	0.118	0.106
CV %	4.3	4.6	5.4	4.8

Calibrador (N = 16)

Valores medios	1ra tanda	2da tanda	3ra tanda	Valor Promedio
DO 450nm	0.286	0.273	0.280	0.280
Desviación estándar	0.027	0.023	0.026	0.025
CV %	9.3	8.5	9.1	9.0
Co/M	1.6	1.7	1.6	1.6

La variabilidad mostrada en las tablas no dió como resultado una clasificación errónea de las muestras.

Nota importante:
Los datos de rendimiento se obtuvieron siguiendo el paso de lectura descrito en la sección M, punto 8.

S. LIMITACIONES.

La contaminación bacteriana de las muestras o la inactivación por calor pueden modificar los valores de absorbancia con la consiguiente alteración de los niveles del analito. Este ensayo es adecuado solo para el análisis de muestras individuales y no para mezclas.
El diagnóstico de una enfermedad infecciosa no se debe formular en base al resultado de un solo ensayo, sino que es necesario tomar en consideraciòn la historia clínica y la sintomatología del paciente así como otros datos diagnósticos.

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Todos los productos de diagnóstico in vitro fabricados por la empresa son controlados por un sistema certificado de control de calidad aprobado por un organismo notificado para el marcado CE. Cada lote se somete a un control de calidad y se libera al mercado únicamente si se ajusta a las especificaciones técnicas y criterios de aceptación de la CE.

Fabricante:
Dia.Pro Diagnostic Bioprobes S.r.l.
Via G. Carducci n° 27 – Sesto San Giovanni (Mi) – Italia

CE
0318

HCV Ab

Version 4.0 Enzyme Immunoassay for the determination of anti Hepatitis C Virus antibody in human serum and plasma

- for “in vitro” diagnostic use only -

DIA.PRO
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REF CVAB.CE
96,192,480,960 Tests

HCV Ab

A. INTENDED USE

Version 4.0 Enzyme ImmunoAssay (ELISA) for the determination of antibodies to Hepatitis C Virus in human plasma and sera. The kit is intended for the screening of blood units and the follow-up of HCV-infected patients.
For "in vitro" diagnostic use only.

B. INTRODUCTION

The World Health Organization (WHO) define Hepatitis C infection as follows:

"Hepatitis C is a viral infection of the liver which had been referred to as parenterally transmitted "non A, non B hepatitis" until identification of the causative agent in 1989. The discovery and characterization of the hepatitis C virus (HCV) led to the understanding of its primary role in post-transfusion hepatitis and its tendency to induce persistent infection.

HCV is a major cause of acute hepatitis and chronic liver disease, including cirrhosis and liver cancer. Globally, an estimated 170 million persons are chronically infected with HCV and 3 to 4 million persons are newly infected each year. HCV is spread primarily by direct contact with human blood. The major causes of HCV infection worldwide are use of unscreened blood transfusions, and re-use of needles and syringes that have not been adequately sterilized. No vaccine is currently available to prevent hepatitis C and treatment for chronic hepatitis C is too costly for most persons in developing countries to afford. Thus, from a global perspective, the greatest impact on hepatitis C disease burden will likely be achieved by focusing efforts on reducing the risk of HCV transmission from nosocomial exposures (e.g. blood transfusions, unsafe injection practices) and high-risk behaviours (e.g. injection drug use).

Hepatitis C virus (HCV) is one of the viruses (A, B, C, D, and E), which together account for the vast majority of cases of viral hepatitis. It is an enveloped RNA virus in the *flaviviridae* family which appears to have a narrow host range. Humans and chimpanzees are the only known species susceptible to infection, with both species developing similar disease. An important feature of the virus is the relative mutability of its genome, which in turn is probably related to the high propensity (80%) of inducing chronic infection. HCV is clustered into several distinct genotypes which may be important in determining the severity of the disease and the response to treatment.

The incubation period of HCV infection before the onset of clinical symptoms ranges from 15 to 150 days. In acute infections, the most common symptoms are fatigue and jaundice; however, the majority of cases (between 60% and 70%), even those that develop chronic infection, are a symptomatic. About 80% of newly infected patients progress to develop chronic infection. Cirrhosis develops in about 10% to 20% of persons with chronic infection, and liver cancer develops in 1% to 5% of persons with chronic infection over a period of 20 to 30 years. Most patients suffering from liver cancer who do not have hepatitis B virus infection have evidence of HCV infection. The mechanisms by which HCV infection leads to liver cancer are still unclear. Hepatitis C also exacerbates the severity of underlying liver disease when it coexists with other hepatic conditions. In particular, liver disease progresses more rapidly among persons with alcoholic liver disease and HCV infection. HCV is spread primarily by direct contact with human blood. Transmission through blood transfusions that are not screened for HCV infection, through the reuse of inadequately sterilized needles, syringes or other medical equipment, or through needle-sharing among drug-users, is well documented. Sexual and perinatal transmission may also occur, although less frequently. Other modes of transmission such as social, cultural, and behavioural practices using percutaneous procedures (e.g. ear and body piercing, circumcision, tattooing) can occur if inadequately sterilized equipment is used. HCV is not spread by sneezing, hugging, coughing, food or water, sharing eating utensils, or casual contact.

In both developed and developing countries, high risk groups include injecting drug users, recipients of unscreened blood, haemophiliacs, dialysis patients and persons with multiple sex partners who engage in unprotected sex. In developed countries, it is estimated that 90% of persons with chronic HCV infection are current and former injecting drug users and those with a history of transfusion of unscreened blood or blood products. In many developing countries, where unscreened blood and blood products are still being used, the major means of transmission are unsterilized injection equipment and unscreened blood transfusions. In addition, people who use traditional scarification and circumcision practices are at risk if they use or re-use unsterilized tools.

WHO estimates that about 170 million people, 3% of the world's population, are infected with HCV and are at risk of developing liver cirrhosis and/or liver cancer. The prevalence of HCV infection in some countries in Africa, the Eastern Mediterranean, South-East Asia and the Western Pacific (when prevalence data are available) is high compared to some countries in North America and Europe.

Diagnostic tests for HCV are used to prevent infection through screening of donor blood and plasma, to establish the clinical diagnosis and to make better decisions regarding medical management of a patient. Diagnostic tests commercially available today are based on Enzyme immunosorbent assays (EIA) for the detection of HCV specific antibodies. EIAs can detect more than 95% of chronically infected patients but can detect only 50% to 70% of acute infections. A recombinant immunoblot assay (RIBA) that identifies antibodies which react with individual HCV antigens is often used as a supplemental test for confirmation of a positive EIA result. Testing for HCV circulating by amplification tests RNA (e.g. polymerase chain reaction or PCR, branched DNA assay) is also being utilized for confirmation of serological results as well as for assessing the effectiveness of antiviral therapy. A positive result indicates the presence of active infection and a potential for spread of the infection and or/the development of chronic liver disease.

Antiviral drugs such as interferon taken alone or in combination with ribavirin, can be used for the treatment of persons with chronic hepatitis C, but the cost of treatment is very high. Treatment with interferon alone is effective in about 10% to 20% of patients. Interferon combined with ribavirin is effective in about 30% to 50% of patients. Ribavirin does not appear to be effective when used alone.

There is no vaccine against HCV. Research is in progress but the high mutability of the HCV genome complicates vaccine development. Lack of knowledge of any protective immune response following HCV infection also impedes vaccine research. It is not known whether the immune system is able to eliminate the virus.

Some studies, however, have shown the presence of virus neutralizing antibodies in patients with HCV infection. In the absence of a vaccine, all precautions to prevent infection must be taken including (a) screening and testing of blood and organ donors; (b) Virus inactivation of plasma derived products; (c) implementation and maintenance of infection control practices in health care settings, including appropriate sterilization of medical and dental equipment; (d) promotion of behaviour change among the general public and health care workers to reduce overuse of injections and to use safe injection practices; and (e) Risk reduction counselling for persons with high-risk drug and sexual practices. "

The genome encodes for structural components, a nucleocapsid protein and two envelope glycoproteins, and functional constituents involved in the virus replication and protein processing.
The nucleocapsid-encoding region seems to be the most conservative among the isolates obtained all over the world.

C. PRINCIPLE OF THE TEST
Microplates are coated with HCV-specific antigens derived from "core" and "ns" regions encoding for conservative and immunodominant antigenic determinants (Core peptide, recombinant NS3, NS4 and NS5 peptides).
The solid phase is first treated with the diluted sample and HCV Ab are captured, if present, by the antigens.
After washing out all the other components of the sample, in the 2nd incubation bound HCV antibodies, IgG and IgM as well, are detected by the addition of polyclonal specific anti hIgG&M antibodies, labelled with peroxidase (HRP).
The enzyme captured on the solid phase, acting on the substrate/chromogen mixture, generates an optical signal that is proportional to the amount of anti HCV antibodies present in the sample. A cut-off value let optical densities be interpreted into HCV antibody negative and positive results.

D. COMPONENTS
Code CVAB.CE contains reagents for 192 tests.

1. Microplate MICROPLATE
n° 2 microplates
12 strips of 8 microwells coated with Core peptide, recombinant NS3, NS4 and NS5 peptides. Plates are sealed into a bag with desiccant.

2. Negative Control CONTROL -
1x4.0ml/vial. Ready to use control. It contains 1% goat serum proteins, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.5% Tween 20, 0.09% Na-azide and 0.045% ProClin 300 as preservatives. The negative control is olive green colour coded.

3. Positive Control CONTROL +
1x4.0ml/vial. Ready to use control. It contains 1% goat serum proteins, human antibodies positive to HCV, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.5% Tween 20, 0.09% Na-azide and 0.045% ProClin 300 as preservatives. The Positive Control is blue colour coded.

4. Calibrator CAL ….
n° 2 vials. Lyophilized calibrator. To be dissolved with the volume of EIA grade water reported on the label. It contains foetal bovine serum proteins, human antibodies to HCV whose content is calibrated on the NIBSC Working Standard code 99/588-003-WI, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.3 mg/ml gentamicine sulphate and 0.045% ProClin 300 as preservatives.

Note: The volume necessary to dissolve the content of the vial may vary from lot to lot. Please use the right volume reported on the label .

5. Wash buffer concentrate WASHBUF 20X
2x60ml/bottle. 20x concentrated solution. Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2, 0.05% Tween 20 and 0.045% ProClin 300.

6. Enzyme Conjugate CONJ
2x16ml/vial. Ready to use and pink/red colour coded reagent. It contains Horseradish Peroxidase conjugated goat polyclonal antibodies to human IgG and IgM, 5% BSA, 10 mM Tris buffer pH 6.8+/-0.1, 0.045% ProClin 300 and 0.02% gentamicine sulphate as preservatives.

7. Chromogen/Substrate SUBS TMB
2x16ml/vial. Ready-to-use component. It contains 50 mM citrate-phosphate buffer pH 3.5-3.8, 4% dimethylsulphoxide, 0.03% tetra-methyl-benzidine or TMB and 0.02% hydrogen peroxide or H2O2.
Note: To be stored protected from light as sensitive to strong illumination.

8. Assay Diluent DILAS
1x15ml/vial. 10 mM tris buffered solution pH 8.0 +/-0.1 containing 0.045% ProClin 300 for the pre-treatment of samples and controls in the plate, blocking interference.

9. Sulphuric Acid H_2SO_4 0.3 M
1x32ml/bottle. It contains 0.3 M H_2SO_4 solution.
Attention: Irritant (H315; H319; P280; P302+P352; P332+P313; P305+P351+P338; P337+P313; P362+P363)

10. Sample Diluent: DILSPE
2x50ml/bottle. It contains 1% goat serum proteins, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.5% Tween 20, 0.09% Na-azide and 0.045% ProClin 300 as preservatives. To be used to dilute the sample.
Note: The diluent changes colour from olive green to dark bluish green in the presence of sample.

11. Plate sealing foils **n° 4**

12. Package insert **n° 1**

Important note: Only upon specific request , Dia.Pro can supply reagents for 96, 480, 960 tests , as reported below**:**

1. Microplate	n°1	n°5	n°10
2.NegativeControl	1x2.0ml/vial	1x10ml/vial	1x20.ml/vial
3.PositiveControl	1x2.0ml/vial	1x10ml/vial	1x20.ml/vial
4.Calibrator	n° 1 vial	n° 5 vials	n° 10 vials
5.Wash buff conc	1x60ml/bottle	5x60ml/bottles	4x150ml/bottles
6.Enz. Conjugate	1x16ml/vial	2x40ml/bottles	4x40ml/bottles
7.Chromog/Subs	1x16ml/vial	2x40ml/bottles	4x40ml/bottles
8.Assay Diluent	1x8ml/vial	1x40ml/bottle	1x80ml/bottle
9.Sulphuric Acid	1x15ml/vial	2x40ml/bottle	2x80ml/bottles
10.SampleDiluent	1x50ml/vial	5x50ml/bottles	4x125ml/bottles
11.Plate seal foils	n° 2	n° 10	n° 20
12. Pack. insert	n° 1	n° 1	n° 1
Number of tests	**96**	**480**	**960**
Code	**CVAB.CE.96**	**CVAB.CE.480**	**CVAB.CE.960**

E. MATERIALS REQUIRED BUT NOT PROVIDED

1. Calibrated Micropipettes (200ul and 10ul) and disposable plastic tips.
2. EIA grade water (bidistilled or deionised, charcoal treated to remove oxidizing chemicals used as disinfectants).
3. Timer with 60 minute range or higher.
4. Absorbent paper tissues.
5. Calibrated ELISA microplate thermostatic incubator capable to provide a temperature of +37°C.
6. Calibrated ELISA microwell reader with 450nm (reading) and with 620-630nm (blanking) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.

F. WARNINGS AND PRECAUTIONS

1. The kit has to be used by skilled and properly trained technical personnel only, under the supervision of a medical doctor responsible of the laboratory.
2. When the kit is used for the screening of blood units and blood components, it has to be used in a laboratory certified and qualified by the national authority in that field (Ministry of Health or similar entity) to carry out this type of analysis.
3. All the personnel involved in performing the assay have to wear protective laboratory clothes, talc-free gloves and glasses. The use of any sharp (needles) or cutting (blades) devices should be avoided. All the personnel involved should be trained in biosafety procedures, as recommended by the Center for Disease Control, Atlanta, U.S. and reported in the National Institute of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
4. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.
5. The laboratory environment should be controlled so as to avoid contaminants such as dust or air-born microbial agents, when opening kit vials and microplates and when performing the test. Protect the Chromogen/Substrate from strong light and avoid vibration of the bench surface where the test is undertaken.
6. Upon receipt, store the kit at 2..8°C into a temperature controlled refrigerator or cold room.
7. Do not interchange components between different lots of the kits. It is recommended that components between two kits of the same lot should not be interchanged.
8. Check that the reagents are clear and do not contain visible heavy particles or aggregates. If not, advise the laboratory supervisor to initiate the necessary procedures for kit replacement.
9. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each sample. Do not reuse disposable tips.
10. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one. Do not reuse disposable tips.
11. Do not use the kit after the expiration date stated on the external container and internal (vials) labels.
12. Treat all specimens as potentially infective. All human serum specimens should be handled at Biosafety Level 2, as recommended by the Center for Disease Control, Atlanta, U.S. in compliance with what reported in the Institutes of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
13. The use of disposable plastic-ware is recommended in the preparation of the liquid components or in transferring components into automated workstations, in order to avoid cross contamination.
14. Waste produced during the use of the kit has to be discarded in compliance with national directives and laws concerning laboratory waste of chemical and biological substances. In particular, liquid waste generated from the washing procedure, from residuals of controls and from samples has to be treated as potentially infective material and inactivated before waste. Suggested procedures of inactivation are treatment with a 10% final concentration of household bleach for 16-18 hrs or heat inactivation by autoclave at 121°C for 20 min..
15. Accidental spills from samples and operations have to be adsorbed with paper tissues soaked with household bleach and then with water. Tissues should then be discarded in proper containers designated for laboratory/hospital waste.
16. The Sulphuric Acid is an irritant. In case of spills, wash the surface with plenty of water
17. Other waste materials generated from the use of the kit (example: tips used for samples and controls, used microplates) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

G. SPECIMEN: PREPARATION AND RECOMMANDATIONS

1.Blood is drawn aseptically by venipuncture and plasma or serum is prepared using standard techniques of preparation of samples for clinical laboratory analysis. No influence has been observed in the preparation of the sample with citrate, EDTA and heparin.
2. Avoid any addition of preservatives to samples; especially sodium azide as this chemical would affect the enzymatic activity of the conjugate, generating false negative results.
3. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results. When the kit is used for the screening of blood units, bar code labeling and electronic reading is strongly recommended.
4. Haemolysed (red) and visibly hyperlipemic ("milky") samples have to be discarded as they could generate false results. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they could give rise to false results.
5.Sera and plasma can be stored at +2°…+8°C in primary collection tubes for up to five days after collection.
Do not freeze primary tubes of collection. For longer storage periods, sera and plasma samples, carefully removed from the primary collection tube, can be stored frozen at –20°C for several months. Any frozen samples should not be frozen/thawed more than once as this may generate particles that could affect the test result.
6. If particles are present, centrifuge at 2.000 rpm for 20 min or filter using 0.2-0.8u filters to clean up the sample for testing.

H. PREPARATION OF COMPONENTS AND WARNINGS

A study conducted on an opened kit has not pointed out any relevant loss of activity up to 6 re-use of the device and up to 6 months.

1. Microplates:
Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant is not turned to dark green, indicating a defect of manufacturing.
In this case call Dia.Pro's customer service.
Unused strips have to be placed back into the aluminium pouch, in presence of desiccant supplied, firmly zipped and stored at +2°..8°C.
When opened the first time, residual strips are stable till the indicator of humidity inside the desiccant bag turns from yellow to green.

2. Negative Control:
Ready to use. Mix well on vortex before use.

3. Positive Control:
Ready to use. Mix well on vortex before use. Handle this component as potentially infective, even if HCV, eventually present in the control, has been chemically inactivated.

4. Calibrator:
Dissolve carefully the content of the lyophilised vial with the volume of EIA grade water reported on its label.
Mix well on vortex before use.
Handle this component as potentially infective, even if HCV, eventually present in the control, has been chemically inactivated.
***Note**: When dissolved the Calibrator is not stable. Store in aliquots at –20°C.*

5. Wash buffer concentrate:
The 20x concentrated solution has to be diluted with EIA grade water up to 1200 ml and mixed gently end-over-end before use. As some salt crystals may be present into the vial, take care to dissolve all the content when preparing the solution.
In the preparation avoid foaming as the presence of bubbles could give origin to a bad washing efficiency.
***Note:** Once diluted, the wash solution is stable for 1 week at +2..8° C.*

6. Enzyme conjugate:
Ready to use. Mix well on vortex before use.
Be careful not to contaminate the liquid with oxidizing chemicals, air-driven dust or microbes.
If this component has to be transferred use only plastic, possibly sterile disposable containers.

7. Chromogen/Substrate:
Ready to use. Mix well on vortex before use.
Be careful not to contaminate the liquid with oxidizing chemicals, air-driven dust or microbes.
Do not expose to strong illumination, oxidizing agents and metallic surfaces.
If this component has to be transferred use only plastic, possible sterile disposable container.

8. Assay Diluent:
Ready to use. Mix well on vortex before use.

9. Sulphuric Acid:
Ready to use. Mix well on vortex before use.
Attention: Irritant (H315; H319; P280; P302+P352; P332+P313; P305+P351+P338; P337+P313; P362+P363).

Precautionary **P statements:**
P280 – Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352 – IF ON SKIN: Wash with plenty of soap and water.
P332 + P313 – If skin irritation occurs: Get medical advice/attention.
P305 + P351 + P338 – IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337 + P313 – If eye irritation persists: Get medical advice/attention.
P362 + P363 - Take off contaminated clothing and wash it before reuse.

10. Sample Diluent:
Ready to use. Mix well on vortex before use.

I. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

1. Micropipettes have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (household alcohol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample. They should also be regularly maintained in order to show a precision of 1% and a trueness of +/-2%. Decontamination of spills or residues of kit components should also be carried out regularly.
2. The ELISA incubator has to be set at +37°C (tolerance of +/- 0.5°C) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water baths are suitable for the incubations, provided that the instrument is validated for the incubation of ELISA tests.
3. The **ELISA washer** is extremely important to the overall performances of the assay. The washer must be carefully validated in advance, checked for the delivery of the right dispensation volume and regularly submitted to maintenance according to the manufacturer's instructions for use. In particular the washer, at the end of the daily workload, has to be extensively cleaned out of salts with deionized water. Before use, the washer has to be extensively primed with the diluted Washing Solution.
   The instrument weekly has to be submitted to decontamination according to its manual (NaOH 0.1 M decontamination suggested).
   5 washing cycles (aspiration + dispensation of 350ul/well of washing solution + 20 sec soaking = 1 cycle) are sufficient to ensure the assay with the declared performances. If soaking is not possible add one more cycle of washing.
   An incorrect washing cycle or salt-blocked needles are the major cause of false positive reactions.
4. Incubation times have a tolerance of $\pm$5%.
5. The ELISA microplate reader has to be equipped with a reading filter of 450nm and with a second filter of 620-630nm, mandatory for blanking purposes. Its standard performances should be (a) bandwidth $\leq$ 10 nm; (b) absorbance range from 0 to $\geq$ 2.0; (c) linearity to $\geq$ 2.0; (d) repeatability $\geq$ 1%. Blanking is carried out on the well identified in the section "Assay Procedure". The optical system of the reader has to be calibrated regularly to ensure that the correct optical density is measured. It should be regularly maintained according to the manufacturer 's instructions.
6. When using an ELISA automated work station, all critical steps (dispensation, incubation, washing, reading, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the section O "Internal Quality Control". The assay protocol has to be installed in the operating system of the unit and validated as for the washer and the reader. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set. Particular attention must be paid to avoid carry over by the needles used for dispensing and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells. The use of ELISA automated work stations is recommended for blood screening when the number of samples to be tested exceed 20-30 units per run.
7. When using automatic devices, in case the vial holder of the instrument does not fit with the vials supplied in the kit, transfer the solution into appropriate containers and label them with the same label peeled out from the original vial. This operation is important in order to avoid mismatching contents of vials, when transferring them. When the test is over, return the secondary labeled containers to 2..8°C, firmly capped.
8. Dia.Pro's customer service offers support to the user in the setting and checking of instruments used in combination with the kit, in order to assure compliance with the requirements described. Support is also provided for the installation of new instruments to be used with the kit.

L. PRE ASSAY CONTROLS AND OPERATIONS

1. Check the expiration date of the kit printed on the external label of the kit box. Do not use if expired.
2. Check that the liquid components are not contaminated by naked-eye visible particles or aggregates. Check that the Chromogen/Substrate is colorless or pale blue by aspirating a small volume of it with a sterile transparent plastic pipette. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box. Check that the

aluminum pouch, containing the microplate, is not punctured or damaged.
3. Dilute all the content of the 20x concentrated Wash Solution as described above.
4. Dissolve the Calibrator as described above.
5. Allow all the other components to reach room temperature (about 1 hr) and then mix as described.
6. Set the ELISA incubator at +37°C and prepare the ELISA washer by priming with the diluted washing solution, according to the manufacturers instructions. Set the right number of washing cycles as reported in the specific section.
7. Check that the ELISA reader has been turned on at least 20 minutes before reading.
8. If using an automated workstation, turn it on, check settings and be sure to use the right assay protocol.
9. Check that the micropipettes are set to the required volume.
10. Check that all the other equipment is available and ready to use.
11. In case of problems, do not proceed further with the test and advise the supervisor.

M. ASSAY PROCEDURE

The assay has to be carried out according to what reported below, taking care to maintain the same incubation time for all the samples in testing.

Automated assay:

In case the test is carried out automatically with an ELISA system, we suggest to make the instrument aspirate 200 ul Sample Diluent and then 10 ul sample.
All the mixture is then carefully dispensed directly into the appropriate sample well of the microplate. Before the next sample is aspirated, needles have to be duly washed to avoid any cross-contamination among samples.
Do not dilute controls/calibrator as they are ready to use.
Dispense 200 ul controls/calibrator in the appropriate control/calibration wells.

Important Note: *Visually monitor that samples have been diluted and dispensed into appropriate wells. This is simply achieved by checking that the colour of dispensed samples has turned to dark bluish-green while the colour of the negative control has remained olive green.*

For the next operations follow the operative instructions reported below for the Manual Assay.
It is strongly recommended to check that the time lap between the dispensation of the first and the last sample will be calculated by the instrument and taken into consideration by delaying the first washing operation accordingly.

Manual assay:

1. Place the required number of Microwells in the microwell holder. Leave the 1st well empty for the operation of blanking.
2. Dispense 200 ul of Negative Control in triplicate, 200 ul Calibrator in duplicate and 200 ul Positive Control in single in proper wells. Do not dilute Controls and Calibrator as they are pre-diluted, ready to use !
3. Add 200 ul of Sample Diluent (DILSPE) to all the sample wells; then dispense 10 ul sample in each properly identified well. Mix gently the plate, avoiding overflowing and contaminating adjacent wells, in order to fully disperse the sample into its diluent.

Important note: *Check that the colour of the Sample Diluent, upon addition of the sample, changes from light green to dark bluish green, monitoring that the sample has been really added.*

4. Dispense 50 ul Assay Diluent (DILAS) into all the controls/calibrator and sample wells. Check that the color of samples has turned to dark blue.
5. Incubate the microplate for **45 min at +37°C**.

Important note: *Strips have to be sealed with the adhesive sealing foil, supplied, only when the test is carried out manually. Do not cover strips when using ELISA automatic instruments.*

6. Wash the microplate with an automatic washer by delivering and aspirating 350ul/well of diluted washing solution as reported previously (section I.3).
7. Pipette 100µl Enzyme Conjugate into each well, except the 1st blanking well, and cover with the sealer. Check that this pink/red coloured component has been dispensed in all the wells, except A1.

Important note: *Be careful not to touch the plastic inner surface of the well with the tip filled with the Enzyme Conjugate. Contamination might occur.*

8. Incubate the microplate for **45 min at +37°C**.
9. Wash microwells as in step 6.
10. Pipette 100µl Chromogen/Substrate mixture into each well, the blank well included. Then incubate the microplate at **room temperature (18-24°C) for 15 minutes**.

Important note: *Do not expose to strong direct illumination. High background might be generated.*

11. Pipette 100µl Sulphuric Acid into all the wells using the same pipetting sequence as in step 10 to stop the enzymatic reaction. Addition of acid will turn the positive control and positive samples from blue to yellow/brown.
12. Measure the colour intensity of the solution in each well, as described in section I.5, at 450nm filter (reading) and at 620-630nm (background subtraction), blanking the instrument on A1 (mandatory).

Important notes:

1. *Ensure that no finger prints are present on the bottom of the microwell before reading. Finger prints could generate false positive results on reading.*
2. *Reading has to be carried out just after the addition of the Stop Solution and anyway not any longer than 20 minutes after its addition. Some self oxidation of the chromogen can occur leading to high background.*
3. *Shaking at 350 ±150 rpm during incubation has been proved to increase the sensitivity of the assay of about 20%.*
4. *The Calibrator (CAL) does not affect the cut-off calculation and therefore the test results calculation. The Calibrator may be used only when a laboratory internal quality control is required by the management.*

N. ASSAY SCHEME

Method	Operations
Controls & Calibrator Samples Assay Diluent (DILAS)	200 ul 200ul dil.+10ul 50 ul
1st incubation	**45 min**
Temperature	+37°C
Wash step	n° 5 cycles with 20" of soaking OR n° 6 cycles without soaking
Enzyme conjugate	100 ul
2nd incubation	**45 min**
Temperature	+37°C
Wash step	n° 5 cycles with 20" of soaking OR n° 6 cycles without soaking
TMB/H2O2	100 ul
3rd incubation	**15 min**
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm / 620-630nm

An example of dispensation scheme is reported below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S2										
B	NC	S3										
C	NC	S4										
D	NC	S5										
E	CAL	S6										
F	CAL	S7										
G	PC	S8										
H	S1	S9										

Legenda: BLK = Blank NC = Negative Control
CAL = Calibrator PC = Positive Control S = Sample

O. INTERNAL QUALITY CONTROL

A check is carried out on the controls and the calibrator any time the kit is used in order to verify whether their OD450nm values are as expected and reported in the table below.

Check	Requirements
Blank well	< 0.100 OD450nm value
Negative Control (NC)	< 0.050 mean OD450nm value after blanking
Calibrator	S/Co > 1.1
Positive Control	> 1.000 OD450nm value

If the results of the test match the requirements stated above, proceed to the next section.
If they do not, do not proceed any further and operate as follows:

Problem	Check
Blank well > 0.100 OD450nm	1. that the Chromogen/Sustrate solution has not got contaminated during the assay
Negative Control (NC) > 0.050 OD450nm after blanking	1. that the washing procedure and the washer settings are as validated in the pre qualification study; 2. that the proper washing solution has been used and the washer has been primed with it before use; 3. that no mistake has been done in the assay procedure (dispensation of positive control instead of negative control; 4. that no contamination of the negative control or of their wells has occurred due to positive samples, to spills or to the enzyme conjugate; 5. that micropipettes haven't got contaminated with positive samples or with the enzyme conjugate 6. that the washer needles are not blocked or partially obstructed.
Calibrator S/Co < 1.1	1. that the procedure has been correctly executed; 2. that no mistake has been done in its distribution (ex.: dispensation of negative control instead of control serum) 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the calibrator has occurred.
Positive Control < 1.000 OD450nm	1. that the procedure has been correctly executed; 2. that no mistake has been done in the distribution of controls (dispensation of negative control instead of positive control. In this case, the negative control will have an OD450nm value > 0.150, too. 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the positive control has occurred.

Should these problems happen, after checking, report any residual problem to the supervisor for further actions.

P. CALCULATION OF THE CUT-OFF

The tests results are calculated by means of a cut-off value determined with the following formula on the mean OD450nm value of the Negative Control (NC):

NC + 0.350 = Cut-Off (Co)

The value found for the test is used for the interpretation of results as described in the next paragraph.

***Important note**: When the calculation of results is done by the operative system of an ELISA automated work station be sure that the proper formulation is used to calculate the cut-off value and generate the right interpretations of results.*

Q. INTERPRETATION OF RESULTS

Test results are interpreted as ratio of the sample OD450nm and the Cut-Off value (or S/Co) according to the following table:

S/Co	Interpretation
< 0.9	Negative
0.9 - 1.1	Equivocal
> 1.1	Positive

A negative result indicates that the patient has not been infected by HCV or that the blood unit may be transfused.
Any patient showing an equivocal result should be tested again on a second sample taken 1-2 weeks later from the patient and examined. The blood unit should not be transfused.
A positive result is indicative of HCV infection and therefore the patient should be treated accordingly or the blood unit should be discarded.

Important notes:

1. *Interpretation of results should be done under the supervision of the responsible of the laboratory to reduce the risk of judgment errors and misinterpretations.*
2. *Any positive result should be confirmed by an alternative method capable to detect IgG and IgM antibodies (confirmation test) before a diagnosis of viral hepatitis is formulated.*
3. *As proved in the Performance Evaluation of the product, the assay is able to detect seroconversion to anti HCV core antibodies **earlier** than some other commercial kits. Therefore a positive result, not confirmed with these commercial kits, does not have to be ruled out as a false positive result ! The sample has to be anyway submitted to a confirmation test (supplied upon request by DiaPro srl, code CCONF).*
4. *As long as the assay is able to detect also IgM antibodies some discrepant results with other commercial products for the detection of anti HCV antibodies - lacking anti hIgM conjugate in the formulation of the enzyme tracer and therefore missing IgM reactivity - may be present. The real positivity of the sample for antibodies to HCV should be then confirmed by examining also IgM reactivity, important for the diagnosis of HCV infection.*
5. *When test results are transmitted from the laboratory to an informatics centre, attention has to be done to avoid erroneous data transfer.*
6. *Diagnosis of viral hepatitis infection has to be done and released to the patient only by a qualified medical doctor.*

An example of calculation is reported below:

The following data must not be used instead or real figures obtained by the user.
Negative Control: 0.019 – 0.020 – 0.021 OD450nm
Mean Value: 0.020 OD450nm
Lower than 0.050 – Accepted
Positive Control: 2.189 OD450nm
Higher than 1.000 – Accepted
Cut-Off = 0.020+0.350 = 0.370
Calibrator: 0.550 - 0.530 OD450nm
Mean value: 0.540 OD450nm S/Co = 1.4
S/Co higher than 1.1 – Accepted
Sample 1: 0.070 OD450nm
Sample 2: 1.690 OD450nm
Sample 1 S/Co < 0.9 = negative
Sample 2 S/Co > 1.1 = positive

R. PERFORMANCES

Evaluation of Performances has been conducted in accordance to what reported in the Common Technical Specifications or CTS (art. 5, Chapter 3 of IVD Directive 98/79/EC).

1. LIMIT OF DETECTION

The limit of detection of the assay has been calculated by means of the British Working Standard for anti-HCV, NIBSC code 99/588-003-WI. The table below reports the mean OD450nm values of this standard when diluted in negative plasma and then examined.

Dilution	Lot # 1	Lot # 2
Factor	S/Co	S/Co
1 X	**2.0**	**2.0**
2 X	**1.1**	**1.2**
4 X	0.7	0.8
8 X	0.5	0.5
Negative plasma	0.3	0.3

In addition the sample coded Accurun 1 – series 3000 - supplied by Boston Biomedica Inc., USA, has been evaluated "in toto" showing the results below:

CVAB.CE Lot ID	Accurun 1 Series	S/Co
1201	3000	1.5
0602	3000	1.5
1202	3000	1.9

In addition, n° 7 samples, tested positive for HCV Ab with Ortho HCV 3.0 SAVe, code 930820, lot. # EXE065-1, were diluted in HCV Ab negative plasma in order to generate limiting dilutions and then tested again on CVAB.CE, lot. # 1202, and Ortho.
The following table reports the data obtained.

Sample n°	Limit Dilution	CVAB.CE S/Co	Ortho 3.0 S/Co
1	256 X	**1.9**	**1.3**
2	256 X	**1.9**	0.7
3	256 X	**2.4**	**1.0**
4	128 X	**2.5**	**3.2**
5	85 X	**3.3**	**1.4**
6	128 X	**2.2**	0.8
7	135 X	**3.2**	**2.2**

2. DIAGNOSTIC SPECIFICITY AND SENSITIVITY

The Performance Evaluation of the device was carried out in a trial conducted on more than total 5000 samples.

2.1 Diagnostic specificity:

It is defined as the probability of the assay of scoring negative in the absence of specific analyte. In addition to the first study, where a total of 5043 unselected blood donors,(including 1st time donors), 210 hospitalized patients and 162 potentially interfering specimens (other infectious diseases, E.coli antibody positive, patients affected by non viral hepatic diseases, dialysis patients, pregnant women, hemolized, lipemic, etc.) were examined, the diagnostic specificity was recently assessed by testing a total of 2876 negative blood donors on six different lots. A value of specificity of 100% was found.
No false reactivity due to the method of specimen preparation has been observed. Both plasma, derived with different standard techniques of preparation (citrate, EDTA and heparin), and sera have been used to determine the value of specificity.
Frozen specimens have been tested, as well, to check for interferences due to collection and storage.
No interference was observed.

2.2 Diagnostic Sensitivity

It defined as the probability of the assay of scoring positive in the presence of specific analyte.
The diagnostic sensitivity has been assessed externally on a total number of 359 specimens; a diagnostic sensitivity of 100% was found. Internally more than other 50 positive samples were tested, providing a value of diagnostic sensitivity of again 100%.
Positive samples from infections carried out by different genotypes of HCV were tested as well.
Furthermore, most of seroconversion panels available from Boston Biomedica Inc., USA, (PHV) and Zeptometrix, USA, (HCV) have been studied.
Results are reported below for some of them.

Panel	N° samples	DiaPro*	Ortho* **
PHV 901	11	9	9
PHV 904	7	2	4
PHV 905	9	3	4
PHV 906	7	7	7
PHV 907	7	3	2
PHV 908	13	10	8
PHV 909	3	2	2
PHV 910	5	3	3
PHV 911	5	3	3
PHV 912	3	1	1
PHV 913	4	2	2
PHV 914	9	5	5
PHV 915	4	3	0
PHV 916	8	4	3
PHV 917	10	6	6
PHV 918	8	2	0
PHV 919	7	3	3
PHV 920	10	6	6
HCV 10039	5	2	0
HCV 6212	9	6	7
HCV 10165	9	5	4

*Note: * Positive samples detected* *** HCV v.3.0*

Finally the Product has been tested on the panel EFS Ac HCV, lot n° 01/08.03.22C/01/A, supplied by the Etablissement Francais Du Sang (EFS), France, with the following results:

EFS Panel Ac HCV

Sample	Lot # 1 S/Co	Lot # 2 S/Co	Lot # 2 S/Co	Results expected
HCV 1	**2.2**	**2.4**	**2.6**	**positive**
HCV 2	**1.6**	**2.0**	**2.1**	**positive**
HCV 3	**1.5**	**1.7**	**1.6**	**positive**
HCV 4	**5.2**	**6.5**	**5.5**	**positive**
HCV 5	**1.6**	**1.8**	**1.6**	**positive**
HCV 6	0.4	0.4	0.4	negative

3. PRECISION:
It has been calculated on two samples, one negative and one low positive, examined in 16 replicates in three separate runs. Results are reported as follows:

Lot # 1202

Negative Sample (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.094	0.099	0.096	0.096
Std.Deviation	0.008	0.007	0.008	0.007
CV %	8.7	6.6	7.9	7.7

Cal # 2 – 7K (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.396	0.403	0.418	0.406
Std.Deviation	0.023	0.029	0.027	0.026
CV %	5.9	7.1	6.4	6.5
S/Co	1.1	1.1	1.2	1.1

Lot # 0602

Negative Sample (N = 16)

Mean values	1st run	2nd run	3rd run	Average
OD 450nm	0.097	0.096	0.094	0.096
Std.Deviation	0.009	0.010	0.008	0.009
CV %	8.9	10.1	8.4	9.1

Cal # 2 – 7K (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.400	0.395	0.393	0.396
Std.Deviation	0.021	0.025	0.026	0.024
CV %	5.4	6.2	6.6	6.1
S/Co	1.2	1.2	1.1	1.2

Lot # 0602/2

Negative Sample (N = 16)

Mean values	1st run	2nd run	3rd run	Average
OD 450nm	0.087	0.091	0.088	0.089
Std.Deviation	0.009	0.007	0.008	0.008
CV %	10.0	8.2	8.6	8.9

Cal # 2 – 7K (N = 16)

Mean values	1st run	2nd run	3rd run	Average
OD 450nm	0.386	0.390	0.391	0.389
Std.Deviation	0.023	0.021	0.023	0.022
CV %	6.0	5.3	5.8	5.7
S/Co	1.1	1.2	1.2	1.2

The variability shown in the tables above did not result in sample misclassification.

S. LIMITATIONS
Repeatable false positive results, not confirmed by RIBA or similar confirmation techniques, were assessed as less than 0.1% of the normal population.
Frozen samples containing fibrin particles or aggregates after thawing have been observed to generate some false results.

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All the IVD Products manufactured by the company are under the control of a certified Quality Management System approved by an EC Notified Body. Each lot is submitted to a quality control and released into the market only if conforming with the EC technical specifications and acceptance criteria.

Manufacturer:
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CE
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HAV Ab

Competitive Enzyme ImmunoAssay (ELISA) for the determination of antibodies to Hepatitis A Virus in human plasma and sera

- for “in vitro” diagnostic use only -

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REF AVAB.CE
96 Tests

HAV Ab

A. INTENDED USE

Competitive Enzyme ImmunoAssay (ELISA) for the determination of antibodies to Hepatitis A Virus in human plasma and sera. The kit is used for the follow-up of patients infected by HAV. For "in vitro" diagnostic use only.

B. INTRODUCTION

The Center for Disease Control or CDC, Atlanta, USA, defines Hepatitis A Virus as follows:

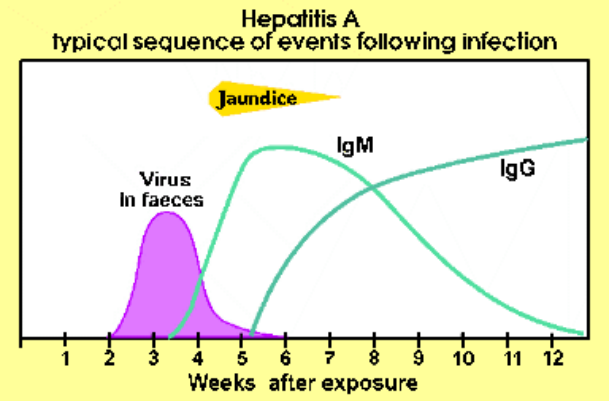
Hepatitis A continues to be one of the most frequently reported vaccine-preventable diseases in the world, despite the licensure of hepatitis A vaccine in 1995. Widespread vaccination of appropriate susceptible populations would substantially lower disease incidence and potentially eliminate indigenous transmission of hepatitis A virus (HAV) infection.

HAV, a 27-nm RNA agent classified as a picornavirus, can produce either asymptomatic or symptomatic infection in humans after an average incubation period of 28 days (range, 15-50 days). The illness caused by HAV infection typically has an abrupt onset of symptoms that can include fever, malaise, anorexia, nausea, abdominal discomfort, dark urine, and jaundice. The likelihood of having symptoms with HAV infection is related to the person's age. In children less than 6 years of age, most (70%) infections are asymptomatic; if illness does occur, it is not usually accompanied by jaundice. Among older children and adults, infection is usually symptomatic, with jaundice occurring in greater than 70% of patients. Signs and symptoms usually last less than 2 months, although 10%-15% of symptomatic persons have prolonged or relapsing disease lasting up to 6 months.
In infected persons, HAV replicates in the liver, is excreted in bile, and is shed in the stool. Peak infectivity of infected persons occurs during the 2-week period before onset of jaundice or elevation of liver enzymes, when the concentration of virus in stool is highest. The concentration of virus in stool declines after jaundice appears. Children and infants can shed HAV for longer periods than adults, up to several months after the onset of clinical illness. Chronic shedding of HAV in feces does not occur; however, shedding can occur in persons who have relapsing illness. Viremia occurs soon after infection and persists through the period of liver enzyme elevation.

Hepatitis A cannot be differentiated from other types of viral hepatitis on the basis of clinical or epidemiologic features alone. Serologic testing to detect immunoglobulin M (IgM) antibody to the capsid proteins of HAV (IgM anti-HAV) is required to confirm a diagnosis of acute HAV infection. In most persons, IgM anti-HAV becomes detectable 5-10 days before the onset of symptoms and can persist for up to 6 months after infection. Immunoglobulin G (IgG) anti-HAV, which appears early in the course of infection, remains detectable for the person's lifetime and confers lifelong protection against the disease. Commercial diagnostic tests are available for the detection of IgM and total (IgM and IgG) anti-HAV in serum.
HAV RNA can be detected in the blood and stool of most persons during the acute phase of infection by using nucleic acid amplification methods, and nucleic acid sequencing has been used to determine the relatedness of HAV isolates.

HAV infection is acquired primarily by the fecal-oral route by either person-to-person contact or ingestion of contaminated food or water. On rare occasions, HAV infection has been transmitted by transfusion of blood or blood products collected from donors during the viremic phase of their infection. In experimentally infected nonhuman primates, HAV has been detected in saliva during the incubation period; however, transmission by saliva has not been demonstrated.
Depending on conditions, HAV can be stable in the environment for months. Heating foods at temperatures greater than 185 F (85 C) for 1 minute or disinfecting surfaces with a 1:100 dilution of sodium hypochlorite (i.e., household bleach) in tap water is necessary to inactivate HAV.

Because most children have asymptomatic or unrecognized infections, they play an important role in HAV transmission and serve as a source of infection for others. In one study of adults without an identified source of infection, 52% of their households included a child less than 6 years old, and the presence of a young child was associated with HAV transmission within the household. In studies where serologic testing of the household contacts of adults without an identified source of infection was performed, 25%-40% of the contacts less than 6 years old had serologic evidence of acute HAV infection (IgM anti-HAV).



C. PRINCIPLE OF THE TEST

The assay is based on the principle of competition where the antibodies in the sample compete with an anti-HAV specific antibody, labeled with HRP, for a fixed amount of antigen on the solid phase.
A purified and inactivated HAV is coated to the microwells.
The patient's serum/plasma is added to the microwell and antibodies to HAV are captured by the solid phase.
After washing, the enzyme conjugate is added and binds to the free HAV antigen, if still present.
The plate is washed to remove unbound conjugate and then the chromogen/substrate is added.
In the presence of peroxidase the colorless substrate is hydrolysed to a coloured end-product, whose optical density may be detected and is inversely proportional to the amount of antibodies to HAV present in the sample.
An additive is added to the sample directly into the well to block interferences able to mask the presence of antibodies, mostly appearing in the follow up of vaccination.

D. COMPONENTS
Each kit contains sufficient reagents to perform 96 tests.

1. Microplate MICROPLATE
8x12 microwell strips coated with purified and inactivated HAV, sealed into a bag with desiccant. Allow the microplate to reach room temperature before opening. Reseal unused strips in the bag with desiccant and store at 2..8°C°.

2. Negative Control: CONTROL -
1x4.0ml/vial. Ready to use. Contains bovine serum proteins, 10 mM phosphate buffer pH 7.4+/-0.1, 0.02% gentamicine sulphate and 0.045% ProClin 300 as preservatives. The negative control is color coded pale yellow.

3. Positive Control: CONTROL +
1x4.0ml/vial. Ready to use. Contains bovine serum proteins, anti HAV antibodies at a concentration higher than 100 WHO mIU/ml, 10 mM phosphate buffer pH 7.4+/-0.1, 0.02% gentamicine sulphate and 0.045% ProClin 300 as preservatives. The positive control is colour coded green.

4. Calibrator: CAL ….
n° 1 vial. Lyophilized. To be dissolved with EIA grade water as reported in the label. Contains bovine serum proteins, anti HAV antibodies at a concentration of about 10 WHO mIU/ml, 10 mM phosphate buffer pH 7.4+/-0.1, 0.02% gentamicine sulphate and 0.045% ProClin 300 as preservatives.
Note: The volume necessary to dissolve the content of the vial may vary from lot to lot. Please use the right volume reported on the label .

5. Wash buffer concentrate: WASHBUF 20X
1x60ml/bottle. 20x concentrated solution. to be diluted up to 1200ml with distilled water before use.
Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2, 0.05% Tween 20 and 0.045% ProClin 300.

6. Enzyme conjugate: CONJ
1x16ml/vial. Ready-to-use solution. Contains Horseradish peroxidase conjugated antibody, specific to HAV, in presence of 10 mM Tris buffer pH 6.8+/-0.1, 2% BSA, 0.045% ProClin 300 and 0.02% gentamicine sulphate as preservatives.
The reagent is colored with a red dye.

7. Chromogen/Substrate: SUBS TMB
1x16ml/vial. Contains a 50 mM citrate-phosphate buffered solution at pH 3.5-3.8, 0.03% tetra-methyl-benzidine or TMB and 0.02% hydrogen peroxide of H_2O_2.
Note: To be stored protected from light as sensitive to strong illumination.

8. Specimen Diluent: DILSPE
1x8ml. Buffered solution suggested to be used in the follow up of vaccination. It contains 0.09% sodium azide and 0.045% ProClin 300 as preservatives. The reagent is color coded dark green.

9. Sulphuric Acid: H_2SO_4 O.3 M
1x15ml/vial. Contains 0.3 M H_2SO_4 solution.
Attention: Irritant (H315; H319; P280; P302+P352; P332+P313; P305+P351+P338; P337+P313; P362+P363)

10. Plate sealing foils **n° 2**

11. Package insert **n° 1**

Upon request:

Calibration Curve: CAL N° …
5x2.0 ml/vial. Ready to use and colour coded standard curve ranging: 0-5-10-50-100 WHO mIU/ml.
(CAL1=0mIU/ml, CAL2=5mIU/ML, CAL3=10mIU/ml, CAL4=50mIU/ml, CAL5=100mIU/ml).
Contains serum proteins, 0.3 mg/ml gentamicine sulphate and 0.045% ProClin 300 GC as preservatives.
Standards are blue colored.

E. MATERIALS REQUIRED BUT NOT PROVIDED
1. Calibrated Micropipettes (150ul, 100ul and 50ul) and disposable plastic tips.
2. EIA grade water (double distilled or deionised, charcoal treated to remove oxidizing chemicals used as disinfectants).
3. Timer with 60 minute range or higher.
4. Absorbent paper tissues.
5. Calibrated ELISA microplate thermostatic incubator (dry or wet) set at +37°C (+/-0.1°C tolerance).
6. Calibrated ELISA microwell reader with 450nm (reading) and with 620-630nm (blanking) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.

F. WARNINGS AND PRECAUTIONS
1. The kit has to be used by skilled and properly trained technical personnel only, under the supervision of a medical doctor responsible of the laboratory.
2. All the personnel involved in performing the assay have to wear protective laboratory clothes, talc-free gloves and glasses. The use of any sharp (needles) or cutting (blades) devices should be avoided. All the personnel involved should be trained in biosafety procedures, as recommended by the Center for Disease Control, Atlanta, U.S. and reported in the National Institute of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
3. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.
4. The laboratory environment should be controlled so as to avoid contaminants such as dust or air-born microbial agents, when opening kit vials and microplates and when performing the test. Protect the Chromogen (TMB) from strong light and avoid vibration of the bench surface where the test is undertaken.
5. Upon receipt, store the kit at 2-8°C into a temperature controlled refrigerator or cold room.
6. Do not interchange components between different lots of the kits. It is recommended that components between two kits of the same lot should not be interchanged.
7. Check that the reagents are clear and do not contain visible heavy particles or aggregates. If not, advise the laboratory supervisor to initiate the necessary procedures.
8. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each sample. Do not reuse disposable tips.
9. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one. Do not reuse disposable tips.
10. Do not use the kit after the expiration date stated on external (primary container) and internal (vials) labels. Treat all specimens as potentially infective. All human serum specimens should be handled at Biosafety Level 2, as recommended by the Center for Disease Control, Atlanta, U.S. in compliance with what reported in the Institutes of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
11. The use of disposable plastic-ware is recommended in the preparation of the washing solution or in transferring components into other containers of automated workstations, in order to avoid contamination.

12. Waste produced during the use of the kit has to be discarded in compliance with national directives and laws concerning laboratory waste of chemical and biological substances. In particular, liquid waste generated from the washing procedure, from residuals of controls and from samples has to be treated as potentially infective material and inactivated. Suggested procedures of inactivation are treatment with a 10% final concentration of household bleach for 16-18 hrs or heat inactivation by autoclave at 121°C for 20 min..
13. Accidental spills have to be adsorbed with paper tissues soaked with household bleach and then with water. Tissues should then be discarded in proper containers designated for laboratory/hospital waste.
14. The Stop Solution is an irritant. In case of spills, wash the surface with plenty of water
15. Other waste materials generated from the use of the kit (example: tips used for samples and controls, used microplates) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

G. SPECIMEN: PREPARATION AND RECOMMANDATIONS
1. Blood is drawn aseptically by venipuncture and plasma or serum is prepared using standard techniques of preparation of samples for clinical laboratory analysis. No influence has been observed in the preparation of the sample with citrate, EDTA and heparin.
2. Avoid any addition of preservatives; especially sodium azide as this chemical would affect the enzymatic activity of the conjugate, generating false negative results.
3. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results. When the kit is used for the screening of blood units, bar code labeling and reading is strongly recommended.
4. Haemolysed and visibly hyperlipemic ("milky") samples have to be discarded as they could generate false results. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they could give rise to false results.
5. Sera and plasma can be stored at +2°…+8°C in primary collection tubes for up to five days after collection.
Do not freeze primary tubes of collection. For longer storage periods, sera and plasma samples, carefully removed from the primary collection tube, can be stored frozen at –20°C for several months. Any frozen samples should not be frozen/thawed more than once as this may generate particles that could affect the test result.
6. If particles are present, centrifuge at 2.000 rpm for 20 min or filter using 0.2-0.8u filters to clean up the sample for testing.

H. PREPARATION OF COMPONENTS AND WARNINGS
A study conducted on opened kit has pointed out no relevant loss of performances up to 3 months from first opening.

1. Antigen coated microwells:
Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant has not turned dark green, indicating a defect in conservation.
In this case, call Dia.Pro's customer service.
Unused strips have to be placed back into the aluminum pouch, with the desiccant supplied, firmly zipped and stored at +2°-8°C.
When opened the first time, unused strips are stable until the humidity indicator inside the desiccant bag turns from yellow to green.

2. Negative Control:
Ready to use. Mix well on vortex before use.

3. Positive Control:
Ready to use. Mix well on vortex before use.

4. Calibrator:
Add the volume of ELISA grade water, reported on the label, to the lyophilized powder; let fully dissolve and then gently mix on vortex. The dissolved calibrator is not stable; store it frozen in aliquots at –20°C.

5. Wash buffer concentrate:
The whole content of the 20x concentrated solution has to be diluted with bi-distilled water up to 1200ml and mixed gently end-over-end before use.
Once diluted, the wash solution is stable for 1 week at 2-8° C.
During preparation avoid foaming as the presence of bubbles could impact on the efficiency of the washing cycles.

6. Enzyme conjugate:
Ready to use. Mix well on vortex before use.
Avoid contamination of the liquid with oxidizing chemicals, dust or microbes. If this component has to be transferred, use only plastic, and if possible, sterile disposable containers.

7. Chromogen/Substrate:
Ready to use. Mix well on vortex before use.
Avoid contamination of the liquid with oxidizing chemicals, air-driven dust or microbes. Do not expose to strong light, oxidizing agents and metallic surfaces.
If this component has to be transferred use only plastic, and if possible, sterile disposable container
8. Specimen Diluent:
Ready to use. Mix well on vortex before use.

9. Sulphuric Acid:
Ready to use. Mix well on vortex before use.
Attention: Irritant (H315; H319; P280; P302+P352; P332+P313; P305+P351+P338; P337+P313; P362+P363).

Legenda:

Warning **H statements:**
H315 – Causes skin irritation.
H319 – Causes serious eye irritation.

Precautionary **P statements:**
P280 – Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352 – IF ON SKIN: Wash with plenty of soap and water.
P332 + P313 – If skin irritation occurs: Get medical advice/attention.
P305 + P351 + P338 – IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337 + P313 – If eye irritation persists: Get medical advice/attention.
P362 + P363 – Take off contaminated clothing and wash it before reuse.

I. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT
1. Micropipettes have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (household alcohol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample. They should also be regularly maintained. Decontamination of spills or residues of kit components should also be carried out regularly. They should also be regularly maintained in order to show a precision of 1% and a trueness of ±2%.
2. The ELISA incubator has to be set at +37°C (tolerance of +/- 0.5°C) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water baths are suitable for the incubations, provided that the instrument is validated for the incubation of ELISA tests.
3. The **ELISA washer** is extremely important to the overall performances of the assay. The washer must be carefully validated in advance, checked for the delivery of the right

dispensation volume and regularly submitted to maintenance according to the manufacturer's instructions for use. In particular the washer, at the end of the daily workload, has to be extensively cleaned out of salts with deionized water. Before use, the washer has to be extensively primed with the diluted Washing Solution.
The instrument weekly has to be submitted to decontamination according to its manual (NaOH 0.1 M decontamination suggested).
5 washing cycles (aspiration + dispensation of 350ul/well of washing solution + 20 sec soaking = 1 cycle) are sufficient to ensure the assay with the declared performances. If soaking is not possible add one more cycle of washing.
An incorrect washing cycle or salt-blocked needles are the major cause of false positive reactions.

4. Incubation times have a tolerance of $\pm$5%.
5. The ELISA microplate reader has to be equipped with a reading filter of 450nm and with a second filter of 620-630nm, mandatory for blanking purposes. Its standard performances should be (a) bandwidth $\leq$ 10 nm; (b) absorbance range from 0 to 4; (c) linearity to 4; (d) repeatability $\geq$ 1%. Blanking is carried out on the well identified in the section "Assay Procedure". The optical system of the reader has to be calibrated regularly to ensure that the correct optical density is measured. It should be regularly maintained according to the manufacturer 's instructions.
6. When using an ELISA automated work station, all critical steps (dispensation, incubation, washing, reading, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the section O "Internal Quality Control". The assay protocol has to be installed in the operating system of the unit and validated as for the washer and the reader. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set. Particular attention must be paid to avoid carry over by the needles used for dispensing and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells. The use of ELISA automated work stations is recommended when the number of samples to be tested exceed 20-30 units per run.
7. Dia.Pro's customer service offers support to the user in the setting and checking of instruments used in combination with the kit, in order to assure compliance with the requirements described. Support is also provided for the installation of new instruments to be used with the kit.

L. PRE ASSAY CONTROLS AND OPERATIONS

1. Check the expiration date of the kit printed on the external label (primary container). Do not use if expired.
2. Check that the liquid components are not contaminated by visible particles or aggregates. Check that the Chromogen/Substrate (TMB+H_2O_2) is colorless or pale blue by aspirating a small volume of it with a sterile plastic pipette. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box (primary container). Check that the aluminum pouch, containing the microplate, is not punctured or damaged.
3. Dilute all the content of the 20x concentrated Wash Solution as described above.
4. Dissolve the Calibrator as described above and gently mix.
5. Allow all the other components to reach room temperature (about 1 hr) and then mix gently on vortex all liquid reagents.
6. Set the ELISA incubator at +37°C and prepare the ELISA washer by priming with the diluted washing solution, according to the manufacturers instructions. Set the right number of washing cycles as reported in the specific section.
7. Check that the ELISA reader is turned on or ensure it will be turned on at least 20 minutes before reading.
8. If using an automated work station, turn on, check settings and be sure to use the right assay protocol.
9. Check that the micropipettes are set to the required volume.
10. Check that all the other equipment is available and ready to use.
11. In case of problems, do not proceed further with the test and advise the supervisor.

M. ASSAY PROCEDURE

The assay has to be carried out according to what reported below, taking care to maintain the same incubation time for all the samples in testing.

1. Place the required number of strips in the microplate holder. Leave A1 well empty for the operation of blanking.
Store the other strips into the bag in presence of the desiccant at +2..8°C, sealed.

2. Dispense 50 ul Specimen Diluent in all the wells identified for samples and controls/calibrator, except for A1.
Then pipette 100 µl of Negative Control in triplicate, 100 µl of Calibrator in duplicate, 100 µl Positive Control in single and then 100 ul of samples.
Check that controls/calibrator and samples have been correctly added. Incubate the microplate at **+37°C for 60 min**.
3. Wash the microplate as reported in section I.3.

4. In all the wells except A1, pipette 100 µl Enzyme Conjugate. Check that the reagent has been correctly added. Incubate the microplate at **+37°C for 60 minutes.**

Important note: *Be careful not to touch the inner surface of the well with the pipette tip when dispensing the Enzyme Conjugate. Contamination might occur.*

5. Wash the microplate as described.

6. Pipette 100 µl TMB/H_2O_2 mixture in each well, the blank wells included. Check that the reagent has been correctly added. Then incubate the microplate at **room temperature for 20 minutes**.
Important note: *Do not expose to strong direct light as a high background might be generated.*

7. Pipette 100 µl Sulphuric Acid into each well to stop the enzymatic reaction using the same pipetting sequence as in step 6. Then measure the color intensity with a microplate reader at 450nm (reading) and at 620-630nm (background subtraction), blanking the instrument on A1 well (mandatory).

Important notes:

1. *Ensure that no finger prints are present on the bottom of the microwell before reading. Finger prints could generate false positive results on reading.*
2. *Reading has should ideally be performed immediately after the addition of the Stop Solution but definitely no longer than 20 minutes afterwards. Some self oxidation of the chromogen can occur leading to a higher background.*

N. ASSAY SCHEME

Specimen Diluent Controls/Calibrator(*) Samples	50 ul 100 ul 100 ul
1st incubation	**60 min**
Temperature	+37°C
Washing step	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
Enzyme Conjugate	100 ul
2nd incubation	**60 min**
Temperature	+37°C
Washing step	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
TMB/H2O2 mix	100 ul
3rd incubation	**20 min**
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm / 620-630

(*) Important Notes:

- *The Calibrator (CAL) does not affect the Cut Off calculation, therefore it does not affect the test's results calculation.*
- *The Calibrator (CAL) used only if a laboratory internal quality control is required by the Management.*

An example of dispensation scheme is reported in the table below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S2										
B	NC	S3										
C	NC	S4										
D	NC	S5										
E	CAL(*)	S6										
F	CAL(*)	S7										
G	PC	S8										
H	S1	S9										

Legenda: BLK = Blank NC = Negative Control
CAL(*) = Calibrator - Not mandatory PC = Positive Control
S = Sample

O. INTERNAL QUALITY CONTROL

A check is performed on the controls any time the kit is used in order to verify whether the expected OD450nm or Co/S values have been matched in the analysis.
Ensure that the following parameters are met:

Parameter	Requirements
Blank well	< 0.100 OD450nm value
Negative Control (NC)	> 0.750 mean OD450nm value after blanking coefficient of variation < 30%
Positive Control	< 0.300 OD450nm value

If the results of the test match the requirements stated above, proceed to the next section.
If they do not, do not proceed any further and perform the following checks:

Problem	Check
Blank well > 0.100 OD450nm	1. that the Chromogen/Substrate solution has not become contaminated during the assay
Negative Control (NC) < 0.750 OD450nm after blanking coefficient of variation > 30%	1. that the washing procedure and the washer settings are as validated in the pre qualification study; 2. that the proper washing solution has been used and the washer has been primed with it before use; 3. that no mistake has been done in the assay procedure (dispensation of positive control instead of negative control; 4. that no contamination of the negative control or of the wells where the control was dispensed has occurred due to positive samples, to spills or to the enzyme conjugate; 5. that micropipettes have not become contaminated with positive samples or with the enzyme conjugate 6. that the washer needles are not blocked or partially obstructed.
Positive Control > 0.300 OD450nm	1. that the procedure has been correctly performed; 2. that no mistake has occurred during the distribution of the control (ex.: dispensation of negative control instead of the positive one); 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the positive control has occurred.

If any of the above problems have occurred, report the problem to the supervisor for further actions.

If Calibrator has used, verify the following data:

Parameter	Requirements
Calibrator 10 mIU/ml (WHO)	Co/S $\geq$ 1.0

If the results of the test doesn't match the requirements stated above, operate as follows:

Problem	Check
Calibrator Co/S < 1.0	1. that the procedure has been correctly performed; 2. that no mistake has occurred during its distribution; 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the calibrator has occurred.

Anyway, if all other parameters (Blank, Negative Control, Positive Control), match the established requirements, the test may be considered valid.

P. CALCULATION OF THE CUT-OFF

The test results are calculated by means of a cut-off value determined with the following formula:

Cut-Off = (NC+PC) / 3

The value found for the test is used for the interpretation of results as described in the next paragraph.

***Important note**: When the calculation of results is performed by the operating system of an ELISA automated work station, make sure that the proper formulation is used to calculate the cut-off value and generate the correct interpretation of results.*

Q. INTERPRETATION OF RESULTS

Test results are interpreted as a ratio of the Cut-Off value and the OD450nm of the sample (or Co/S) according to the following table:

Co/S	Interpretation
< 0.9	Negative
0.9 - 1.1	Equivocal
> 1.1	Positive

A negative result indicates that the patient has not been infected by HAV.
Any patient showing an equivocal result should be retested on a second sample taken 1-2 weeks after the initial sample.
A positive result is indicative of a past or recent HAV infection and therefore the patient should be treated accordingly.

An example of calculation is reported below.

The following data must not be used instead or real figures obtained by the user.

Negative Control: 1.900 – 2.000 – 2.100 OD450nm
Mean Value: 2.000 OD450nm
Higher than 0.750 – Accepted

Positive Control: 0.100 OD450nm
Lower than 0.300 – Accepted

Cut-Off = (2.000 + 0.100) / 3 = 0.700

Calibrator: 0.400-0.360 OD450nm
Mean value: 0.380 OD450nm
Co/S > 1 – Accepted

Sample 1: 0.050 OD450nm
Sample 2: 1.900 OD450nm
Sample 1 Co/S > 1.1 positive
Sample 2 Co/ < 0.9 negative

Important notes:

1. *Interpretation of results should be done under the supervision of the laboratory supervisor to reduce the risk of judgement errors and misinterpretations.*
2. *When test results are transmitted from the laboratory to another facility, attention must be paid to avoid erroneous data transfer.*
3. *Diagnosis of viral hepatitis infection has to be taken by and released to the patient by a suitably qualified medical doctor.*

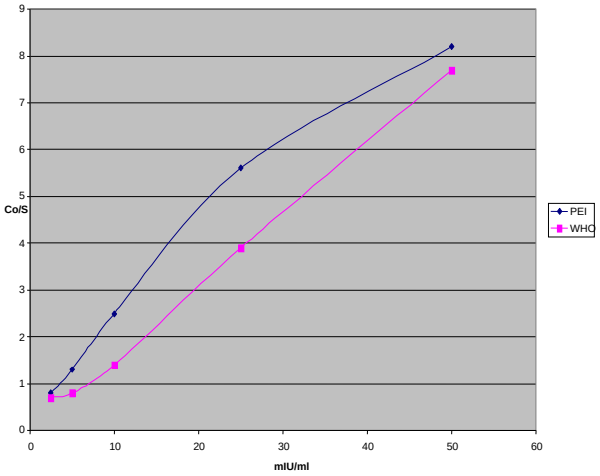
R. PERFORMANCE CHARACTERISTICS

1. Limit of detection

The limit of detection of the assay has been calculated by means of the 2nd International Standard supplied by WHO.
Two control samples, supplied by Boston Biomedica Inc., USA, with code Accurun 52 and 120, were also examined.
The sensitivity shown by the assay is < 10 WHO mIU/ml or < 5 PEI mU/ml.
Results of Quality Control are given in the following table:

WHO mIU/ml	OD450 nm	Co/S	PEI mU/ml	OD450 nm	Co/S
50	0.099	**7.7**	**50**	0.093	**8.2**
25	0.197	**3.9**	**25**	0.137	**5.6**
10	0.543	**1.4**	**10**	0.304	**2.5**
5	0.943	0.8	**5**	0.587	**1.3**
2.5	1.015	0.7	**2.5**	0.949	0.8
Neg. Control	2.217		**Neg. Control**	2.217	
Accurun 52	0.060	**12.7**	**Accurun 120**	0.115	**6.6**

Curves are reported below:



2. Diagnostic sensitivity:

The diagnostic sensitivity has been tested in a clinical trial on panels of samples classified positive by a US FDA approved kit.
An overall value of 100% has been found in the study conducted on a total number of more than 200 samples.
Seroconversion and performance panels have also been studied. Results obtained by examining two panels supplied by Boston Biomedica Inc., USA, are reported below.

Seroconversion Panel: PHT 902

Sample	OD450nm	Co/S	DiaSorin
CTRL (-)	1,968	0,3	
CTRL (+)	0,084	**8,1**	
Calibrator	0,470	**1,5**	
PHT902			
1	1,878	0,4	neg
2	1,501	0,5	neg
3	0,090	**7,6**	**pos**
4	0,123	**5,6**	**pos**
5	0,120	**5,7**	**pos**

Performance Panel: PHT 201

Sample	OD450nm	Co/S	DiaSorin	Sample	OD450nm	Co/S	DiaSorin
1	0,169	**4,0**	**pos**	14	0,139	**4,9**	**pos**
2	0,132	**5,2**	**pos**	15	0,115	**5,9**	**pos**
3	0,143	**4,8**	**pos**	16	0,167	**4,1**	**pos**
4	0,104	**6,6**	**pos**	17	0,086	**8,0**	**pos**
5	0,438	**1,6**	**pos**	18	0,160	**4,3**	**pos**
6	0,121	**5,7**	**pos**	19	0,175	**3,9**	**pos**
7	0,127	**5,4**	**pos**	20	1,772	0,4	neg
8	0,150	**4,6**	**pos**	21	0,090	**7,6**	**pos**
9	0,115	**5,9**	**pos**	22	0,201	**3,4**	**pos**
10	0,094	**7,3**	**pos**	23	0,281	**2,4**	**pos**
11	0,070	**9,8**	**pos**	24	0,134	**5,1**	**pos**
12	1,814	0,4	neg	25	0,142	**4,8**	**pos**
13	0,097	**7,1**	**pos**	Neg	1,780	0.4	neg

3. Diagnostic specificity:

The diagnostic specificity has been determined on panels of negative samples from normal individuals and blood donors, classified negative with a US FDA approved kit.

Both plasma, derived with different standard techniques of preparation (citrate, EDTA and heparin), and sera have been used to determine the specificity. No false reactivity due to the method of specimen preparation has been observed.

Frozen specimens have also been tested to check whether this interferes with the performance of the test. No interference was observed on clean and particle free samples.

Samples derived from patients with different viral (HCV, HDV, HBV, HIV) and non viral pathologies of the liver that may interfere with the test were examined.

No cross reaction were observed.

The Performance Evaluation study conducted in the external reference center on more than 1000 samples has provided a value > 98% .

4. Precision

The mean values obtained from a study conducted on two samples of different anti-HAV reactivity, examined in 16 replicates in three separate runs is reported below:

Test # 1

Sample	Negative	Low Pos.
OD450nm	2.425	0.608
Std. Deviation	0.065	0.023
CV %	2.7	3.9

Test # 2

Sample	Negative	Low Pos.
OD450nm	2.373	0.573
Std. Deviation	0.107	0.034
CV %	4.5	6.0

Test # 3

Sample	Negative	Low Pos.
OD450nm	2.478	0.554
Std. Deviation	0.108	0.023
CV %	4.4	4.2

The variability shown in the tables did not result in sample misclassification.

S. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or heat inactivation of the specimen may affect the absorbance values of the samples with consequent alteration of the level of the analyte.

This test is suitable only for testing single samples and not pooled ones.

Diagnosis of an infectious disease should not be established on the basis of a single test result. The patient's clinical history, symptomatology, as well as other diagnostic data should be considered.

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All the IVD Products manufactured by the company are under the control of a certified Quality Management System in compliance with ISO 13485 rule. Each lot is submitted to a quality control and released into the market only if conforming with the EC technical specifications and acceptance criteria.

Manufactured by
Dia.Pro Diagnostic Bioprobes S.r.l.
Via G. Carducci n° 27 – Sesto San Giovanni (MI) – Italy

CE

Dia.Pro
Diagnostic
BioProbes

EC DECLARATION OF CONFORMITY

MANUFACTURER	DIA.PRO DIAGNOSTIC BIOPROBES S.R.L. VIA G. CARDUCCI N° 27 – 20099 SESTO SAN GIOVANNI (MILANO) – ITALY
PRODUCT	**HAV Ab** CODE: **AVAB.CE** (96 tests)
CLASSIFICATION	GENERAL IVD
CONFORMITY ASSESSMENT ROUTE	SELF CERTIFICATION

WE HEREBY DECLARE THAT THE ABOVE MENTIONED PRODUCT MEETS THE PROVISIONS OF THE COUNCIL DIRECTIVE 98/79/EC FOR IN VITRO DIAGNOSTIC DEVICES.

ISO CERTIFICATE	UNE EN ISO 13485 N° 2013 11 0039 EN, RELEASED BY AEMPS (AGENCIA ESPAÑOLA DE MEDICAMENTOS Y PRODUCTOS SANITARIOS)

PLACE & DATE OF FIRST ISSUE	MILANO – SEPTEMBER 2003
PLACE & DATE OF CURRENT ISSUE	SESTO SAN GIOVANNI (MI) – MARCH 2019
SIGNATURE **Legal Representative** **Dr.ssa Fiorenza Scozzesi**	

Rev:	05/2018

HAV IgM

"Capture" Enzyme Immuno Assay (ELISA) for the determination of IgM class antibodies to Hepatitis A Virus in human plasma and sera

- for "in vitro" diagnostic use only -

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REF AVM.CE
96 tests

HAV IgM

A. INTENDED USE

Enzyme ImmunoAssay (ELISA) for the determination of IgM class antibodies to Hepatitis A Virus in human plasma and sera with the "capture" system. The kit may be used for the identification of the viral agent causing hepatitis in the patient and the follow up of the acute phase of the infection.
For “in vitro” diagnostic use only.

B. INTRODUCTION

The Center for Disease Control or CDC, Atlanta, USA, defines Hepatitis A Virus as follows:

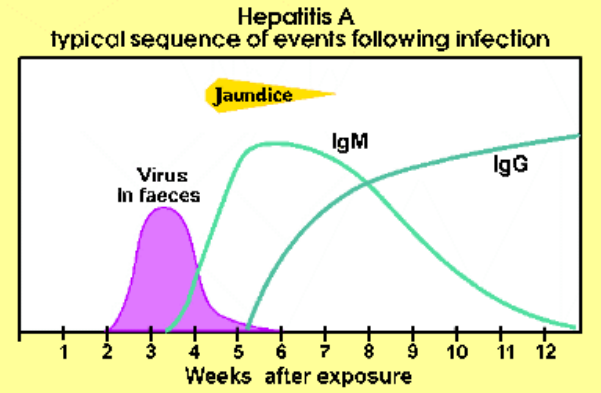
Hepatitis A continues to be one of the most frequently reported vaccine-preventable diseases in the world, despite the licensure of hepatitis A vaccine in 1995. Widespread vaccination of appropriate susceptible populations would substantially lower disease incidence and potentially eliminate indigenous transmission of hepatitis A virus (HAV) infection.

HAV, a 27-nm RNA agent classified as a picornavirus, can produce either asymptomatic or symptomatic infection in humans after an average incubation period of 28 days (range, 15-50 days). The illness caused by HAV infection typically has an abrupt onset of symptoms that can include fever, malaise, anorexia, nausea, abdominal discomfort, dark urine, and jaundice. The likelihood of having symptoms with HAV infection is related to the person's age. In children less than 6 years of age, most (70%) infections are asymptomatic; if illness does occur, it is not usually accompanied by jaundice. Among older children and adults, infection is usually symptomatic, with jaundice occurring in greater than 70% of patients. Signs and symptoms usually last less than 2 months, although 10%-15% of symptomatic persons have prolonged or relapsing disease lasting up to 6 months.
In infected persons, HAV replicates in the liver, is excreted in bile, and is shed in the stool. Peak infectivity of infected persons occurs during the 2-week period before onset of jaundice or elevation of liver enzymes, when the concentration of virus in stool is highest. The concentration of virus in stool declines after jaundice appears. Children and infants can shed HAV for longer periods than adults, up to several months after the onset of clinical illness. Chronic shedding of HAV in feces does not occur; however, shedding can occur in persons who have relapsing illness. Viremia occurs soon after infection and persists through the period of liver enzyme elevation.

Hepatitis A cannot be differentiated from other types of viral hepatitis on the basis of clinical or epidemiologic features alone. Serologic testing to detect immunoglobulin M (IgM) antibody to the capsid proteins of HAV (IgM anti-HAV) is required to confirm a diagnosis of acute HAV infection. In most persons, IgM anti-HAV becomes detectable 5-10 days before the onset of symptoms and can persist for up to 6 months after infection. Immunoglobulin G (IgG) anti-HAV, which appears early in the course of infection, remains detectable for the person's lifetime and confers lifelong protection against the disease. Commercial diagnostic tests are available for the detection of IgM and total (IgM and IgG) anti-HAV in serum.
HAV RNA can be detected in the blood and stool of most persons during the acute phase of infection by using nucleic acid amplification methods, and nucleic acid sequencing has been used to determine the relatedness of HAV isolates.

HAV infection is acquired primarily by the fecal-oral route by either person-to-person contact or ingestion of contaminated food or water. On rare occasions, HAV infection has been transmitted by transfusion of blood or blood products collected from donors during the viremic phase of their infection. In experimentally infected nonhuman primates, HAV has been detected in saliva during the incubation period; however, transmission by saliva has not been demonstrated.
Depending on conditions, HAV can be stable in the environment for months. Heating foods at temperatures greater than 185 F (85 C) for 1 minute or disinfecting surfaces with a 1:100 dilution of sodium hypochlorite (i.e., household bleach) in tap water is necessary to inactivate HAV.

Because most children have asymptomatic or unrecognized infections, they play an important role in HAV transmission and serve as a source of infection for others. In one study of adults without an identified source of infection, 52% of their households included a child less than 6 years old, and the presence of a young child was associated with HAV transmission within the household. In studies where serologic testing of the household contacts of adults without an identified source of infection was performed, 25%-40% of the contacts less than 6 years old had serologic evidence of acute HAV infection (IgM anti-HAV).



C. PRINCIPLE OF THE TEST

The assay is based on the principle of "IgM capture" where IgM class antibodies in the sample are first captured by the solid phase coated with anti hIgM antibody.
After washing out all the other components of the sample and in particular IgG antibodies, the specific IgM captured on the solid phase are detected by the addition of a purified preparation of inactivated HAV, labelled with an antibody conjugated with peroxidase (HRP).
After incubation, microwells are washed to remove unbound conjugate and then the chromogen/substrate is added.
In the presence of peroxidase the colorless substrate is hydrolysed to a colored end-product, whose optical density may be detected and is proportional to the amount of antibodies to HAV present in the sample.

D. COMPONENTS
The kit contains reagents for 96 tests.

1. Microplate: MICROPLATE
12 strips of 8 breakable wells coated with anti human IgM antibody, affinity purified, and sealed into a bag with desiccant. Bring the microplate to room temperature before opening the bag. Unused strips have to be returned into the bag and the bag has to be sealed and stored back to 2..8°C, in presence of the desiccant.

2. Negative Control: CONTROL -
1x4.0 ml/vial. Ready to use control. It contains goat serum proteins, 10 mM tris buffer pH 6.0+/-0.1, 0.1% Tween 20, 0.09% sodium azide and 0.1% Kathon GC as preservatives.
The negative control is colourless.

3. Positive Control: CONTROL +
1x4.0 ml/vial. Ready to use control. It contains anti HAV IgM, goat serum proteins, 10 mM tris buffer pH 6.0+/-0.1, 0.1% Tween 20, 0.09% sodium azide and 0.1% Kathon GC as preservatives. The positive control is green colour coded.

4. Calibrator: CAL ….
N° 1 lyophilized vial. To be dissolved with EIA grade water as reported in the label. It contains anti HAV IgM, 2% BSA, 10 mM tris buffer pH 6.0+/-0.1, 0.09% sodium azide and 0.1% Kathon GC as preservatives.
Note: The volume necessary to dissolve the content of the vial may vary from lot to lot. Please use the right volume reported on the label .

5. Wash buffer concentrate: WASHBUF 20X
1x60ml/bottle. 20x concentrated solution.
Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2, 0.05% Tween 20 and 0.05% Kathon GC.

6. Enzyme conjugate 20X: CONJ
1x0.8 ml/vial. 20X concentrated solution. It contains Horseradish peroxidase conjugated antibody specific to HAV in presence of 10 mM Tris buffer pH 6.8+/-0.1, 2% BSA, 0.1% Kathon GC and 0.02% gentamicine sulphate as preservatives.

7. HAV Antigen: Ag HAV
1x16 ml/vial. Ready-to-use solution. It contains inactivated and stabilised HAV in presence of 10 mM Tris buffer pH 6.8+/-0.1, 2% BSA, 0.1% Kathon GC and 0.02% gentamicine sulphate as preservatives.
The reagent is red colour coded.

8. Specimen Diluent: DILSPE
2x60.0 ml/vial. Proteic buffered solution for the dilution of samples. It contains goat serum proteins, 10 mM tris buffer pH 6.0+/-0.1, 0.1% Tween 20, 0.09% sodium azide and 0.1% Kathon GC as preservatives.
The reagent is blue colour coded.

9. Chromogen/Substrate: SUBS TMB
1x16ml/vial. It contains a 50 mM citrate-phosphate buffered solution at pH 3.5-3.8, 0.03% tetra-methyl-benzidine or TMB and 0.02% hydrogen peroxide of H_2O_2.
Note: To be stored protected from light as sensitive to strong illumination.

10. Sulphuric Acid: H_2SO_4 O.3 M
1x15ml/vial.
It contains 0.3 M H2SO4 solution.
Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

11. Plate sealing foils n° 2

12. Package insert n° 1

E. MATERIALS REQUIRED BUT NOT PROVIDED
1. Calibrated Micropipettes of 10ul, 100ul and 1000ul and disposable plastic tips.
2. EIA grade water (double distilled or deionised, charcoal treated to remove oxidizing chemicals used as disinfectants).
3. Timer with 60 minute range or higher.
4. Absorbent paper tissues.
5. Calibrated ELISA microplate thermostatic incubator (dry or wet) set at +37°C (+/-0.1°C tolerance).
6. Calibrated ELISA microwell reader with 450nm (reading) and with 620-630nm (blanking) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.

F. WARNINGS AND PRECAUTIONS
1. The kit has to be used by skilled and properly trained technical personnel only, under the supervision of a medical doctor responsible of the laboratory.
2. All the personnel involved in performing the assay have to wear protective laboratory clothes, talc-free gloves and glasses. The use of any sharp (needles) or cutting (blades) devices should be avoided. All the personnel involved should be trained in biosafety procedures, as recommended by the Center for Disease Control, Atlanta, U.S. and reported in the National Institute of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
3. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.
4. The laboratory environment should be controlled so as to avoid contaminants such as dust or air-born microbial agents, when opening kit vials and microplates and when performing the test. Protect the Chromogen/Subtrate (TMB & H_2O_2) from strong light and avoid vibration of the bench surface where the test is undertaken.
5. Upon receipt, store the kit at 2-8°C into a temperature controlled refrigerator or cold room.
6. Do not interchange components between different lots of the kits. It is recommended that components between two kits of the same lot should not be interchanged.
7. Check that the reagents are clear and do not contain visible heavy particles or aggregates. If not, advise the laboratory supervisor to initiate the necessary procedures.
8. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each sample.
9. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one.
10. Do not use the kit after the expiration date stated on external (primary container) and internal (vials) labels. Treat all specimens as potentially infective. All human serum specimens should be handled at Biosafety Level 2, as recommended by the Center for Disease Control, Atlanta, U.S. in compliance with what reported in the Institutes of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
11. The use of disposable plastic-ware is recommended in the preparation of the washing solution or in transferring components into other containers of automated workstations, in order to avoid contamination.
12. Waste produced during the use of the kit has to be discarded in compliance with national directives and laws concerning laboratory waste of chemical and biological substances. In particular, liquid waste generated from the washing procedure, from residuals of controls and from samples has to be treated as potentially infective material and

inactivated. Suggested procedures of inactivation are treatment with a 10% final concentration of household bleach for 16-18 hrs or heat inactivation by autoclave at 121°C for 20 min..
13. Accidental spills have to be adsorbed with paper tissues soaked with household bleach and then with water. Tissues should then be discarded in proper containers designated for laboratory/hospital waste.
14. The Stop Solution is an irritant. In case of spills, wash the surface with plenty of water
15. Other waste materials generated from the use of the kit (example: tips used for samples and controls, used microplates) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

G. SPECIMEN: PREPARATION AND RECOMMANDATIONS
1. Blood is drawn aseptically by venepuncture and plasma or serum is prepared using standard techniques of preparation of samples for clinical laboratory analysis. No influence has been observed in the preparation of the sample with citrate, EDTA and heparin.
2. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results. When the kit is used for the screening of blood units, bar code labeling and reading is strongly recommended.
3. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they could give rise to false results.
4. Sera and plasma can be stored at +2°…8°C for up to five days after collection. For longer storage periods, samples can be stored frozen at –20°C for several months. Any frozen samples should not be freeze/thawed more than once as IgM antibodies may get damaged and as this procedure may generate particles that could affect the test result.
5. If particles are present, centrifuge at 2.000 rpm for 20 min or filter using 0.2-0.8u filters to clean up the sample for testing.

H. PREPARATION OF COMPONENTS AND WARNINGS
A study conducted on an opened kit has not pointed out any relevant loss of activity up to 3 months.

1. Antibody coated microwells:
Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant has not turned dark green, indicating a defect in conservation.
In this case, call Dia.Pro's customer service.
Unused strips have to be placed back into the aluminium pouch, with the desiccant supplied, firmly zipped and stored at +2°-8°C. When opened the first time, unused strips are stable until the humidity indicator inside the desiccant bag turns from yellow to green.

2. Negative Control:
Ready to use. Mix well on vortex before use.

3. Positive Control:
Ready to use. Mix well on vortex before use. Handle this component as potentially infectious, even if HAV, eventually present in the control, has been chemically inactivated.

4. Calibrator:
Add the volume of ELISA grade water, reported on the label, to the lyophilised powder; let fully dissolve and then gently mix on vortex. The solution is not stable. Store the Calibrator frozen in aliquots at –20°C.
***Note**: When dissolved the Calibrator is not stable. Store in aliquots at –20°C.*

5. Wash buffer concentrate:
The whole content of the concentrated solution has to be diluted 20x with bidistilled water and mixed gently end-over-end before use.
Once diluted, the wash solution is stable for 1 week at 2-8° C.
During preparation avoid foaming as the presence of bubbles could impact on the efficiency of the washing cycles.
***Note:** Once diluted, the wash solution is stable for 1 week at +2..8° C.*

6. Enzyme conjugate:
20X preparation. Mix well on vortex.
Avoid contamination of the liquid with oxidizing chemicals, dust or microbes when the reagent is aspirated to be used.

7. HAV Antigen:
Ready to use. Mix well on vortex before use.
Handle this component as potentially infectious, even if HAV has been chemically inactivated.

6+7. HAV Antigen/Antibody complex:
About 5-10 min before its use, dilute the 20X concentrated Enzyme Conjugate in the proper volume of HAV Antigen, necessary for the assay. Then mix on vortex carefully.
Example: To run 2 strips, dilute 100 ul Enzyme Conjugate 20X into 2 ml of HAV Antigen.
***Note**: This immunocomplex is not stable; discard the exceeding volume.*

8. Sample Diluent:
Ready to use. Mix well on vortex before use.

9. Chromogen/Substrate:
Ready to use. Mix well on vortex before use.
Avoid contamination of the liquid with oxidizing chemicals, air-driven dust or microbes.
Do not expose to strong light, oxidizing agents and metallic surfaces. If this component has to be transferred use only plastic, and if possible, sterile disposable container.

10. Sulphuric Acid:
Ready to use. Mix well on vortex before use.
Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

Legenda:

Warning **H statements:**
H315 – Causes skin irritation.
H319 – Causes serious eye irritation.

Precautionary **P statements:**
P280 – Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352 – IF ON SKIN: Wash with plenty of soap and water.
P332 + P313 – If skin irritation occurs: Get medical advice/attention.
P305 + P351 + P338 – IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337 + P313 – If eye irritation persists: Get medical advice/attention.
P362 + P363 – Take off contaminated clothing and wash it before reuse.

I. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT
1. Micropipettes have to be calibrated to deliver the correct volume (tolerance +/-5%) required by the assay and must be submitted to regular decontamination (household

alcohol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample. They should also be regularly maintained. Decontamination of spills or residues of kit components should also be carried out regularly. They should also be regularly maintained in order to show a precision of 1% and a trueness of ±2%.

2. The ELISA incubator has to be set at +37°C (tolerance of +/-0.5°C) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water baths are suitable for the incubations, provided that the instrument is validated for the incubation of ELISA tests.
3. The ELISA washer is extremely important to the overall performances of the assay. The washer must be carefully validated and correctly optimized using the kit controls and reference panels, before using the kit for routine laboratory tests. 4-5 washing cycles (aspiration + dispensation of 350ul/well of washing solution = 1 cycle) are sufficient to ensure that the assay performs as expected. A soaking time of 20-30 seconds between cycles is suggested. In order to set correctly their number, it is recommended to run an assay with the kit controls and well characterized negative and positive reference samples, and check to match the values reported below in the section O'. Regular calibration of the volumes delivered by, and maintenance (decontamination and cleaning of needles) of the washer has to be carried out according to the instructions of the manufacturer.
4. Incubation times have a tolerance of ±5%.
5. The ELISA reader has to be equipped with a reading filter of 450nm and with a second filter (620-630nm, strongly recommended) for blanking purposes Blanking is carried out on the well identified in the section "Assay Procedure". The optical system of the reader has to be calibrated regularly to ensure the correct optical density is measured. It should be regularly maintained according to the manufacturer 's instructions.
6. When using an ELISA automated work station, all critical steps (dispensation, incubation, washing, reading, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the section O "Internal Quality Control'. The assay protocol has to be installed in the operating system of the unit and validated as for the washer and the reader. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set. Particular attention must be paid to avoid carry over by the needles used for dispensing and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells. The use of ELISA automated work stations is recommended when the number of samples to be tested exceed 20-30 units per run.
7. Dia.Pro's customer service offers support to the user in the setting and checking of instruments used in combination with the kit, in order to assure compliance with the requirements described. Support is also provided for the installation of new instruments to be used with the kit.

L. PRE ASSAY CONTROLS AND OPERATIONS

1. Check the expiration date of the kit printed on the external label (primary container). Do not use the device if expired.
2. Check that the liquid components are not contaminated by visible particles or aggregates. Check that the Chromogen/Substrate is colourless or pale blue by aspirating a small volume of it with a sterile plastic pipette. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box (primary container). Check that the aluminium pouch, containing the microplate, is not punctured or damaged.
3. Dilute all the content of the 20x concentrated Wash Solution as described above.
4. Dissolve the Calibrator as described above and gently mix.
5. Allow all the other components to reach room temperature (about 1 hr) and then mix gently on vortex all liquid reagents.
6. Set the ELISA incubator at +37°C +/-0.1°C and prepare the ELISA washer by priming with the diluted washing solution, according to the manufacturers instructions. Set the right number of washing cycles as found in the validation of the instrument for its use with the kit.
7. Check that the ELISA reader is turned on or ensure it will be turned on at least 20 minutes before reading.
8. If using an automated work station, turn on, check settings and be sure to use the right assay protocol.
9. Check that the micropipettes are set to the required volume.
10. Check that all the other equipment is available and ready to use.
11. In case of problems, do not proceed further with the test and advise the supervisor.

M. ASSAY PROCEDURE

The assay has to be carried out according to what reported below, taking care to maintain the same incubation time for all the samples in testing.

1. Dilute samples 1:101 by dispensing first 10 µl sample and then 1 ml Sample Diluent into a dilution tube; mix gently on vortex.

2. Place the required number of Microwells in the microwell holder. Leave the 1st well empty for the operation of blanking.

3. Dispense 100 µl Negative Control in triplicate, 100 µl Positive Control in single and 100 µl Calibrator in duplicate in proper wells. Do not dilute controls and the calibrator as they are ready to use !

4. Dispense 100 µl diluted samples in the proper sample wells and then check that all the samples wells are blue coloured and that controls and calibrator have been dispensed.
5. Incubate the microplate for **60 min at +37°C** .

Important note: *Strips have to be sealed with the adhesive sealing foil, supplied, only when the test is carried out manually. Do not cover strips when using ELISA automatic instruments.*

6. About 5-10 minutes before use, prepare the HAV Antigen/Antibody immunocomplex as described previously.

7. Wash the microplate with an automatic washer as reported previously (section I.3).

8. Pipette 100 µl HAV Antigen/Antibody complex into each well, except the 1st blanking well, and cover with the sealer. Check that all wells are red coloured, except A1.

Important note: *Be careful not to touch the plastic inner surface of the well with the tip filled with the Enzyme Conjugate. Contamination might occur.*

9. Incubate the microplate for **60 min at +37°C** .

10. Wash microwells as in step 7.

11. Pipette 100 µl Chromogen/Substrate mixture into each well, the blank well included. Then incubate the microplate at **room temperature (18-24°C) for 20 minutes**.

Important note: *Do not expose to strong direct illumination. High background might be generated.*

12. Pipette 100 µl Sulphuric Acid into all the wells to stop the enzymatic reaction using the same pipetting sequence as in step 10. Addition of acid will turn the positive control and positive samples from blue to yellow.

13. Measure the colour intensity of the solution in each well, as described in section I.5, at 450nm filter (reading) and at 620-630nm (background subtraction, strongly recommended), blanking the instrument on A1.

Important notes:

1. *If the second filter is not available ensure that no finger prints are present on the bottom of the microwell before reading at 450nm. Finger prints could generate false positive results on reading.*
2. *Reading has to be carried out just after the addition of the Stop Solution and anyway not any longer than 20 minutes after its addition. Some self oxidation of the chromogen can occur leading to high background.*

N. ASSAY SCHEME

Controls&Calibrator (*) samples diluted 1:101	100 ul
1st incubation	**60 min**
Temperature	+37°C
Washing	4-5 cycles
HAV & Tracer	100 ul
2nd incubation	**60 min**
Temperature	+37°C
Washing	4-5 cycles
TMB/H2O2 mix	100 ul
3rd incubation	**20 min**
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm

(*) Important Notes:

- *The Calibrator (CAL) does not affect the Cut Off calculation, therefore it does not affect the test's results calculation.*
- *The Calibrator (CAL) used only if a laboratory internal quality control is required by the Management.*

An example of dispensation scheme is reported in the table below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S2										
B	NC	S3										
C	NC	S4										
D	NC	S5										
E	CAL(*)	S6										
F	CAL(*)	S7										
G	PC	S8										
H	S1	S9										

Legenda: BLK = Blank NC = Negative Control
CAL(*) = Calibrator - Not mandatory PC = Positive Control S = Sample

O. INTERNAL QUALITY CONTROL

A check is performed on the controls any time the kit is used in order to verify whether the expected OD450nm or S/Co values have been matched in the analysis.
Ensure that the following parameters are met:

Parameter	Requirements
Blank well	< 0.100 OD450nm value
Negative Control mean value (NC)	< 0.150 OD450nm value after blanking coefficient of variation < 30%
Positive Control	> 0.500 OD450nm

If the results of the test match the requirements stated above, proceed to the next section.
If they do not, do not proceed any further and perform the following checks:

Problem	Check
Blank well > 0.100 OD450nm	1. that the Chromogen/Substrate solution has not become contaminated during the assay
Negative Control (NC) > 0.150 OD450nm after blanking coefficient of variation > 30%	1. that the washing procedure and the washer settings are as validated in the pre qualification study; 2. that the proper washing solution has been used and the washer has been primed with it before use; 3. that no mistake has been done in the assay procedure (dispensation of positive control instead of negative control; 4. that no contamination of the negative control or of the wells where the control was dispensed has occurred due to positive samples, to spills or to the enzyme conjugate; 5. that micropipettes have not become contaminated with positive samples or with the enzyme conjugate 6. that the washer needles are not blocked or partially obstructed.
Positive Control < 0.500 OD450nm	1. that the procedure has been correctly performed; 2. that no mistake has occurred during the distribution of the control (dispensation of negative control instead of positive control). 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the positive control has occurred.

If any of the above problems have occurred, report the problem to the supervisor for further actions.

If Calibrator has used, verify the following data:

Parameter	Requirements
Calibrator	S/Co > 1

If the results of the test doesn't match the requirements stated above, operate as follows:

Problem	Check
Calibrator S/Co < 1	1. that the procedure has been correctly performed; 2. that no mistake has occurred during its distribution (e.g.: dispensation of negative control instead) 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the calibrator has occurred.

Anyway, if all other parameters (Blank, Negative Control, Positive Control), match the established requirements, the test may be considered valid.

P. CALCULATION OF THE CUT-OFF

The test results are calculated by means of the mean OD450nm value of the Negative Control (NC) and a mathematical calculation, in order to define the following cut-off formulation:

Cut-Off = NC + 0.250

The value found for the test is used for the interpretation of results as described in the next paragraph.

***Important note**: When the calculation of results is performed by the operating system of an ELISA automated work station, ensure that the proper formulation is used to calculate the cut-off value and generate the correct interpretation of results.*

Q. INTERPRETATION OF RESULTS

Test results are interpreted as a ratio of the sample OD450nm and the Cut-Off value (or S/Co) according to the following table:

S/Co	Interpretation
< 0.8	Negative
0.8 – 1.2	Equivocal
> 1.2	Positive

A negative result indicates that the patient is not undergoing an acute infection by HAV.
Any patient showing an equivocal result, should be re-tested by examining a second sample after 1-2 weeks from first testing.
A positive result is indicative of an HAV infection event and therefore the patient should be treated accordingly.

An example of calculation is reported below:

The following data must not be used instead or real figures obtained by the user.

Negative Control: 0.050 – 0.060 – 0.070 OD450nm
Mean Value: 0.060 OD450nm
Lower than 0.150 – Accepted
Positive Control: 2.189 OD450nm
Higher than 0.500 – Accepted

Cut-Off = 0.060+0.250 = 0.310

Calibrator: 0.550 - 0.530 OD450nm
Mean value: 0.540 OD450nm S/Co = 1.7
S/Co higher than 1.0 – Accepted

Sample 1: 0.070 OD450nm
Sample 2: 1.690 OD450nm
Sample 1 S/Co < 0.8 = negative
Sample 2 S/Co > 1.2 = positive

Important notes:

1. *Interpretation of results should be done under the supervision of the laboratory supervisor to reduce the risk of judgment errors and misinterpretations.*
2. *Any positive result should be confirmed by an alternative method (confirmation test) before a diagnosis of viral hepatitis is confirmed.*
3. *When test results are transmitted from the laboratory to another facility, attention must be paid to avoid erroneous data transfer.*
4. *Diagnosis of viral hepatitis infection has to be taken by and released to the patient by a suitably qualified medical doctor.*

R. PERFORMANCE CHARACTERISTICS

1. Limit of detection

In absence of a defined international standard for HAV IgM, the limit of detection of the assay has been calculated by means of the following preparations:

1. Accurun # 121 supplied by Boston Biomedica Inc. – USA
2. Accurun # 51 supplied by Boston Biomedica Inc., USA

These preparation were prepared according to the manufacturer's instructions, diluted in Sample Diluent (1:100) and then further diluted in Sample Diluent to generate a limiting curve (accurun # 121).
Results of Quality Control are given in the following table:

Preparation	Dilutions	S/Co
Accurun # 121	1:100	**5.4**
	1:200	**4.1**
	1:400	**2.8**
	1:800	**1.9**
	1:1600	1.0
Accurun # 51	1:100	**4.2**

2. Diagnostic Sensitivity:

The diagnostic sensitivity has been tested on panels of samples classified positive by a US FDA approved kit.
Positive samples were collected from patients carrying HAV acute infection, confirmed by clinical symptoms and analysis.

An overall value of 100% has been found in the study conducted on a total number of more than 100 samples.
A seroconversion panel has also been studied.

Results obtained by examining a preparation supplied by Boston Biomedica Inc., USA, are reported below.

Seroconversion Panel : PHT 902

Sample	OD450nm	S/Co	DiaSorin Refer.	
			S/Co	Score
CTRL (-)	0,048	0,2		
CTRL (+)	1,736	**5,8**		
PHT902				
1	0,037	0,1	0,3	neg
2	0,042	0,1	0,3	neg
3	1,956	**6,6**	**6,8**	**pos**
4	1,988	**6,7**	**6,7**	**pos**
5	0,669	**2,2**	**1,5**	**pos**

3. Diagnostic Specificity:

The diagnostic specificity has been determined on panels of specimens, negative with the reference kit, derived from normal individuals and blood donors of European origin.
Both plasma, derived with different standard techniques of preparation (citrate, EDTA and heparin), and sera have been used to determine the specificity. No false reactivity due to the method of specimen preparation has been observed.

Frozen specimens have also been tested to check whether this interferes with the performance of the test. No interference was observed on clean and particle free samples.
Samples derived from patients with different viral (HCV, HDV, HBV, HEV) and non viral pathologies of the liver that may interfere with the test were examined.
No cross reaction were observed.
The Performance Evaluation study conducted in a qualified external reference centre on more than 500 samples has provided a value > 98%.

3. Precision:
It has been calculated on two samples, one negative and one low positive, examined in 16 replicates in three separate runs. Results are reported as follows:

Test # 1

Sample	Negative	Low Pos.
OD450nm	0.058	0.719
Std. Deviation	0.008	0.052
CV %	14.3	7.2

Test # 2

Sample	Negative	Low Pos.
OD450nm	0.048	0.709
Std. Deviation	0.007	0.063
CV %	13.9	8.9

Test # 3

Sample	Negative	Low Pos.
OD450nm	0.050	0.713
Std. Deviation	0.007	0.055
CV %	13.4	7.7

S. LIMITATIONS
False positivity has been assessed as less than 2% of the normal population, mostly due to high titers of RF.
Frozen samples containing fibrin particles or aggregates may generate false positive results.
Bacterial contamination or heat inactivation of the specimen may affect the absorbance values of the samples with consequent alteration of the level of the analyte.
This test is suitable only for testing single samples and not pooled ones.
Diagnosis of an infectious disease should not be established on the basis of a single test result. The patient's clinical history, symptomatology, as well as other diagnostic data should be considered.

REFERENCES
1. Dienstag J.L.. "Hepatitis A Virus : identification, characterization and epidemiologic investigations". Progress in liver desease VI, Popper E., Schaffner F. (eds), pp 343-370, New York, Gruner and Stratton, 1979.
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3. Parry J.V., (1981) "Hepatitis A infection: guidelines for the development of satisfactory assays for laboratory diagnosis". The Institute of Medical Laboratory Sciences, 38, 303-311.
4. Lindberg J., Frosner G., Hansson B.G. et al.
"Serologic markers of hepatitis A and B in chronic active hepatitis". Scandinavian Journal of Gastroenterology, 13:525-527, 1978.
5. Barbara J.A., Howell D.R., Briggs M., Parry J.V.. "Post transfusion hepatitis A". Lancet (1982), 1-738.
6. Zachoval R., Dienstag J.L., Purcell R.H. "Tests for hepatitis A virus antigen and antibody" in "Hepatitis A". Gerety R.J. (Ed), pp 33-46, Orlando, Academic Press, Inc. 1984

All the IVD Products manufactured by the company are under the control of a certified Quality Management System in compliance with ISO 13485 rule. Each lot is submitted to a quality control and released into the market only if conforming with the EC technical specifications and acceptance criteria.

Manufacturer:
Dia.Pro Diagnostic Bioprobes Srl
Via G. Carducci n° 27 – Sesto San Giovanni (MI) – Italy

CE

Dia.Pro
Diagnostic
BioProbes

EC DECLARATION OF CONFORMITY

MANUFACTURER	DIA.PRO DIAGNOSTIC BIOPROBES S.R.L. VIA G. CARDUCCI N° 27 – 20099 SESTO SAN GIOVANNI (MILANO) – ITALY
PRODUCT	**HAV IgM** CODE: **AVM.CE** (96 tests)
CLASSIFICATION	GENERAL IVD
CONFORMITY ASSESSMENT ROUTE	SELF CERTIFICATION

WE HEREBY DECLARE THAT THE ABOVE MENTIONED PRODUCT MEETS THE PROVISIONS OF THE COUNCIL DIRECTIVE 98/79/EC FOR IN VITRO DIAGNOSTIC DEVICES.

ISO CERTIFICATE	UNE EN ISO 13485 N° 2013 11 0039 EN, RELEASED BY AEMPS (AGENCIA ESPAÑOLA DE MEDICAMENTOS Y PRODUCTOS SANITARIOS)

PLACE & DATE OF FIRST ISSUE	MILANO – SEPTEMBER 2003
PLACE & DATE OF CURRENT ISSUE	SESTO SAN GIOVANNI (MI) – MARCH 2019
SIGNATURE **Legal Representative** **Dr.ssa Fiorenza Scozzesi**	

Rev:	05/2018

Notified Body 1023
INSTITUTE FOR TESTING AND CERTIFICATION, Inc.,
třída Tomáše Bati 299, Louky, 763 02 Zlín, Czech Republic

EC Certificate - Full Quality Assurance System
No. 11 0040 QS/NB

The quality system of manufacturer

Federal Budget Institute of Science "Central Research Institute for Epidemiology"

3a Novogireevskaya Street, Moscow 111123, Russia

has been certified as meeting the requirements of

Directive 98/79/EC

on in vitro diagnostic medical devices, Annex IV excluding (4, 6)

for the following product category(ies):

AmpliSens® PCR kits

The Notified Body No. 1023 declares that the aforementioned manufacturer has implemented a quality assurance system for design, manufacture and final inspection of the respective devices / device categories in accordance with IVDD Annex IV. This quality assurance system conforms to the requirements of this Directive and is subjected to periodical surveillance. For placing on the market of List A devices covered by this certificate, an EC Design-Examination Certificate according to Annex IV (Section 4) is required.

Valid from: 2022-04-28
Valid until: 2025-05-26
First Issued: 2011-01-24
Revision: k

NOTIFIED BODY 1023 INSTITUTE FOR TESTING AND CERTIFICATION

Date: 2022-04-28

Mgr. Jiří Heš
Representative of the Notified Body No. 1023

Notified Body 1023
INSTITUTE FOR TESTING AND CERTIFICATION, Inc.,
třída Tomáše Bati 299, Louky, 763 02 Zlín, Czech Republic

Annex to EC Certificate No. 11 0040 QS/NB

issued for manufacturer:

Federal Budget Institute of Science "Central Research Institute for Epidemiology"
3a Novogireevskaya Street, Moscow 111123, Russia

Product(s):

Name:	**AmpliSens® *Rubella virus*-FRT PCR kit**
Trade name(s):	-
Model(s):	variant FRT-50 F
Classification:	List B
GMDN:	30793
Name:	**AmpliSens® *Toxoplasma gondii*-FRT PCR kit**
Trade name(s):	-
Model(s):	variant FRT-50 F
Classification:	List B
GMDN:	52428
Name:	**AmpliSens® *CMV*-FEP PCR kit**
Trade name(s):	-
Model(s):	variant FEP (0.2-ml tubes)
Classification:	List B
GMDN:	30798

Date: 2022-04-28
Revision: k

Mgr. Jiří Heš
Representative of the Notified Body No. 1023

Notified Body 1023
INSTITUTE FOR TESTING AND CERTIFICATION, Inc.,
třída Tomáše Bati 299, Louky, 763 02 Zlín, Czech Republic

Annex to EC Certificate No. 11 0040 QS/NB

issued for manufacturer:

Federal Budget Institute of Science "Central Research Institute for Epidemiology"
3a Novogireevskaya Street, Moscow 111123, Russia

Name:	**AmpliSens® *CMV*-FRT PCR kit**
Trade name(s):	-
Model(s):	variant FRT-100 F
Classification:	List B
GMDN:	30798
Name:	**AmpliSens® *HSV* / *CMV*-MULTIPRIME-FRT PCR kit**
Trade name(s):	-
Model(s):	variant FRT-100 F
Classification:	List B
GMDN:	61348
Name:	**AmpliSens® *CMV*-screen/monitor-FRT PCR kit**
Trade name(s):	-
Model(s):	variant FRT-100 F
Classification:	List B
GMDN:	30798

NOTIFIED BODY 1023 · INSTITUTE FOR TESTING AND CERTIFICATION · 4

Date: 2022-04-28
Revision: k

Mgr. Jiří Heš
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Notified Body 1023
INSTITUTE FOR TESTING AND CERTIFICATION, Inc.,
třída Tomáše Bati 299, Louky, 763 02 Zlín, Czech Republic

Annex to EC Certificate No. 11 0040 QS/NB

issued for manufacturer:

Federal Budget Institute of Science "Central Research Institute for Epidemiology"
3a Novogireevskaya Street, Moscow 111123, Russia

Name:	**AmpliSens® *EBV / CMV / HHV6*-screen-FRT PCR kit**
Trade name(s):	-
Model(s):	variant FRT-100 F
Classification:	List B
GMDN:	61348
Name:	**AmpliSens® *Chlamydia trachomatis*-FEP PCR kit**
Trade name(s):	-
Model(s):	variant FEP (0.2-ml tubes)
Classification:	List B
GMDN:	30677
Name:	**AmpliSens® *Chlamydia trachomatis*-FRT PCR kit**
Trade name(s):	-
Model(s):	variant FRT, variant FRT-100 F
Classification:	List B
GMDN:	30677

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Date: 2022-04-28
Revision: k

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INSTITUTE FOR TESTING AND CERTIFICATION, Inc.,
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Annex to EC Certificate No. 11 0040 QS/NB

issued for manufacturer:

Federal Budget Institute of Science "Central Research Institute for Epidemiology"
3a Novogireevskaya Street, Moscow 111123, Russia

Name:	**AmpliSens® *C.trachomatis / Ureaplasma / M.genitalium*-MULTIPRIME-FEP PCR kit**
Trade name(s):	-
Model(s):	variant FEP (0.2 ml tubes)
Classification:	List B
GMDN:	50409
Name:	**AmpliSens® *C.trachomatis / Ureaplasma / M.genitalium*-MULTIPRIME-FRT PCR kit**
Trade name(s):	-
Model(s):	variant FRT-100 F
Classification:	List B
GMDN:	50409
Name:	**AmpliSens® *C.trachomatis / Ureaplasma / M.hominis*-MULTIPRIME-FEP PCR kit**
Trade name(s):	-
Model(s):	variant FEP (0.2 ml tubes)
Classification:	List B
GMDN:	50409

NOTIFIED BODY 1023 · INSTITUTE FOR TESTING AND CERTIFICATION

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INSTITUTE FOR TESTING AND CERTIFICATION, Inc.,
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Annex to EC Certificate No. 11 0040 QS/NB

issued for manufacturer:

Federal Budget Institute of Science "Central Research Institute for Epidemiology"
3a Novogireevskaya Street, Moscow 111123, Russia

Name:	**AmpliSens® *C.trachomatis / Ureaplasma / M.hominis*-MULTIPRIME-FRT PCR kit**
Trade name(s):	-
Model(s):	variant FRT-100 F
Classification:	List B
GMDN:	50409
Name:	**AmpliSens® *C.trachomatis / Ureaplasma / M.genitalium / M.hominis*-MULTIPRIME-FRT PCR kit**
Trade name(s):	-
Model(s):	variant FRT-100 F
Classification:	List B
GMDN:	50409
Name:	**AmpliSens® *N.gonorrhoeae / C.trachomatis / M.genitalium / T.vaginalis*-MULTIPRIME-FRT PCR kit**
Trade name(s):	-
Model(s):	variant FRT-100 F
Classification:	List B
GMDN:	50409

Date: 2022-04-28
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Annex to EC Certificate No. 11 0040 QS/NB

issued for manufacturer:

Federal Budget Institute of Science "Central Research Institute for Epidemiology"
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Name:	**AmpliSens® *N.gonorrhoeae / C.trachomatis / M.genitalium*-MULTIPRIME-FRT PCR kit**
Trade name(s):	-
Model(s):	variant FRT-100 F
Classification:	List B
GMDN:	50409
Name:	**AmpliSens® Genoscreen HLA B*5701-FRT PCR kit**
Trade name(s):	-
Model(s):	variant FRT
Classification:	List B
GMDN:	56403
Name:	**AmpliSens® *Mycoplasma pneumoniae / Chlamydophila pneumoniae*-FEP PCR kit**
Trade name(s):	-
Model(s):	variant FEP (0.2 ml tubes)
Classification:	List B
GMDN:	58957

Date: 2022-04-28
Revision: k

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Annex to EC Certificate No. 11 0040 QS/NB

issued for manufacturer:

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Name:	**AmpliSens® *Mycoplasma pneumoniae* / *Chlamydophila pneumoniae*-FRT PCR kit**
Trade name(s):	-
Model(s):	variant FRT-100 F
Classification:	List B
GMDN:	58957
Name:	**AmpliSens® *T.vaginalis* / *N.gonorrhoeae* / *C.trachomatis*-MULTIPRIME-FRT PCR kit**
Trade name(s):	-
Model(s):	variant FRT-100 F
Classification:	List B
GMDN:	61144

Facility(ies):

Federal Budget Institute of Science "Central Research Institute for Epidemiology"
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NOTIFIED BODY 1023 INSTITUTE FOR TESTING AND CERTIFICATION

Date: 2022-04-28
Revision: k

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Notified Body 1023
INSTITUTE FOR TESTING AND CERTIFICATION, Inc.,
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Annex to EC Certificate No. 11 0040 QS/NB

issued for manufacturer:

Federal Budget Institute of Science "Central Research Institute for Epidemiology"
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Certificate History:

Revision	Date	Reference Number	Action
	2011-01-24	813600111	Certification
a	2011-07-21	813600161	Change of manufacturer name
b	2012-02-13	343601304	Product scope extension
c	2014-05-13	343602568	Product scope extension
d	2016-01-15	813600504a	Prolongation of certificate validity
e	2016-06-17	813600504	Re-certification process
f	2016-08-29	343603690	Change of manufacturer facility address
g	2017-11-30	343603888	Changes of product compositions, packaging and quality system documentation
h	2018-10-31	813600754	Change of product labelling, shelf life extension and quality system documentation
i	2019-05-09	813600859	Product shelf life extension
j	2021-04-27	813601045	Re-certification process

Date: 2022-04-28
Revision: k

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Annex to EC Certificate No. 11 0040 QS/NB

issued for manufacturer:

Federal Budget Institute of Science "Central Research Institute for Epidemiology"
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Revision	Date	Reference Number	Action
k	2022-04-28	813601141	Extension of the certificate validity regarding to REGULATION (EU) 2022/112 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL, dated 25th January 2022

NOTIFIED BODY 1023 INSTITUTE FOR TESTING AND CERTIFICATION

Date: 2022-04-28
Revision: k

Mgr. Jiří Heš
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FEDERAL SERVICE FOR SUPERVISION OF CONSUMER RIGHTS PROTECTION AND HUMAN WELFARE
FEDERAL BUDGET INSTITUTE OF SCIENCE
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CE

EC DECLARATION OF CONFORMITY

Directive 98/79/EC of the European Parliament and of the Council of 27th of October 1998 on *In Vitro* Diagnostic Medical Devices

Federal Budget Institute of Science "Central Research Institute for Epidemiology" hereby under own responsibility declares that the products covered by the declaration conform with Essential Requirements listed in Annex I of EC Directive 98/79/EC (IVD Directive). Supporting documentation is retained under the premises of the manufacturer.

The quality management system meets the requirements of the standard EN ISO 13485 "Medical devices – Quality management systems – Requirements for regulatory purposes" and is certified by Institute for testing and certification, Inc. (certificate No. 21 0023 SJ, valid until 26.04.2024).

Manufacturer:	Federal Budget Institute of Science "Central Research Institute for Epidemiology"
Authorized Representative:	Ecoli Dx, s.r.o. Purkyňova 74/2 Praha 1, 110 00 Czech Republic Tel: +420 325 209 912 Cell: +420 739 802 523 E-mail: ecoli@ecoli.sk
Product Name:	Annex for this Declaration
Description:	Reagent kits for qualitative detection and quantification of DNA (RNA) of different infectious agents or HLA B*5701 DNA in human specimens
Classification:	Article 9, paragraph 3 of EC Council Directive 98/79/EC on *in Vitro* Diagnostic Devices Annex II List B IVDs (According to EC Declaration of Conformity List)
Conformity Assessment Route:	Annex IV (IVDD) Full QA System
Notified Body:	Institute for testing and certification, Inc. třida Tomáše Bati 299 Louky, 763 02 Zlin, Czech Republic E-mail: itc@itczlin.cz Notified Body No. 1023
EC Certificate:	No. 11 0040 QS/NB revision k, valid until 2025-05-26
Place, Date of Issue:	Zlin, Czech Republic, 2022-04-28

Signed _____

Full name: Vasiliy G. Akimkin
Title: Director

Valid from 2022-04-28

Valid until 2025-05-26

№№	Description	Model(s)
1.	**AmpliSens® *Rubella virus*-FRT** PCR kit	variant FRT-50 F
2.	**AmpliSens® *Toxoplasma gondii*-FRT** PCR kit	variant FRT-50 F
3.	**AmpliSens® *CMV*-FEP** PCR kit	variant FEP (0.2-ml tubes)
4.	**AmpliSens® *CMV*-FRT** PCR kit	variant FRT-100 F
5.	**AmpliSens® *HSV* / *CMV*-MULTIPRIME-FRT** PCR kit	variant FRT-100 F
6.	**AmpliSens® *CMV*-screen/monitor-FRT** PCR kit	variant FRT-100 F
7.	**AmpliSens® *EBV* / *CMV* / *HHV*6-screen-FRT** PCR kit	variant FRT-100 F
8.	**AmpliSens® *Chlamydia trachomatis*-FEP** PCR kit	variant FEP (0.2-ml tubes)
9.	**AmpliSens® *Chlamydia trachomatis*-FRT** PCR kit	variant FRT variant FRT-100 F
10.	**AmpliSens® *C.trachomatis* / *Ureaplasma* / *M.genitalium*-MULTIPRIME-FEP** PCR kit	variant FEP (0.2-ml tubes)
11.	**AmpliSens® *C.trachomatis* / *Ureaplasma* / *M.genitalium*-MULTIPRIME-FRT** PCR kit	variant FRT-100 F
12.	**AmpliSens® *C.trachomatis* / *Ureaplasma* / *M.hominis*-MULTIPRIME-FEP** PCR kit	variant FEP (0.2-ml tubes)
13.	**AmpliSens® *C.trachomatis* / *Ureaplasma* / *M.hominis*-MULTIPRIME-FRT** PCR kit	variant FRT-100 F
14.	**AmpliSens® *C.trachomatis* / *Ureaplasma* / *M.genitalium* / *M.hominis*-MULTIPRIME-FRT** PCR kit	variant FRT-100 F
15.	**AmpliSens® *N.gonorrhoeae* / *C.trachomatis* / *M.genitalium* / *T.vaginalis*-MULTIPRIME-FRT** PCR kit	variant FRT-100 F
16.	**AmpliSens® *N.gonorrhoeae* / *C.trachomatis* / *M.genitalium*-MULTIPRIME-FRT** PCR kit	variant FRT-100 F
17.	**AmpliSens® Genoscreen HLA B*5701-FRT** PCR kit	variant FRT
18.	**AmpliSens® *Mycoplasma pneumoniae* / *Chlamydophila pneumoniae*-FEP** PCR kit	variant FEP (0.2-ml tubes)
19.	**AmpliSens® *Mycoplasma pneumoniae* / *Chlamydophila pneumoniae*-FRT** PCR kit	variant FRT-100 F
20.	**AmpliSens® *T.vaginalis* / *N.gonorrhoeae* / *C.trachomatis*-MULTIPRIME-FRT** PCR kit	variant FRT-100 F

FEDERAL SERVICE FOR SUPERVISION OF CONSUMER RIGHTS PROTECTION AND HUMAN WELFARE
FEDERAL BUDGET INSTITUTE OF SCIENCE
«CENTRAL RESEARCH INSTITUTE FOR EPIDEMIOLOGY»
111123, Moscow, 3A, Novogireevskaya street, Tel.: +7 495 974 96 42, Fax: +7 495 305 54 23,
e-mail: obtk@pcr.ru

CE

EC DECLARATION OF CONFORMITY

Directive 98/79/EC of the European Parliament and of the Council of 27th of October 1998 on In Vitro Diagnostic Medical Devices

Federal Budget Institute of Science "Central Research Institute for Epidemiology" hereby under own responsibility declares that the products covered by the declaration conform with Essential Requirements listed in Annex I of EC Directive 98/79/EC (IVD Directive). Supporting documentation is retained under the premises of the manufacturer.

The quality management system meets the requirements of the standard EN ISO 13485 "Medical devices – Quality management systems – Requirements for regulatory purposes" and is certified by Institute for testing and certification, Inc. (certificate No. 21 0023 SJ, valid until 26.04.2024).

Manufacturer:	Federal Budget Institute of Science "Central Research Institute for Epidemiology"
Authorised Representative:	Ecoli Dx, s.r.o Purkyňova 74/2 Praha 1, 110 00 Czech Republic Tel: +420 325 209 912 Cell: +420 739 802 523 Email: ecoli@ecoli.sk
Product Name:	Annex for this Declaration
Description:	Reagent kits for qualitative detection and quantification of DNA (RNA) of different infectious agents
Classification:	Article 9, paragraph 1 of EC Council Directive 98/79/EC on *in Vitro* Diagnostic Devices
Conformity Assessment Route:	Annex III (IVDD)

Signed _____ Valid from 25.05.2022

Full name: Vasiliy G. Akimkin
Title: Director

№№	Description	Product Code (for reference only)
1.	**AmpliSens® All bacto-screen-FRT** PCR kit	H-2631-1-CE H-2632-1-4-CE
2.	**AmpliSens® All-screen-FRT** PCR kit	R-B45(RG,iQ)-CE
3.	**AmpliSens® All viro-screen-FRT** PCR kit	H-2761-1-CE
4.	**AmpliSens® ARVI-screen-FRT** PCR kit	R-V57-100-F(RG,iQ,Dt)-CE
5.	**AmpliSens® Ascaridosis-FRT** PCR kit	H-1971-1-CE
6.	**AmpliSens® *Bacillus anthracis*-FRT** PCR kit	R-B41(RG)-CE
7.	**AmpliSens® *Bordetella* multi-FRT** PCR kit	R-B84-100-F(RG,iQ,Dt)-CE
8.	**AmpliSens® *Borrelia burgdorferi sensu lato*-FRT** PCR kit	R-B37(RG)-CE
9.	**AmpliSens® *Borrelia miyamotoi*-FRT** PCR kit	H-2791-1-CE H-2792-1-4-CE
10.	**AmpliSens® BRCA1-FRT** PCR kit	S-3901-1-CE
11.	**AmpliSens® *Brucella* spp.-FRT** PCR kit	R-B10(RG)-CE
12.	**AmpliSens® *C.albicans* / *C.glabrata* / *C.krusei*-MULTIPRIME-FRT** PCR kit	R-F3-F(RG,iQ)-CE
13.	**AmpliSens® *Candida albicans*-FEP** PCR kit	F1-100-R0,2-FEP-CE
14.	**AmpliSens® *Candida albicans*-FRT** PCR kit	R-F1-F(RG,iQ)-CE
15.	**AmpliSens® *CCHFV*-FRT** PCR Kit	R-V22-50-F(RG,iQ,Mx,Dt)-CE
16.	**AmpliSens® *Corynebacterium diphtheriae* / tox-genes-FRT** PCR kit	H-2842-1-CE H-2843-1-4-CE
17.	**AmpliSens® *Cov*-Bat-FRT** PCR kit	H-2242-1-CE
18.	**AmpliSens® COVID-19-FL** PCR kit	H-4094-1-1-CE
19.	**AmpliSens® *Coxiella burnetii*-FRT** PCR kit	R-B85-50-F(RG,iQ,Mx,Dt)-CE
20.	**AmpliSens® *Cryptococcus neoformans*-FRT** PCR kit	R-F4-F(RG,iQ)-CE
21.	**AmpliSens® *Dengue virus* type-FRT** PCR kit	R-V63(RG,CFX)-CE
22.	**AmpliSens® *Dengue virus*-FRT** PCR kit	H-2391-1-CE H-2392-1-4-CE
23.	**AmpliSens® *EBOV* Zaire-FRT** PCR kit	R-V69-50-F-CE
24.	**AmpliSens® *EBV*-screen/monitor-FRT** PCR kit	R-V9-100-S(RG,iQ,Mx)-CE
25.	**AmpliSens® *Enterovirus* 71-FRT** PCR kit	R-V64-F-CE
26.	**AmpliSens® *Enterovirus*-FRT** PCR kit	R-V16(RG)-CE
27.	**AmpliSens® *Enterovirus* / *Parechovirus*-FRT** PCR kit	H-3751-1-2-CE
28.	**AmpliSens® ESBL CTX-M-FRT** PCR kit	HN-3571-1-CE
29.	**AmpliSens® Escherichioses-FRT** PCR kit	R-B62(RG,iQ)-CE
30.	**AmpliSens® F2/F5-SNP-FRT** PCR kit	S-3451-1-CE S-3452-1-4-CE
31.	**AmpliSens® FiloA-screen-FRT** PCR kit	H-2781-1-4-CE
32.	**AmpliSens® Florocenosis / Aerobes-FRT** PCR kit	R-B88-100-FT-CE
33.	**AmpliSens® Florocenosis / Bacterial vaginosis-FRT** PCR kit	R-B74-100-FT(RG)-CE
34.	**AmpliSens® Florocenosis / *Candida*-FRT** PCR kit	R-F5-100-FT(RG,CFX)-CE
35.	**AmpliSens® Florocenosis / *Mycoplasma*-FRT** PCR kit	R-B75-100-FT(RG,iQ,Mx)-CE
36.	**AmpliSens® *Giardia lamblia*-FRT** PCR kit	H-2821-1-CE H-2822-1-4-CE

№№	Description	Product Code (for reference only)
37.	**AmpliSens® *Gardnerella vaginalis*-FEP** PCR kit	B7-100-R0,2-FEP-CE
38.	**AmpliSens® *Gardnerella vaginalis*-FRT** PCR kit	R-B7-F(RG,iQ)-CE
39.	**AmpliSens® Genoscreen-*IL*28B-FRT** PCR kit	R-O5-100-F(RG,iQ,Dt,CFX)-CE
40.	**AmpliSens® *HAV*-FRT** PCR kit	R-V4(RG,iQ)-CE
41.	**AmpliSens® *Helicobacter pylori*-FRT** PCR kit	R-B9(RG,iQ)-CE H-2831-1-CE H-2832-1-4-CE
42.	**AmpliSens® Hemochromatosis-FRT** PCR kit	S-2451-1-CE S-2452-1-4-CE
43.	**AmpliSens® *HGV*-FRT** PCR kit	R-V2-50-F(RG,iQ,Mx,Dt)-CE
44.	**AmpliSens® *HHV*6-screen-titre-FRT** PCR kit	R-V10-T(RG,iQ,Mx)-CE
45.	**AmpliSens® *HHV*7-screen/monitor-FRT** PCR kit	H-2431-1-1-CE
46.	**AmpliSens® *HHV*8-screen/monitor-FRT** PCR kit	H-3581-1-1-CE H-3582-1-14-CE
47.	**AmpliSens® *HPV* 16/18-FRT** PCR kit	R-V12-100-CE R-V12-F-CE R-V12(RG,iQ,Mx)-CE
48.	**AmpliSens® *HPV* 6/11-FRT** PCR kit	R-V11-100-CE R-V11-Mod(RG,iQ,Mx)-CE R-V11(RG,iQ,Mx)-CE
49.	**AmpliSens® *HPV* HCR genotype-FRT** PCR kit	R-V25(RG,iQ,Mx)-CE
50.	**AmpliSens® *HPV* HCR genotype-titre-FRT** PCR kit	R-V67-F-CE H-2261-1-13-CE
51.	**AmpliSens® *HPV* HCR screen-EPh** PCR kit	V31-100F-CE
52.	**AmpliSens® *HPV* HCR screen-FEP** PCR kit	V31-3x-FEP-CE V31-FEP-CE
53.	**AmpliSens® *HPV* HCR screen-titre-14-FRT** PCR kit	H-2311-1-13-CE
54.	**AmpliSens® *HPV* HCR screen-titre-FRT** PCR kit	R-V31-T-2x(RG,iQ,SC)-CE R-V31-T-4x(RG,iQ,Mx)-CE R-V31-F-CE
55.	**AmpliSens® *HSV* I, II-FRT** PCR kit	R-V8-F(RG,iQ)-CE
56.	**AmpliSens® *HSV*-typing-FEP** PCR kit	V38-100-R0,2-FEP-CE
57.	**AmpliSens® *HSV*-typing-FRT** PCR kit	R-V38-F(RG,iQ)-CE
58.	**AmpliSens® *Human enterovirus*-FEP** PCR kit	H-2771-2-2-CE H-2772-2-CE
59.	**AmpliSens® *Human enterovirus*-FRT** PCR kit	H-2771-1-2-CE H-2773-1-CE H-2773-1-4-CE
60.	**AmpliSens® *Influenza virus* A/H1-swine-FEP** PCR kit	V55-50-R0,2-FEP-CE
61.	**AmpliSens® *Influenza virus* A/H1-swine-FRT** PCR kit	R-V55(RG)-CE R-V55-F(SC)-CE
62.	**AmpliSens® *Influenza virus* A-type-FRT** PCR kit	R-V54-100-F(RG,iQ,Dt,SC)-CE
63.	**AmpliSens® *Influenza virus* A-type-H5, H7, H9-FRT** PCR kit	R-V66-F-CE
64.	**AmpliSens® *Influenza virus* A H5N1-FRT** PCR kit	R-V33(SC)-CE
65.	**AmpliSens® *Influenza virus* A/B-FRT** PCR kit	R-V36-100-F-Mod(RG,iQ,Dt, CFX,SC)-CE
66.	**AmpliSens® *Influenza virus* B-type-FRT** PCR kit	H-3991-1-23-CE H-3992-1-3-CE
67.	**AmpliSens® *JCV-BKV* screen-monitor-FRT** PCR kit	H-2441-1-1-CE

№№	Description	Product Code (for reference only)
68.	**AmpliSens® *Legionella pneumophila*-FEP** PCR kit	B50-R0,2-FEP-CE
69.	**AmpliSens® *Legionella pneumophila*-FRT** PCR kit	R-B50(RG)-CE
70.	**AmpliSens® *Leptospira*-FRT** PCR kit	R-B49(RG,iQ)-CE
71.	**AmpliSens® Leucosis Quantum *M-bcr*-FRT** PCR kit	TR-O1(RG,iQ,Mx,A)-CE
72.	**AmpliSens® *Listeria monocytogenes*-screen/monitor-FRT** PCR kit	H-2161-1-1-CE
73.	**AmpliSens® MDR A.b.-OXA-FRT PCR kit**	HN-3871-1-CE HN-3872-1-4-CE
74.	**AmpliSens® MDR KPC/OXA-48-FRT** PCR kit	R-C2(RG,CFX)-CE
75.	**AmpliSens® MDR MBL-FRT** PCR kit	R-C1(RG,CFX)-CE
76.	**AmpliSens® MDR MCR-1-FRT** PCR kit	HN-4171-1-CE HN-4172-1-4-CE
77.	**AmpliSens® MDR VRE-FRT** PCR kit	HN-3891-1-CE HN-3892-1-4-CE
78.	**AmpliSens® *MRSA*-screen-titre-FRT** PCR kit	R-B78-100-FT(RG,iQ)-CE
79.	**AmpliSens® *MTC*-diff-FRT** PCR kit	R-B80(RG,iQ,Dt,SC)-CE
80.	**AmpliSens® MTC-MDR-FRT** PCR kit	H-3611-1-CE H-3612-1-4-CE
81.	**AmpliSens® *MTC*-FEP** PCR kit	B57-FEP-CE
82.	**AmpliSens® *MTC*-FRT** PCR kit	R-B57(RG,iQ,SC,Dt)-CE
83.	**AmpliSens® MTHFR-SNP-FRT** PCR kit	S-3721-1-CE S-3722-1-4-CE
84.	**AmpliSens® *Mycoplasma genitalium*-FEP** PCR kit	B4-100-R0,2-FEP-CE
85.	**AmpliSens® *Mycoplasma genitalium*-FRT** PCR kit	R-B4(RG)-CE R-B4-F(RG,iQ)-CE
86.	**AmpliSens® *Mycoplasma hominis*-FEP** PCR kit	B3-100-R0,2-FEP-CE
87.	**AmpliSens® *Mycoplasma hominis*-FRT** PCR kit	R-B3(RG)-CE R-B3-F(RG,iQ)-CE
88.	**AmpliSens® *M.genitalium*-ML/FQ-Resist-FRT** PCR kit	H-3971-1-CE
89.	**AmpliSens® *N.meningitidis* / *H.influenzae* / *S.pneumonia*-FRT** PCR kit	R-B25(RG,iQ)-CE
90.	**AmpliSens® *Neisseria gonorrhoeae*-screen-FEP** PCR kit	B51-100-R0,2-FEP-CE
91.	**AmpliSens® *Neisseria gonorrhoeae*-screen-FRT** PCR kit	R-B51(RG)-CE R-B51-F(RG,iQ)-CE
92.	**AmpliSens® NmABCW-FRT** PCR kit	H-3861-1-3-CE
93.	**AmpliSens® *Norovirus* GI / GII-FRT** PCR kit	H-2751-1-3-CE
94.	**AmpliSens® *Parvovirus* B19-FRT** PCR kit	R-V49(RG,iQ,Mx)-CE
95.	**AmpliSens *Plasmodium* spp. / *P.falciparum* / *P.vivax*-FRT** PCR kit	H-3981-1-CE H-3982-1-4-CE
96.	**AmpliSens® *Pneumocystis jirovecii* (*carinii*)-FRT** PCR kit	R-F2-Mod(RG,iQ,Mx)-CE
97.	**AmpliSens® Pneumo-quantum-FRT** PCR kit	H-2811-1-1-CE H-2812-1-14-CE
98.	**AmpliSens® *Poliovirus*-FRT** PCR kit	R-V58(RG,iQ)-CE
99.	**BRCA-screen** kit	S-1619-6-CE
100.	**PEERO-prep** reagent kit for sample preparation	K15-1611-40-CE
101.	**AmpliSens® Pyroscreen PHARMA-screen-Imatinib kit**	S-16121-6-CE

№№	Description	Product Code (for reference only)
102.	**AmpliSens® Pyroscreen PHARMA-screen-transport kit**	S-16119-6-CE
103.	**AmpliSens® Pyroscreen PHARMA-screen-Warfarin kit**	S-16120-6-CE
104.	**AmpliSens® *Rickettsia conorii*-FRT** PCR kit	H-2741-1-CE H-2742-1-4-CE
105.	**AmpliSens® *Rickettsia* spp. *SFG*-FRT PCR kit**	H-3741-1-CE H-3742-1-4-CE
106.	**AmpliSens® *Rotavirus* / *Norovirus* / *Astrovirus*-FRT** PCR kit	R-V40(RG,iQ)-CE
107.	**AmpliSens® SARS-CoV-2-IT** reagent kit	H-4121-10-CE
108.	**AmpliSens® SARS-CoV-2-N501Y-IT** reagent kit	H-4161-10-CE
109.	**AmpliSens® *Shigella* spp. and *EIEC* / *Salmonella* spp. / *Campylobacter* spp.-FRT** PCR kit	R-B44(RG,iQ)-CE
110.	**AmpliSens® *Streptococcus agalactiae*-screen-titre-FRT** PCR kit	R-B77-100-FT(RG,iQ)-CE
111.	**AmpliSens® *Streptococcus pyogenes*-screen/monitor-FRT** PCR kit	H-2171-1-1-CE H-2172-1-14-CE
112.	**AmpliSens® *T.vaginalis* / *N.gonorrhoeae*-MULTIPRIME-FRT** PCR kit	R-B65-F(RG,iQ)-CE
113.	**AmpliSens® TBE-FRT** PCR kit	R-V52(RG)-CE
114.	**AmpliSens® *TBEV*, *B.burgdorferi sl*, *A.phagocytophilum*, *E.chaffeensis* / *E.muris*-FRT** PCR kit	R-V59(RG,iQ,Mx,Dt)-CE
115.	**AmpliSens® *Treponema pallidum*-FRT** PCR kit	R-B20-F(RG,iQ)-CE
116.	**AmpliSens® *Trichomonas vaginalis*-EPh** PCR kit	B6-100-R0,2-CE
117.	**AmpliSens® *Trichomonas vaginalis*-FEP** PCR kit	B6-100-R0,2-FEP-CE
118.	**AmpliSens® *Trichomonas vaginalis*-FRT** PCR kit	R-B6-F(RG,iQ)-CE
119.	**AmpliSens® *U.parvum* / *U.urealyticum*-FEP** PCR kit	B19-100-R0,2-FEP-CE
120.	**AmpliSens® *U.parvum* / *U.urealyticum*-FRT** PCR kit	R-B19(RG)-CE R-B19-F(RG,iQ)-CE
121.	**AmpliSens® *Ureaplasma* spp.-FRT** PCR kit	R-B2(RG)-CE R-B2-F(RG,iQ)-CE
122.	**AmpliSens® *Ureaplasma* spp.-screen-titre-FRT** PCR kit	R-B2-100-FT(RG,iQ,Mx)-CE
123.	**AmpliSens® *Vibrio cholerae*-FRT** PCR kit	R-B53(RG)-CE
124.	**AmpliSens® *VZV*-FRT** PCR kit	R-V61-50-F(RG)-CE
125.	**AmpliSens® WNV-FRT** PCR kit	R-V53(RG,iQ,Mx)-CE
126.	**AmpliSens® *Yellow fever virus*-FRT** PCR kit	H-2461-1-CE H-2462-1-4-CE
127.	**AmpliSens® *Yersinia enterocolitica* / *Y.pseudotuberculosis*-FRT** PCR kit	R-B64(RG,iQ)-CE
128.	**AmpliSens® *Yersinia pestis*-FRT** PCR kit	R-B79(RG,iQ,Dt)-CE
129.	**AmpliSens® *Zika virus*-FRT** PCR kit	H-2411-1-CE

Signed _____

Full name: Vasiliy Akimkin
Title: Director

Management Systems Certification Body
Institut pro testování a certifikaci, a.s.
třída Tomáše Bati 299, Louky, 763 02 Zlín, Czech Republic
www.itczlin.cz

CERTIFICATE

No. 21 0023 SJ

We confirm on the basis of a performed audit that company

Federal Budget Institute of Science "Central Research Institute for Epidemiology"

3a, Novogireevskaya str., 111123 Moscow, Russian Federation
Company VAT No.: 7720024671

has implemented and documented a functional quality management system in compliance with the requirements of the standard

EN ISO 13485:2016

Covering the following activities:

Design and development, manufacturing and final control of *in vitro* diagnostic medical devices

The Certificate is issued on the basis of the results mentioned in Audit Report No. 233404948/2021. The Certificate validity is conditioned by positive results of surveillance audits, which the certified company committed to undergo.
During use of the Certificate the Certificate Holder undertakes to follow the Rules of Use of the Certificate. This document is publicly available on www.itczlin.cz

S 3002

Date of Issue: 27. 04. 2021
Valid until: 26. 04. 2024

Date of the first certification awarding: 20. 05. 2015

Ing. Pavel Vaněk
Head of Certification Body

УТВЕРЖДЕНА
Приказом Росздравнадзора
от 12.02.2010г. № 965-Пр/10

УТВЕРЖДАЮ
Директор Федерального государственного учреждения науки «Центральный научно-исследовательский институт эпидемиологии» Федеральной службы по надзору в сфере защиты прав потребителей и благополучия человека
_____ В.И.Покровский
«10» 12 2009 г.

ИНСТРУКЦИЯ

по применению набора реагентов для выявления и дифференциации ДНК бактерий рода *Шигелла* (*Shigella* spp.) и энтероинвазивных *E.coli* (*EIEC*), *Сальмонелла* (*Salmonella* spp.), термофильных *Кампилобактерий* (*Campylobacter* spp.) в объектах окружающей среды и клиническом материале методом полимеразной цепной реакции (ПЦР) с гибридизационно-флуоресцентной детекцией

«АмплиСенс® *Shigella* spp. и *EIEC* / *Salmonella* spp. / *Campylobacter* spp.-FL»

ОГЛАВЛЕНИЕ

СПИСОК СОКРАЩЕНИЙ

В настоящей инструкции применяются следующие сокращения и обозначения:

ВКО	- внутренний контрольный образец
ОКО	- отрицательный контрольный образец
В-	- отрицательный контроль этапа экстракции ДНК/РНК
ПКО	- положительный контрольный образец
ПЦР	- полимеразная цепная реакция
ФГУН ЦНИИЭ Роспотребнадзора	- федеральное государственное учреждение науки «Центральный научно-исследовательский институт эпидемиологии» Федеральной службы по надзору в сфере защиты прав потребителей и благополучия человека
FEP	- детекция по «конечной точке»
FRT	- детекция в режиме «реального времени»

НАЗНАЧЕНИЕ

Набор реагентов **«АмплиСенс® *Shigella* spp. и *EIEC* / *Salmonella* spp. / *Campylobacter* spp.-FL»** предназначен для выявления и дифференциации ДНК бактерий рода *Шигелла* (*Shigella* spp.) и энтероинвазивных *E.coli* (*EIEC*), *Сальмонелла* (*Salmonella* spp.), термофильных *Кампилобактерий* (*Campylobacter* spp.) в объектах окружающей среды и клиническом материале методом полимеразной цепной реакции (ПЦР) с гибридизационно-флуоресцентной детекцией.

Для экстракции ДНК используются наборы реагентов, рекомендованные ФГУН ЦНИИЭ Роспотребнадзора («ДНК-сорб-В» или «РИБО-преп»). При экстракции ДНК из исследуемых образцов используется только РНК-элюент, входящий в состав набора реагентов «АмплиСенс® *Shigella* spp. и *EIEC* / *Salmonella* spp. / *Campylobacter* spp.-FL».

ВНИМАНИЕ! Результаты ПЦР-исследования учитываются в комплексной диагностике заболевания.

ПРИНЦИП МЕТОДА

Выявление ДНК *Шигелла* (*Shigella* spp.) и энтероинвазивных *E.coli* (*EIEC*), *Сальмонелла* (*Salmonella* spp.), термофильных *Кампилобактерий* (*Campylobacter* spp.) с гибридизационно-флуоресцентной детекцией включает в себя следующие этапы: экстракция (выделение) ДНК из образцов клинического материала, амплификацию участка ДНК данного микроорганизма и гибридизационно-флуоресцентную детекцию, которая производится либо непосредственно в ходе ПЦР (вариант FRT), либо после ее завершения (вариант FEP).

Экстракция ДНК из клинического материала проводится в присутствии внутреннего контрольного образца (**ВКО-FL**), который позволяет контролировать выполнение процедуры исследования для каждого образца. Пробы ДНК используются для амплификации участка ДНК перечисленных выше возбудителей при помощи специфичных к этому участку ДНК-праймеров и фермента Taq-полимеразы. В составе реакционной смеси присутствуют флуоресцентно-меченые олигонуклеотидные зонды, которые гибридизуются с комплементарным участком амплифицируемой ДНК-мишени, в результате чего происходит нарастание интенсивности флуоресценции. Это позволяет регистрировать накопление специфического продукта амплификации путем измерения интенсивности флуоресцентного сигнала. Детекция флуоресцентного сигнала при использовании варианта FEP осуществляется после окончания ПЦР с помощью флуоресцентного ПЦР-детектора, а при использовании варианта FRT – непосредственно в ходе ПЦР с помощью амплификатора с системой детекции флуоресцентного сигнала в режиме «реального времени».

ВАРИАНТЫ И ФОРМЫ ВЫПУСКА НАБОРА РЕАГЕНТОВ

Набор реагентов выпускается в 1 варианте

Вариант FEP/FRT

Набор реагентов выпускается в 1 форме комплектации:

Форма 1 включает комплект реагентов «ПЦР-комплект» вариант FEP/FRT-50 F.

Форма комплектации 1 предназначена для проведения амплификации и дифференциации ДНК шигелл (*Shigella* spp.) и энтероинвазивных *E.coli* (*EIEC*), сальмонелл (*Salmonella* spp.), термофильных кампилобактерий (*Campylobacter* spp.) с гибридизационно-флуоресцентной детекцией в режиме «реального времени» или по «конечной точке». Для проведения полного ПЦР-исследования необходимо использовать комплекты реагентов для экстракции ДНК, рекомендованные ФГУН ЦНИИЭ Роспотребнадзора.

Для элюции ДНК на этапе выделения должен использоваться только РНК-элюент, входящий в данный ПЦР-комплект.

АНАЛИТИЧЕСКИЕ ХАРАКТЕРИСТИКИ

Аналитическая чувствительность

Патоген	Вид клинического материала	Комплект для экстракции ДНК	Комплект для амплификации и детекции	Аналитическая чувствительность
Шигелла (*Shigella* spp.) и энтероинвазивные *E.coli* (*EIEC*)	Фекалии	**«РИБО-преп»**	«ПЦР-комплект» вариант FEP/FRT-50 F	$1x10^3$ ГЭ/мл
Сальмонелла (*Salmonella* spp.)	Фекалии	**«РИБО-преп»**	«ПЦР-комплект» вариант FEP/FRT-50 F	$1x10^3$ ГЭ/мл
Термофильные *Кампилобактерии* (*Campylobacter* spp.)	фекалии	**«РИБО-преп»**	«ПЦР-комплект» вариант FEP/FRT-50 F	$1x10^3$ ГЭ/мл

Аналитическая специфичность

Специфичность набора реагентов проверялась на следующих штаммах микроорганизмов:

Коллекция ГИСК им. Л.А. Тарасевича штаммы энтеровирусов (*Coxsakie* B1, B2, B3, B4, B5, B6; *Polio (Sabin*) I, II, III). Также тестировались аденовирусы серогрупп 5 и 7; вирусы гриппа А (H13N2, H9N2, H8N4, H2N3, H4N6, H11N6, H12N5, H3N8, H1N1, H6N2, H10N7, H5N1), В, риновирусы, RS вирусы, аденовирусы человека – 3, 5, 7, 37, 40 типов.

Коллекция ФГУ ВГНКИ: *Salmonella enteritidis* S-6, *Salmonella choleraesuis* 370, *Salmonella typhimurium* 371, *Salmonella dublin* 373, *Salmonella typhi* C1, *Salmonella abortusovis* 372, *Salmonella gallinarum-pullorum*, *Shigella flexneri* 851b, *Campylobacter fetus subsp*. *fetus* 25936, *Campylobacter jejuni subsp. jejuni* 43435, *Clebsiella* K 65 SW4, *Listeria monocitogenes* УСХЧ 19, *Listeria monocitogenes* УСХЧ 52, *Proteus vulgaris* 115/98, *Pseudomonas aeruginosa* ДН c1, *Staphilococcus aureus* 653, *Staphilococcus aureus* 29112, *Morganella Morganii* 619 c 01, *Enterobacter faecalis* 356.

Коллекция Центра контроля и профилактики заболеваний (CDC, США): 44 изолята норовирусов различных генетических кластеров 1 и 2 генотипа, 40 штаммов ротавирусов различных [P]G типов, 19 штаммов астровирусов 1, 2, 4, 5, 8 серотипов и 15 штаммов аденовирусов различных типов и следующие бактериальные штаммы (см. табл. 1).

Таблица 1

Панель бактериальных агентов Центра контроля и профилактики заболеваний (CDC, США)

Strain ID	Organism	Strain ID	Organism
K2033	*Salmonella* Ser. Grumpensis	K2015	*Salmonella* Ser. Oranienburg
K1806	*Salmonella* Ser. Newport	AM01144	*Salmonella* Ser. Newport
K2077	*Salmonella* Ser. Enteriditis	K1810	*Salmonella* Ser. Anatum
83-99	*Salmonella* Ser. Typhimurium	K1991	*Salmonella* Ser. Typhimurium
PS505	*Shigella boydii*	K1898	*Salmonella* Ser. Heidelberg
PS408	*Shigella sonnei*	PS555	*Shigella boydii*
B4003	*Shigella sonnei*	F6446	*Shigella dysenteriae*
PS801	*Shigella dysenteriae*	S821X1	*Shigella dysenteriae* type 1
C898	*Shigella dysenteriae* type1	S177X1	*Shigella dysenteriae* type 1
F2035	*Shigella flexneri*	S3314	*Shigella dysenteriae* type 2
E2539-C1	Enterotoxigenic *Escherichia coli* (ETEC)	PS071	*Shigella flexneri*
H10407	Enterotoxigenic *Escherichia coli* (ETEC)	PS050	*Shigella flexneri*
F1008	Enterotoxigenic *Escherichia coli* (ETEC)	F7862	*Shigella flexneri*
EDL 933	Shiga-toxin *E. coli* (STEC)	TX1	Enterotoxigenic *Escherichia coli* (ETEC)
3543-01	Shiga-toxin *E. coli* (STEC)	3525-01	Shiga-toxin *Escherichia coli* (STEC)
4752-71	*Proteus vulgaris*	25922	*Escherichia coli* O6:H1
QA/QC	*Citrobacter freundii*	621-64	*Citrobacter freundii*
QA/QC	*Aeromonas*	3910-68	*Aeromonas spp.*
3043-74	*Serratia marcescens*	E9113	*Vibrio cholerae*
QA/QC	*Serratia marcescens*	501-83	*Edwardsiella spp.*
F7894	*Vibrio vulnificus*	587-82	*Providencia stuartii*
F8515	*Yersinia enterocolitica*	27853	*Pseudomonas aeruginosa*
F8510	*Yersinia enterocolitica*	D4989	*Helicobacter cineadi*
K4299	*Vibrio parahaemolyticus*	D6827	*Helicobacter pullorum*
F9835	*Vibrio parahaemolyticus*	D5127	*Helicobacter pylori*
K2023	*Salmonella* Ser. Kentucky	D2686	*Arcobacter butzleri*
K1684	*Salmonella* O-1, 4, 12 gr. B	-	-

При проведении тестирования данных панелей, а также образцов ДНК человека неспецифических реакций выявлено не было.

МЕРЫ ПРЕДОСТОРОЖНОСТИ

Работа должна проводиться в лаборатории, выполняющей молекулярно-биологические (ПЦР) исследования клинического материала на наличие возбудителей инфекционных болезней, с соблюдением санитарно-эпидемических правил СП 1.3.2322-08 «Безопасность работы с микроорганизмами III – IV групп патогенности (опасности) и возбудителями паразитарных болезней», СП 2.1.7.728-99 «Правила сбора, хранения и удаления отходов лечебно-профилактических учреждений» и методических указаний МУ 1.3.1888-04 «Организация работы

при исследованиях методом ПЦР материала, инфицированного патогенными биологическими агентами III – IV групп патогенности».

При работе всегда следует выполнять следующие требования:

- Следует рассматривать исследуемые образцы как инфекционно-опасные, организовывать работу и хранение в соответствии с СП 1.3.2322-08 «Безопасность работы с микроорганизмами III – IV групп патогенности (опасности) и возбудителями паразитарных болезней».
- Убирать и дезинфицировать разлитые образцы или реактивы, используя дезинфицирующие средства в соответствии СП 1.3.2322-08 «Безопасность работы с микроорганизмами III – IV групп патогенности (опасности) и возбудителями паразитарных болезней».
- Удалять неиспользованные реактивы в соответствии с требованиями СП 2.1.7.728-99 «Правила сбора, хранения и удаления отходов лечебно-профилактических учреждений».

ВНИМАНИЕ! При удалении отходов после амплификации (пробирок, содержащих продукты ПЦР) недопустимо открывание пробирок и разбрызгивание содержимого, поскольку это может привести к контаминации продуктами ПЦР лабораторной зоны, оборудования и реагентов.

- Применять набор строго по назначению, согласно данной инструкции.
- Допускать к работе с набором только специально обученный персонал.
- Не использовать набор по истечении срока годности.
- Избегать контакта с кожей, глазами и слизистой оболочкой. При контакте немедленно промыть пораженное место водой и обратиться за медицинской помощью.
- Листы по безопасности материалов (MSDS – material safety data sheet) доступны по запросу.

ДОПОЛНИТЕЛЬНЫЕ МАТЕРИАЛЫ И ОБОРУДОВАНИЕ

1. Комплект реагентов для выделения ДНК – «ДНК-сорб-В» (ТУ 9398-036-01897593-2009), «РИБО-преп» (ТУ 9398-071-01897593-2008) или другие рекомендованные ФГУН ЦНИИЭ Роспотребнадзора.
2. Дополнительные материалы и оборудование для экстракции ДНК – согласно инструкции к комплекту реагентов для

выделения ДНК.

3. Бокс абактериальной воздушной среды (ПЦР-бокс).
4. Центрифуга/вортекс.
5. Автоматические дозаторы переменного объема (от 5 до 20 мкл, при работе с «ПЦР-комплектом» вариант FEP/FRT-50 F – от 5 до 20 мкл, от 20 до 200 мкл).
6. Одноразовые наконечники с фильтром до 100 мкл в штативах.
7. Штативы для микропробирок объемом 0,2 мл или 0,5 мл (в соответствии с используемыми комплектами реагентов).
8. Холодильник от 2 до 8 °С с морозильной камерой не выше минус 16 °С для выделенных проб ДНК.
9. Отдельный халат, шапочки, обувь и одноразовые перчатки по МУ 1.3.1888-04.
10. Емкость для сброса наконечников.

<u>При детекции «по конечной точке»:</u>

11. Программируемый амплификатор (например, «Терцик» («ДНК-Технология», Россия), «Gradient Palm Cycler» («Corbett Research», Австралия), «MAXYGENE» («Axygen», США), «GeneAmp PCR System 2700» («Applied Biosystems») или аналогичные).
12. Флуоресцентный ПЦР-детектор (например, «АЛА-1/4» («BioSan», Латвия), «Джин» («ДНК-Технология», Россия) или аналогичные).
13. Одноразовые полипропиленовые пробирки для ПЦР (плоская крышка, нестрипованные) на 0,2 или 0,5 мл:
    а) объемом 0,2 мл (например, «Axygen», США) – для амплификаторов, адаптированных для ПЦР-пробирок 0,2 мл («Gradient Palm Cycler», «GeneAmp PCR System 2700», «MAXYGENE» и др.);
    б) объемом 0,5 мл (например, «Axygen», США) – для амплификаторов, адаптированных для ПЦР-пробирок 0,5 мл («Терцик» и др.).

<u>При детекции в режиме «реального времени»:</u>

14. Программируемый амплификатор с системой детекции флуоресцентного сигнала в режиме «реального времени» (например, «Rotor-Gene» 3000/6000 («Corbett Research», Австралия), «Rotor-Gene Q» («Qiagen», Германия), «iQ5» («Bio-Rad», США), «Mx3000P» («Stratagene», США), «ДТ-96»

(«ДНК-Технология», Россия) или аналогичные).

15. Одноразовые полипропиленовые пробирки для ПЦР:
    а) на 0,2 мл (плоская крышка, нестрипованные), (например, «Axygen», США) для постановки в ротор на 36 пробирок – для приборов для ПЦР в реальном времени с детекцией через дно пробирки (например, «Rotor-Gene»).
    б) на 0,2 мл (куполообразная крышка) (например, «Axygen», США) – для приборов для ПЦР в реальном времени с детекцией через крышку (например, «iQ5», «Mx3000P»).

ВЗЯТИЕ, ТРАНСПОРТИРОВАНИЕ И ХРАНЕНИЕ ИССЛЕДУЕМОГО МАТЕРИАЛА

Перед началом работы следует ознакомиться с методическими рекомендациями «Взятие, транспортировка, хранение клинического материала для ПЦР-диагностики», разработанными ФГУН ЦНИИЭ Роспотребнадзора, Москва, 2008 г.

Материалом для исследования служат образцы фекалий, концентраты образцов воды, подготовленные в соответствии с МУК 4.2.2029-05. «Методические указания по санитарно-вирусологическому контролю водных объектов».

ПОДГОТОВКА ИССЛЕДУЕМОГО МАТЕРИАЛА К ЭКСТРАКЦИИ ДНК

Концентраты образцов воды не требуют специальной подготовки для экстракции ДНК. Подготовка образцов фекалий проводится в соответствии с методическими рекомендациями «Взятие, транспортировка, хранение клинического материала для ПЦР-диагностики», разработанными ФГУН ЦНИИЭ Роспотребнадзора, Москва, 2008 г.

ВАРИАНТ FEP

СОСТАВ

Комплект реагентов «ПЦР-комплект» вариант FEP/FRT-50 F - комплект реагентов для амплификации и дифференциации ДНК бактерий рода *Шигелла* (*Shigella* spp.) и энтероинвазивных *E.coli* (*EIEC*), *Сальмонелла* (*Salmonella* spp.), термофильных *Кампилобактерий* (*Campylobacter* spp.) с гибридизационно-флуоресцентной детекцией **включает:**

Реактив	*Описание*	*Объем (мл)*	*Кол-во*
ПЦР-смесь-1-FEP/FRT *Shigella* spp. / *Salmonella* spp.	Прозрачная бесцветная жидкость	0,6	1 пробирка
ПЦР-смесь-1-FEP/FRT *Campylobacter* spp. / STI	Прозрачная бесцветная жидкость	0,6	1 пробирка
ПЦР-смесь-2-FRT	Прозрачная бесцветная жидкость	0,3	2 пробирки
Полимераза (TaqF)	Прозрачная бесцветная жидкость	0,02	2 пробирки
ДНК-буфер	Прозрачная бесцветная жидкость	0,5	1 пробирка
ПКО ДНК *Shigella sonnei* / *Salmonella*	Прозрачная бесцветная жидкость	0,1	1 пробирка
ПКО ДНК *Campylobacter jejuni* / STI	Прозрачная бесцветная жидкость	0,1	1 пробирка
Минеральное масло для ПЦР	Бесцветная вязкая жидкость	4,0	1 флакон

Комплект реагентов рассчитан на проведение 55 реакций амплификации, включая контроли.

К комплекту реагентов прилагаются контрольные образцы этапа выделения и РНК-элюент для экстракции ДНК:

Реактив	*Описание*	*Объем (мл)*	*Кол-во*
ВКО-FL	Прозрачная бесцветная жидкость	1,0	1 пробирка
ОКО	Прозрачная бесцветная жидкость	1,6	1 пробирка
РНК-элюент	Прозрачная бесцветная жидкость	1,2	5 пробирок

ПРОВЕДЕНИЕ ПЦР-ИССЛЕДОВАНИЯ

ПЦР-исследование состоит из следующих этапов:

- Экстракция (выделение) ДНК из исследуемых образцов.
- Амплификация ДНК.
- Флуоресцентная детекция продуктов амплификации по «конечной точке».

- Интерпретация результатов.

ЭКСТРАКЦИЯ ДНК ИЗ ИССЛЕДУЕМЫХ ОБРАЗЦОВ

Экстракцию ДНК провести в соответствии с инструкцией к используемому комплекту реагентов для экстракции ДНК из клинического материала («ДНК-сорб-В», «РИБО-преп» или другие комплекты реагентов, рекомендованные ФГУН ЦНИИЭ Роспотребнадзора). Экстракция ДНК из каждого клинического образца проводится в присутствии внутреннего контрольного образца (**ВКО-FL**).

ВНИМАНИЕ! При экстракции ДНК из исследуемых образцов используется только РНК-элюент, входящий в состав набора реагентов **«АмплиСенс® *Shigella* spp. и *EIEC* / *Salmonella* spp. / *Campylobacter* spp.-FL»**.

ПРОВЕДЕНИЕ АМПЛИФИКАЦИИ

Общий объем реакционной смеси – 25 мкл, включая объем пробы ДНК – 10 мкл.

А. Подготовка пробирок для проведения амплификации

Выбор пробирок для амплификации зависит от используемого амплификатора.

Для внесения в пробирки реагентов, проб ДНК и контрольных образцов используются одноразовые наконечники с фильтрами.

1. Компоненты реакционных смесей следует смешивать непосредственно перед проведением анализа. Смешивать реагенты из расчета на необходимое число реакций, включающее тестирование исследуемых и контрольных образцов, необходимо согласно **расчетной таблице** (см. табл. 2). Следует учитывать, что **для тестирования даже одного исследуемого или контрольного образца ДНК необходимо проводить постановку всех контролей этапа ПЦР (положительного контроля (К+), отрицательного контроля (К-) и двух пробирок «Фон» для каждого типа смеси)**. Рекомендуется смешивать реагенты для четного числа реакций с целью более точного дозирования.
2. Отобрать необходимое количество пробирок с учетом количества исследуемых, контрольных образцов ДНК и

пробирок «Фон». Тип пробирок, стрипов или планшетов выбрать в зависимости от используемого прибора.

3. Для приготовления реакционных смесей и смесей для пробирок «Фон» необходимо в отдельной стерильной пробирке смешать одну из **ПЦР-смесей-1 (ПЦР-смесь-1-FEP/FRT *Shigella* spp. / *Salmonella* spp., или ПЦР-смесь-1-FEP/FRT *Campylobacter* spp. / STI), ПЦР-смесь-2-FRT** (см. табл. 2). Тщательно перемешать смеси на вортексе и осадить капли с крышек пробирок.
4. Приготовить 4 пробирки **«Фон»** (по две для каждого типа реакционной смеси). Для этого внести по **15 мкл** приготовленных смесей (без полимеразы (TaqF)) каждой в две пробирки **«Фон»**, добавить по **10 мкл ДНК-буфера**, перемешать пипетированием. Сверху раскапать по **1 капле минерального масла для ПЦР** (примерно 25 мкл).
5. В оставшиеся части реакционных смесей добавить **полимеразу (TaqF)** в количестве см. табл. 2. Тщательно перемешать смесь на вортексе и осадить капли с крышки пробирки.

ВНИМАНИЕ! Количество добавляемой в реакционную смесь полимеразы (TaqF), указанное в табл. 2, приведено с учетом уже отобранных 30 мкл реакционной смеси для двух пробирок «Фон».

6. Внести в оставшиеся пробирки по 15 мкл готовых реакционных смесей. Сверху раскапать по 1 капле минерального масла для ПЦР (примерно 25 мкл).

Таблица 2

Схема приготовления реакционных смесей для ПЦР с детекцией по «конечной точке»

		Объем реагентов на указанное количество реакций		
Объем реагента на одну реакцию (мкл)		**10.00**	**5.00**	**0.50**
Число исследуемых образцов	**Число реакций[1]**	**ПЦР-смесь-1-FEP/FRT, мкл**	**ПЦР-смесь-2-FRT, мкл**	**Полимераза (TaqF), мкл**
2	**8**	**80**	**40**	**3.0**
4	**10**	**100**	**50**	**4.0**
6	**12**	**120**	**60**	**5.0**
8	**14**	**140**	**70**	**6.0**
10	**16**	**160**	**80**	**7.0**
12	**18**	**180**	**90**	**8.0**
14	**20**	**200**	**100**	**9.0**
16	**22**	**220**	**110**	**10.0**
18	**24**	**240**	**120**	**11.0**
20	**26**	**260**	**130**	**12.0**
22	**28**	**280**	**140**	**13.0**
24	**30**	**300**	**150**	**14.0**
26	**32**	**320**	**160**	**15.0**
28	**34**	**340**	**170**	**16.0**

ВНИМАНИЕ! Количество добавляемой полимеразы (TaqF) указано с вычетом двух пробирок «Фон».

7. Используя наконечники с фильтром, в пробирки с реакционной смесью добавить по **10 мкл ДНК-проб**, выделенных из исследуемых или контрольных проб этапа выделения ДНК. Неиспользованные остатки реакционной смеси выбросить.

ВНИМАНИЕ! При добавлении ДНК-проб, выделенных с помощью комплекта реагентов «ДНК-сорб-В», необходимо избегать попадания сорбента в реакционную смесь для ПЦР.

8. Поставить **контрольные реакции амплификации:**
   а) **отрицательный контроль (К-)** – внести в пробирки с реакционной смесью **10 мкл ДНК-буфера;**
   б) **положительный контроль (К+ *Shigella/Salmonella*)** – внести в пробирки **10 мкл ПКО ДНК *Shigella sonnei* / *Salmonella*** для **ПЦР-смеси-1-FEP/FRT *Shigella* spp. / *Salmonella* spp;**

[1] Число исследуемых образцов + контроль этапа выделения ДНК + 2 контроля этапа ПЦР + 2 пробирки «Фон» + запас на один образец (N+1+2+2+1, где N-количество клинических образцов).

в) **положительный контроль (К+ *Campylobacter*/STI)** – внести в пробирки **10 мкл ПКО ДНК *Campylobacter jejuni* / STI** для **ПЦР-смеси-1-FEP/FRT *Campylobacter* spp. / STI.**

Б. Проведение амплификации

ВНИМАНИЕ! Пробы амплифицировать сразу после соединения реакционной смеси, ДНК-пробы и контролей! Время внесения проб в реакционную смесь и запуск реакции на приборе не должно превышать 10-15 мин.

Запустить на амплификаторе программу.

Таблица 3

Программа амплификации

	Амплификаторы с активным регулированием[2]			Амплификаторы с активным регулированием[3]			Амплификаторы с матричным регулированием[4]		
цикл	температура	время	циклы	температура	время	циклы	температура	время	циклы
0	**95°C**	пауза		**95°C**	пауза		**95°C**	пауза	
1	**95 °C**	**15 мин**	**1**	**95 °C**	**15 мин**	**1**	**95 °C**	**15 мин**	**1**
2	**95 °C**	**10 с**	**42**	**95 °C**	**10 с**	**42**	**95 °C**	**1 мин**	**42**
	60 °C	**10 с**		**60 °C**	**25 с**		**60 °C**	**1 мин**	
	72 °C	**10 с**		**72 °C**	**25 с**		**72 °C**	**1 мин**	
3	**72 °C**	**1 мин**	**1**	**72 °C**	**1 мин**	**1**	**72 °C**	**1 мин**	**1**
4	**10 °C**	хранение		**10 °C**	хранение		**10 °C**	хранение	

1. По окончании выполнения программы приступить к флуоресцентной детекции.

ФЛУОРЕСЦЕНТАЯ ДЕТЕКЦИЯ ПРОДУКТОВ АМПЛИФИКАЦИИ ПО «КОНЕЧНОЙ ТОЧКЕ»

Детекция проводится с помощью флуоресцентного ПЦР-детектора (согласно инструкции к используемому прибору) путем измерения интенсивности флуоресцентного сигнала по двум каналам.

[2] Например, «Терцик» («ДНК-Технология»), «GeneAmp PCR System 2400» («Perkin Elmer»)
[3] Например, «Gradient Palm Cycler» («Corbett Research»), «GeneAmp PCR System 2700» («Perkin Elmer»)
[4] Например, «MiniCycler», «PTC-100» («MJ Research»), «Uno-2» («Biometra»)

Таблица 4

Схема соответствия тестируемых патогенов и каналов флуоресцентной детекции

Канал детекции	ПЦР-смесь-1 FEP/FRT *Shigella* spp. / *Salmonella* spp.	ПЦР-смесь-1-FEP/FRT *Campylobacter* spp. / STI
FAM/Green	ДНК *Shigella* spp.	ДНК *Campylobacter* spp.
JOE/Yellow/HEX	ДНК *Salmonella* spp.	ВКО

ВНИМАНИЕ! До проведения детекции в программном обеспечении ПЦР-детектора должны быть внесены и сохранены соответствующие настройки – см. вкладыш к ПЦР-комплекту.

ИНТЕРПРЕТАЦИЯ РЕЗУЛЬТАТОВ

Полученные результаты интерпретируют на основании данных об уровне флуоресцентного сигнала относительно фона по соответствующим каналам для контрольных образцов и проб ДНК, выделенных из клинических образцов. Интерпретация производится автоматически с помощью программного обеспечения используемого прибора. Принцип интерпретации результатов представлен в табл. 5.

Таблица 5

Интерпретация результатов ПЦР-исследования

ПЦР-смесь-1	Результат по уровню флуоресценции		Результат
	FAM/Green	JOE/Yellow/HEX	
ПЦР-смесь-1-FEP/FRT *Shigella* spp. / *Salmonella* spp.	Выше порогового значения положительного результата	Ниже порогового значения отрицательного результата	В пробе выявлена ДНК *Shigella* spp.
	Ниже порогового значения отрицательного результата	Выше порогового значения положительного результата	В пробе выявлена ДНК *Salmonella* spp.
	Ниже порогового значения отрицательного результата	Ниже порогового значения отрицательного результата	В пробе не выявлена ДНК *Shigella* spp. и ДНК *Salmonella* spp.[5]
	Выше порогового значения положительного результата	Выше порогового значения положительного результата	В пробе выявлена ДНК *Shigella* spp. и ДНК *Salmonella* spp.

[5] при значении флуоресценции Выше порогового значения по каналу HEX при использовании **ПЦР-смеси-1-FEP/FRT *Campylobacter* / STI.**

ПЦР-смесь-1	Результат по уровню флуоресценции		Результат
	FAM/Green	JOE/Yellow/HEX	
ПЦР-смесь-1-FEP/FRT *Campylobacter* spp. / STI	Выше порогового значения положительного результата	Ниже порогового значения отрицательного результата	В пробе выявлена ДНК *Campylobacter* spp.
	Ниже порогового значения отрицательного результата	Выше порогового значения положительного результата	В пробе не выявлена ДНК *Campylobacter* spp.
	Ниже порогового значения отрицательного результата	Ниже порогового значения отрицательного результата	Проба требует повторного перевыделения и тестирования на всех ПЦР-смесях 1
	Выше порогового значения положительного результата	Выше порогового значения положительного результата	В пробе выявлена ДНК *Campylobacter* spp.

Если значение уровня флуоресценции для пробы находится между пороговыми значениями положительного и отрицательного результата он расценивается как **невалидный** или **сомнительный**, и требует повторения ПЦР-исследование соответствующего исследуемого образца.

Результат ПЦР-исследования считается достоверным, если получены правильные результаты для положительного и отрицательного контролей амплификации и отрицательного контроля выделения ДНК, в соответствии с табл. 7.

Таблица 6

Результаты для контролей различных этапов ПЦР-исследования

ПЦР-смесь-1	Контроль	Контролируемый этап	Канал для флуорофора FAM	Канал для флуорофора JOE
ПЦР-смесь-1-FEP/FRT *Shigella* spp. / *Salmonella* spp.	В-	Экстракция ДНК	Ниже порогового значения отрицательного результата	Ниже порогового значения отрицательного результата
	К-	ПЦР	Ниже порогового значения отрицательного результата	Ниже порогового значения отрицательного результата
	К+ *Shigella/Salmonella*	ПЦР	Выше порогового значения положительного результата	Выше порогового значения положительного результата

ВАРИАНТ FEP

ПЦР-смесь-1	Контроль	Контролируемый этап	Канал для флуорофора FAM	Канал для флуорофора JOE
ПЦР-смесь-1-FEP/FRT *Campylobacter* spp. / STI	B-	Экстракция ДНК	Ниже порогового значения отрицательного результата	Выше порогового значения положительного результата
	K-	ПЦР	Ниже порогового значения отрицательного результата	Ниже порогового значения отрицательного результата
	K+ *Campylobacter*/ STI	ПЦР	Выше порогового значения положительного результата	Выше порогового значения положительного результата

ВНИМАНИЕ!

1. Если для положительного контроля ПЦР (К+) сигнал по каналам JOE/Yellow/HEX и FAM/Green ниже порогового значения положительного результата необходимо повторить амплификацию и детекцию для всех образцов, в которых сигнал по каналам JOE/Yellow/HEX и FAM/Green был ниже порогового значения положительного результата на соответствующем типе ПЦР-смеси-1*.*
2. Если для отрицательного контроля экстракции ДНК (В-) (кроме канала JOE/Yellow/HEX для ПЦР-смеси-1-FEP/FRT *Campylobacter* STI и/или отрицательного контроля ПЦР (К-) сигнал по каналам JOE/Yellow/HEX или FAM/Green выше порогового значения положительного результата необходимо повторить ПЦР-исследование для всех образцов, в которых обнаружена ДНК соответствующих патогенов, начиная с этапа выделения (экстракции) ДНК.

ВАРИАНТ FRT

СОСТАВ

Комплект реагентов «ПЦР-комплект» вариант FEP/FRT-50 F - комплект реагентов для амплификации и дифференциации ДНК бактерий рода *Шигелла* (*Shigella* spp.) и энтероинвазивных *E.coli* (*EIEC*), *Сальмонелла* (*Salmonella* spp.), термофильных *Кампилобактерий* (*Campylobacter* spp.) с гибридизационно-флуоресцентной детекцией **включает:**

Реактив	*Описание*	*Объем (мл)*	*Кол-во*
ПЦР-смесь-1-FEP/FRT *Shigella* spp. / *Salmonella* spp.	Прозрачная бесцветная жидкость	0,6	1 пробирка
ПЦР-смесь-1-FEP/FRT *Campylobacter* spp. / STI	Прозрачная бесцветная жидкость	0,6	1 пробирка
ПЦР-смесь-2-FRT	Прозрачная бесцветная жидкость	0,3	2 пробирки
Полимераза (TaqF)	Прозрачная бесцветная жидкость	0,02	2 пробирки
ДНК-буфер	Прозрачная бесцветная жидкость	0,5	1 пробирка
ПКО ДНК *Shigella sonnei* / *Salmonella*	Прозрачная бесцветная жидкость	0,1	1 пробирка
ПКО ДНК *Campylobacter jejuni* / STI	Прозрачная бесцветная жидкость	0,1	1 пробирка
Минеральное масло для ПЦР	Бесцветная вязкая жидкость	4,0	1 флакон

Комплект реагентов рассчитан на проведение 55 реакций амплификации, включая контроли.

К комплекту реагентов прилагаются контрольные образцы этапа выделения и РНК-элюент для экстракции ДНК:

Реактив	*Описание*	*Объем, мл*	*Кол-во*
ВКО-FL	Прозрачная бесцветная жидкость	1,0	1 пробирка
ОКО	Прозрачная бесцветная жидкость	1,6	1 пробирка
РНК-элюент	Прозрачная бесцветная жидкость	1,2	5 пробирок

ПРОВЕДЕНИЕ ПЦР-ИССЛЕДОВАНИЯ

ПЦР-исследование состоит из следующих этапов:

- Экстракция (выделение) ДНК из исследуемых образцов.
- Амплификация ДНК с флуоресцентной детекцией в режиме

«реального времени».
- Интерпретация результатов.

ЭКСТРАКЦИЯ ДНК ИЗ ИССЛЕДУЕМЫХ ОБРАЗЦОВ

Экстракцию ДНК провести в соответствии с инструкцией к используемому комплекту реагентов для выделения ДНК из клинического материала («ДНК-сорб-В», «РИБО-преп» или другие комплекты реагентов, рекомендованные ФГУН ЦНИИЭ Роспотребнадзора). Экстракция ДНК из каждого клинического образца проводится в присутствии внутреннего контрольного образца (**ВКО-FL**).

ВНИМАНИЕ! При экстракции ДНК из исследуемых образцов используется только РНК-элюент, входящий в состав набора реагентов **«АмплиСенс® *Shigella* spp. и *EIEC* / *Salmonella* spp. / *Campylobacter* spp.-FL».**

ПРОВЕДЕНИЕ АМПЛИФИКАЦИИ С ДЕТЕКЦИЕЙ В РЕЖИМЕ «РЕАЛЬНОГО ВРЕМЕНИ»

Общий объем реакционной смеси – 25 мкл, включая объем пробы ДНК – 10 мкл.

А. Подготовка пробирок для амплификации

Выбор пробирок для амплификации зависит от используемого амплификатора с системой детекции в режиме «реального времени».

Для внесения в пробирки реагентов, проб ДНК и контрольных образцов используются одноразовые наконечники с фильтрами.

1. Компоненты реакционных смесей следует смешивать непосредственно перед проведением анализа. Смешивать реагенты из расчета на необходимое число реакций, включающее тестирование исследуемых и контрольных образцов, необходимо согласно **расчетной таблице** (см. табл. 7). Следует учитывать, что **для тестирования даже одного исследуемого образца ДНК необходимо проводить постановку всех контролей этапа ПЦР (положительного контроля (К+) и отрицательного контроля (К-) для каждого типа смеси).** Рекомендуется смешивать реагенты для четного числа реакций с целью более точного дозирования.
2. Отобрать необходимое количество пробирок с учетом

количества исследуемых и контрольных образцов ДНК. Тип пробирок, стрипов или планшета выбрать в зависимости от используемого прибора.

3. Для приготовления реакционных смесей необходимо в отдельной стерильной пробирке смешать одну из **ПЦР-смесей-1 (ПЦР-смесь-1-FEP/FRT *Shigella* spp./ *Salmonella* spp.,** или **ПЦР-смесь-1-FEP/FRT *Campylobacter* spp./STI)**, **ПЦР-смесь-2-FRT**, **полимеразу (TaqF)** (см. табл. 7). Тщательно перемешать смеси на вортексе и осадить капли с крышек пробирок.
4. Внести в отобранные пробирки по **15 мкл** готовых реакционных смесей.

Таблица 7

Схема приготовления реакционных смесей для ПЦР с детекцией в режиме «реального времени»

		Объем реактивов на указанное количество реакций		
Объем реагента на одну реакцию (мкл)		**10.00**	**5.00**	**0.50**
Число исследуемых образцов	**Число реакций[6]**	**ПЦР-смесь-1-FEP/FRT, мкл**	**ПЦР-смесь-2-FRT, мкл**	**Полимераза (TaqF), мкл**
2	**6**	**60**	**30**	**3.0**
4	**8**	**80**	**40**	**4.0**
6	**10**	**100**	**50**	**5.0**
8	**12**	**120**	**60**	**6.0**
10	**14**	**140**	**70**	**7.0**
12	**16**	**160**	**80**	**8.0**
14	**18**	**180**	**90**	**9.0**
16	**20**	**200**	**100**	**10.0**
18	**22**	**220**	**110**	**11.0**
20	**24**	**240**	**120**	**12.0**
22	**26**	**260**	**130**	**13.0**
24	**28**	**280**	**140**	**14.0**
26	**30**	**300**	**150**	**15.0**
28	**32**	**320**	**160**	**16.0**

5. Используя наконечники с фильтром, в пробирки с реакционной смесью добавить по **10 мкл ДНК-проб**, выделенных из исследуемых или контрольных проб этапа выделения ДНК. Неиспользованные остатки реакционной смеси выбросить.

[6] Число исследуемых образцов + контроль этапа выделения ДНК + 2 контроля этапа ПЦР + запас на один образец (N+1+2+1, где N-количество клинических образцов).

ВНИМАНИЕ! При добавлении ДНК-проб, выделенных с помощью комплекта реагентов «ДНК-сорб-В», необходимо избегать попадания сорбента в реакционную смесь для ПЦР.

6. Поставить **контрольные реакции амплификации:**
   а) **отрицательный контроль (К-)** – внести в пробирки с реакционной смесью **10 мкл ДНК-буфера;**
   б) **положительный контроль (К+ *Shigella/Salmonella*)** – внести в пробирки **10 мкл ПКО ДНК *Shigella sonnei* / *Salmonella*** для **ПЦР-смеси-1-FEP/FRT *Shigella* spp. / *Salmonella* spp;**
   в) **положительный контроль (К+ *Campylobacter*/STI)** – внести в пробирки **10 мкл ПКО ДНК *Campylobacter jejuni* / STI** для **ПЦР-смеси-1-FEP/FRT *Campylobacter* spp. / STI.**

Б. Проведение амплификации с детекцией в режиме «реального времени»

1. Запрограммировать прибор (амплификатор с системой детекции в режиме «реального времени») для выполнения соответствующей программы амплификации и детекции флуоресцентного сигнала (см. табл. 8, 9 и Методические Рекомендации по применению набора реагентов **«АмплиСенс® *Shigella* spp. и *EIEC* / *Salmonella* spp. / *Campylobacter* spp.-FL»**).

Таблица 8

Программа амплификации приборов роторного типа[7]

Этап	Температура, °С	Продолжительность этапа	Измерение флуоресценции	Количество циклов
1	95	15 мин	–	1
2	95	10 с	–	45
	60	25 с	FAM/Green, JOE/Yellow	
	72	10 с	–	

[7] Например, «RotorGene 3000» и «RotorGene 6000» («Corbett Research», Австралия)

Таблица 9

Программа амплификации для приборов планшетного типа[8]

Этап	Температура, °С	Продолжительность этапа	Измерение флуоресценции	Количество циклов
1	**95**	**15 мин**	–	**1**
2	**95**	**10 с**	–	**45**
	60	**25 с**	FAM, HEX	
	72	**10 с**	–	

Детекция флуоресцентного сигнала назначается по двум каналам - для флуорофоров FAM/Green и JOE/Yellow/HEX (при одновременном проведении других тестов назначается детекция и по другим используемым каналам).

2. Установить пробирки в ячейки реакционного модуля прибора.
3. Запустить выполнение программы амплификации с детекцией флуоресцентного сигнала.
4. По окончании выполнения программы приступить к анализу и учету результатов.

АНАЛИЗ И ИНТЕРПРЕТАЦИЯ РЕЗУЛЬТАТОВ

Анализ результатов поводят с помощью программного обеспечения используемого прибора для проведения ПЦР с детекцией в режиме «реального времени». Анализируют кривые накопления флуоресцентного сигнала по двум каналам FAM/Green и JOE/Yellow/HEX.

Результаты интерпретируются на основании наличия (или отсутствия) пересечения кривой флуоресценции с установленной на соответствующем уровне пороговой линией, что определяет наличие (или отсутствие) для данной пробы ДНК значения порогового цикла «*Ct*» в соответствующей графе в таблице результатов.

Результаты интерпретируются в соответствии с табл. 10.

[8] Например, «iQ5» («BioRad», США), «Mx3000P» («Cepheid», США)

Таблица 10

Интерпретация результатов ПЦР-исследования

Канал детекции	ПЦР-смесь-1 FEP/FRT *Shigella* spp. / *Salmonella* spp.	ПЦР-смесь-1-FEP/FRT *Campylobacter* / STI
FAM/Green	Определено значение меньше граничного – обнаружена ДНК *Shigella* spp.	Определено значение меньше граничного – обнаружена ДНК *Campylobacter* spp.
	Значение отсутствует или больше граничного – ДНК *Shigella* spp. не обнаружена[9]	Значение отсутствует или больше граничного – ДНК *Campylobacter* spp. не обнаружена[9]
JOE/ Yellow/HEX	Определено значение меньше граничного – обнаружена ДНК *Salmonella* spp.	Определено значение меньше граничного – результаты тестирования образца валидны
	Значение отсутствует или больше граничного – ДНК *Salmonella* spp. не обнаружена[9]	Значение отсутствует или больше граничного – результаты тестирования образца невалидны[10]

ВНИМАНИЕ! Граничные значения *Ct* указаны во вкладыше к ПЦР-комплекту.

Результат ПЦР-исследования считается достоверным, если получены правильные результаты для положительного и отрицательного контролей амплификации и отрицательного контроля выделения ДНК, в соответствии с таблицей оценки результатов контрольных реакций (табл. 11).

Таблица 11

Результаты для контролей различных этапов ПЦР-исследования

ПЦР-смесь-1	Контроль	Контроли-руемый этап	Канал FAM/Green	Канал JOE/Yellow/HEX
ПЦР-смесь-1-FEP/FRT *Shigella* spp. / *Salmonella* spp.	В-	Экстракция ДНК	Значение отсутствует или больше граничного	Значение отсутствует или больше граничного
	К-	ПЦР	Значение отсутствует или больше граничного	Значение отсутствует или больше граничного
	К+ *Shigella* / *Salmonella*	ПЦР	Определено значение меньше граничного	Определено значение меньше граничного

[9] При значении *Ct* по каналу JOE/ Yellow/HEX для ПЦР-смеси-1-FEP/FRT *Campylobacter* / STI меньше граничного.

[10] Если значение *Ct* по каналу JOE/ Yellow/HEX для ПЦР-смесь-1-FEP/FRT *Campylobacter* / STI отсутствует или больше граничного, то отрицательный результат анализа при использовании других ПЦР-смесей-1 считается невалидным и необходимо провести повторный ПЦР-анализ данного исследуемого образца, начиная с этапа выделения.

ВАРИАНТ FRT

ПЦР-смесь-1	Контроль	Контроли-руемый этап	Канал FAM/Green	Канал JOE/Yellow/HEX
ПЦР-смесь-1-FEP/FRT *Campylobacter* spp. / STI	B-	Экстракция ДНК	Значение отсутствует или больше граничного	Определено значение меньше граничного
	K-	ПЦР	Значение отсутствует или больше граничного	Значение отсутствует или больше граничного
	K+ *Campylobacter*/ STI	ПЦР	Определено значение меньше граничного	Определено значение меньше граничного

ВНИМАНИЕ!

1. Если для положительного контроля ПЦР (К+) сигнал по каналу JOE/Yellow/HEX и FAM/Green больше граничного значения, необходимо повторить амплификацию и детекцию для всех образцов, в которых сигнал по каналу JOE/Yellow/HEX и FAM/Green был больше граничного значения на соответствующем типе ПЦР-смеси-1.
2. Если для отрицательного контроля экстракции ДНК (В-) (кроме канала JOE/Yellow/HEX для ПЦР-смеси-1-FEP/FRT *Campylobacter* / STI) и/или отрицательного контроля ПЦР (К-) сигнал по каналу JOE/Yellow/HEX или FAM/Green меньше граничного значения, необходимо повторить ПЦР-исследование для всех образцов, в которых обнаружена ДНК соответствующих патогенов, начиная с этапа выделения (экстракции) ДНК.

СРОК ГОДНОСТИ. УСЛОВИЯ ТРАНСПОРТИРОВАНИЯ И ХРАНЕНИЯ

Срок годности. 9 мес. Набор реагентов с истекшим сроком годности применению не подлежит.

Транспортирование. Набор реагентов транспортировать при температуре от 2 до 8 °С не более 5 сут. «ПЦР-комплект» вариант FEP/FRT-50 F при получении разукомплектовать в соответствии с указанными температурами хранения.

Хранение. Набор реагентов хранить при температуре от 2 до 8 °С (кроме ПЦР-смеси-1 FEP/FRT *Shigella* spp. / *Salmonella* spp., ПЦР-смеси-1-FEP/FRT *Campylobacter* spp. / STI, ПЦР-смеси-2-FRT и полимеразы (TaqF)). ПЦР-смесь-1-FEP/FRT *Shigella* spp. / *Salmonella* spp., ПЦР-смесь-1-FEP/FRT *Campylobacter* spp. / STI, ПЦР-смесь-2-FRT и полимеразу (TaqF) хранить при температуре не выше минус 16 °С.

Условия отпуска. Для лечебно-профилактических и санитарно-профилактических учреждений.

Рекламации на качество набора реагентов **«АмплиСенс®** ***Shigella*** **spp. и** ***EIEC / Salmonella*** **spp. /** ***Campylobacter*** **spp.-FL»** направлять в адрес ФГУН Государственный научно-исследовательский институт стандартизации и контроля медицинских биологических препаратов им. Л.А. Тарасевича Роспотребнадзора (119002 г. Москва, пер. Сивцев Вражек, д. 41), тел./факс (499) 241-39-22, а также на предприятие-изготовитель ФГУН ЦНИИЭ Роспотребнадзора (111123 г. Москва, ул. Новогиреевская, д. 3а), тел. (495) 974-96-42, факс (495) 305-54-23 e-mail: obtk@pcr.ru) и в отдел по работе с рекламациями и организации обучения тел. (495) 925-05-54, факс (495) 916-18-18, e-mail: products@pcr.ru).

Заведующий НПЛ
ФГУН ЦНИИЭ Роспотребнадзора — Е.Н. Родионова

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«УТВЕРЖДАЮ»
Директор Федерального бюджетного учреждения науки «Центральный научно-исследовательский институт эпидемиологии» Федеральной службы по надзору в сфере защиты прав потребителей и благополучия человека
_____ В.И.Покровский
«15» февраля 2012 г.

«УТВЕРЖДАЮ»
Директор Федерального казенного учреждения здравоохранения Российского научно-исследовательского противочумного института «Микроб» Федеральной службы по надзору в сфере защиты прав потребителей и благополучия человека
_____ В.В. Кутырев
«15» февраля 2012 г.

ИНСТРУКЦИЯ

по применению набора реагентов
для выявления ДНК *Vibrio cholerae* и
идентификации патогенных штаммов *Vibrio cholerae* в
биологическом материале и объектах окружающей среды
методом полимеразной цепной реакции (ПЦР) с
гибридизационно-флуоресцентной детекцией

«АмплиСенс® *Vibrio cholerae*-FL»

АмплиСенс®

Федеральное бюджетное учреждение науки
«Центральный научно-исследовательский
институт эпидемиологии»,
Российская Федерация, 111123,
город Москва, улица Новогиреевская, дом 3а

IVD

ОГЛАВЛЕНИЕ

СПИСОК СОКРАЩЕНИЙ

В настоящей инструкции применяются следующие сокращения и обозначения:

ВК+	- положительный контроль амплификации образца ВКО
ВКО	- внутренний контрольный образец
ДНК	- дезоксирибонуклеиновая кислота
К–	- отрицательный контроль ПЦР
К+	- положительный контроль ПЦР
ОКО	- отрицательный контрольный образец
ОК	- отрицательный контроль экстракции
ПКО	- положительный контрольный образец
ПЦР	- полимеразная цепная реакция
ФБУН ЦНИИ Эпидемиологии Роспотребнадзора	- федеральное бюджетное учреждение науки «Центральный научно-исследовательский институт эпидемиологии» Федеральной службы по надзору в сфере защиты прав потребителей и благополучия человека
FRT	- флуоресцентная детекция в режиме «реального времени»

НАЗНАЧЕНИЕ

Набор реагентов **«АмплиСенс® *Vibrio cholerae*-FL»** предназначен для выявления ДНК *Vibrio cholerae* (по наличию последовательности hly), идентификации патогенных штаммов *Vibrio cholerae* (по наличию основных факторов вирулентности – ctxA, tcpA) и для определения принадлежности к серогруппам O1 (по наличию амплификации мишени wbeT) и O139 (по наличию амплификации мишени wbfR) в биологическом материале и объектах окружающей среды методом ПЦР с гибридизационно-флуоресцентной детекцией.

ВНИМАНИЕ! Результаты ПЦР-исследования учитываются в комплексной диагностике заболевания[1].

ПРИНЦИП МЕТОДА

Выявление *Vibrio cholerae* методом полимеразной цепной реакции (ПЦР) с гибридизационно-флуоресцентной детекцией включает в себя три этапа: экстракцию ДНК из образцов биологического материала и объектов окружающей среды, амплификацию участка ДНК *Vibrio cholerae* и гибридизационно-флуоресцентную детекцию, которая производится непосредственно в ходе ПЦР. Экстракция ДНК проводится в присутствии внутреннего контрольного образца (ВКО *Vibrio cholerae*), который позволяет контролировать выполнение процедуры исследования для каждого образца. Затем с полученными пробами проводится реакция амплификации

[1] В соответствии с Директивой Европейского Союза 98/79/EC.

участков ДНК *Vibrio cholerae* при помощи специфичных к этому участку ДНК праймеров и фермента Taq-полимеразы. В составе реакционной смеси присутствуют флуоресцентно-меченые олигонуклеотидные зонды, которые гибридизуются с комплементарными участками амплифицируемых ДНК-мишеней, в результате чего происходит нарастание интенсивности флуоресценции. Это позволяет регистрировать накопление специфических продуктов амплификации путем измерения интенсивности флуоресцентных сигналов. Детекция флуоресцентных сигналов происходит непосредственно в ходе ПЦР с помощью амплификатора с системой детекции флуоресцентного сигнала в режиме «реального времени».

Постановка реакций осуществляется в формате «мультиплекс» в двух пробирках, с использованием «горячего старта»: «Скрин» – амплификация мишеней ctxA (FAM), tcpA (ROX) и ВКО (JOE), «Тип» – амплификация мишеней hly (JOE) – холерные вибрионы всех серогрупп, wbeT (FAM) – принадлежность к серогруппе O1, wbfR (ROX) – принадлежность к серогруппе O139. Для интерпретации результатов необходима постановка обеих реакций «Скрин» и «Тип».

ФОРМАТЫ И ФОРМЫ ВЫПУСКА НАБОРА РЕАГЕНТОВ

Набор реагентов выпускается в 1 формате.

Формат FRT

Набор реагентов выпускается в 3 формах комплектации:

Форма 1 включает комплекты реагентов «ДНК-сорб-В» вариант 50, «ПЦР-комплект» вариант FRT.

Форма 2 включает комплект реагентов «ПЦР-комплект» вариант FRT.

Форма 3 включает наборы реагентов оптом, расфасованные по отдельным реагентам, с маркировкой реагентов на их оптовой фасовке.

Форма комплектации 1 предназначена для проведения полного ПЦР-исследования, включающего экстракцию ДНК из биологического материала или объектов окружающей среды и амплификацию ДНК *Vibrio cholerae* с гибридизационно-флуоресцентной детекцией в режиме «реального времени».

Форма комплектации 2 предназначена для проведения амплификации ДНК *Vibrio cholerae* с гибридизационно-флуоресцентной детекцией в режиме «реального времени». Для проведения полного ПЦР-исследования необходимо

использовать комплекты реагентов для экстракции РНК/ДНК, рекомендованные ФБУН ЦНИИ Эпидемиологии Роспотребнадзора.

Форма комплектации 3 предназначена для производственных целей для последующей маркировки на языке заказчика и комплектации по наборам.

ВНИМАНИЕ! Форма комплектации 3 используется только в соответствии с регламентом, утвержденным ФБУН ЦНИИ Эпидемиологии Роспотребнадзора.

АНАЛИТИЧЕСКИЕ ХАРАКТЕРИСТИКИ

Аналитическая чувствительность

Вид клинического материала	Комплект для экстракции РНК/ДНК	Аналитическая чувствительность
Фекалии нативные	«ДНК-сорб-В» – **для всех типов материала,** «РИБО-преп» - для фекалий водянистой консистенции	1×10^3 ГЭ/мл[2] 1×10^3 м.к./мл[3]
Мазки со слизистой прямой кишки		
Рвотные массы		
Секционный материал		
Вода после предварительной фильтрации		
Смывы с объектов окружающей среды		
Пептонная вода после посева биологического материала или пищевых продуктов		
Культуры микроорганизмов		

Примечание – Данная чувствительность достигается при соблюдении нижеизложенных правил подготовки исследуемого материала при следовании данной инструкции.

Аналитическая специфичность

Специфическая активность набора реагентов доказана при исследовании штаммов *V.cholerae*: Р-1, КМ-569, 10588, КМ 26, М045, 17 полевых изолятов *V.cholerae* О1 серогруппы, выделенных в 1991, 1994 и 1999 годах, 15 полевых изолятов *V.cholerae* других серогрупп, выделенных в 2000, 2001 и 2002 годах (из коллекции Противочумной станции Украины), 42 изолята, выделенных от людей и из объектов окружающей среды за 1965-2004 гг из Государственной коллекции патогенных бактерий ФГУЗ РосНИПЧИ «Микроб».

[2] Чувствительность выражается в геномных эквивалентах (ГЭ) возбудителя в 1 мл пробы.
[3] Чувствительность выражается в микробных клетках (м.к.) возбудителя в 1 мл пробы.

Отсутствие перекрестной реакции при определении принадлежности к серогруппе О1 и О139 доказано при тестировании штаммов *V.cholerae*, относящихся к различным серогруппам: О2-О9, О11-О14, О16-О33, О35, О36, О39-О63, О65-О69, О71, О73-О75, О77, О79-О82 из Государственной коллекции патогенных бактерий (РосНИПЧИ «Микроб»).

Показано отсутствие неспецифических реакций компонентов набора в отношении ДНК близкородственных микроорганизмов, представителей нормальной микрофлоры и ряда других возбудителей кишечных инфекций: *Vibrio parahaemolyticus*, *Vibrio alginolyticus*, *Vibrio anguillarum*, *Vibrio mimicus*, *Vibrio splendidus*, *Vibrio fluvialis*, *Vibrio proteolyticus*, *Escherichia coli*, *Salmonella enteritidis*, *Salmonella typhi, Shigella flexneri*, *Shigella sonnei*, *Campylobacter fetus*, *Campylobacter jejuni*, *Klebsiella pneumonia*, *Listeria monocytogenes*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Morganella morganii*, *Enterobacter faecalis*, *Aeromonas*, *Plesiomonas shidel*, *Commomonas*, а также кДНК/ДНК человека.

При исследовании 100 образцов фекалий людей без энтеритов и 50 образцов фекалий людей с энтеритами различной бактериальной и вирусной этиологии ложноположительных результатов не выявлено.

МЕРЫ ПРЕДОСТОРОЖНОСТИ

Работа с исследуемым материалом, подозрительным на зараженность микроорганизмами I–II групп патогенности, должна проводиться с соблюдением санитарно-эпидемиологических правил СП 1.3.1285-03 «Безопасность работы с микроорганизмами I–II групп патогенности (опасности)», утвержденными Главным государственным санитарным врачом Российской Федерации – Первым заместителем Министра здравоохранения Российской Федерации Г.Г. Онищенко 12 марта 2003 г., СанПиН 2.1.7.2790-10 «Санитарно-эпидемиологические требования к обращению с медицинскими отходами» и методических указаний МУ 1.3. 2569-09 «Организация работы лабораторий, использующих методы амплификации нуклеиновых кислот при работе с материалом, содержащим микроорганизмы I–IV групп патогенности».

При работе всегда следует выполнять следующие требования:

- Следует рассматривать исследуемые образцы как

инфекционно-опасные, организовывать работу и хранение в соответствии с СП 1.3.1285-03 «Безопасность работы с микроорганизмами I–II групп патогенности (опасности)».
- Убирать и дезинфицировать разлитые образцы или реактивы, используя дезинфицирующие средства в соответствии СП 1.3.1285-03 «Безопасность работы с микроорганизмами I–II групп патогенности (опасности)».
- Лабораторный процесс должен быть однонаправленным. Анализ проводится в отдельных помещениях (зонах). Работу следует начинать в Зоне Выделения, продолжать в Зоне Амплификации и Детекции. Не возвращать образцы, оборудование и реактивы в зону, в которой была проведена предыдущая стадия процесса.
- Удалять неиспользованные реактивы в соответствии с СанПиН 2.1.7.2790-10 «Санитарно-эпидемиологические требования к обращению с медицинскими отходами».

ВНИМАНИЕ! При удалении отходов после амплификации (пробирок, содержащих продукты ПЦР), недопустимо открывание пробирок и разбрызгивание содержимого, поскольку это может привести к контаминации продуктами ПЦР лабораторной зоны, оборудования и реагентов.

- Применять набор строго по назначению, согласно данной инструкции.
- Допускать к работе с набором только специально обученный персонал.
- Не использовать набор по истечении срока годности.
- Использовать одноразовые перчатки, лабораторные халаты, защищать глаза во время работы с образцами и реактивами. Тщательно вымыть руки по окончании работы.
- Избегать контакта с кожей, глазами и слизистой оболочкой. При контакте немедленно промыть пораженное место водой и обратиться за медицинской помощью.
- Листы безопасности материалов (MSDS – material safety data sheet) доступны по запросу.

ДОПОЛНИТЕЛЬНЫЕ МАТЕРИАЛЫ И ОБОРУДОВАНИЕ

1. Мертиолят натрия, 0,1 % раствор. Для приготовления 0,1 % раствора мертиолята натрия 0,1 г мертиолята растворяют в 100 мл стерильного 0,9 % раствора хлорида натрия. Полученный 0,1 % раствор мертиолята хранят во флаконе из темного стекла не более 3 месяцев при температуре от 2

до 8 °С.

2. Комплект реагентов для выделения ДНК «ДНК-сорб-В» (ТУ 9398-003-01897593-2008) или комплект реагентов для выделения ДНК/РНК «РИБО-преп» (ТУ 9398-071-01897593-2008) при работе с формой комплектации 2.
3. Дополнительные материалы и оборудование для экстракции ДНК – согласно инструкции к комплекту реагентов для выделения ДНК.
4. Бокс абактериальной воздушной среды (ПЦР-бокс).
5. Центрифуга/вортекс.
6. Автоматические дозаторы переменного объема (от 5 до 20 мкл и от 20 до 200 мкл).
7. Одноразовые наконечники с фильтром на 100 и 200 мкл в штативах.
8. Штативы для пробирок объемом 0,2 мл.
9. Холодильник от 2 до 8 °С с морозильной камерой не выше минус 16 °С для выделенных проб ДНК.
10. Отдельный халат, шапочки, обувь и одноразовые перчатки по МУ 1.3.2569-09.
11. Емкость для сброса наконечников.
12. Одноразовые полипропиленовые тонкостенные пробирки для ПЦР объемом 0,2 мл с плоской крышкой (например, Axygen, США).
13. Программируемый амплификатор с системой детекции флуоресцентного сигнала в режиме «реального времени» (например, Rotor-Gene 3000/6000 (Corbett Research, Австралия), Rotor-Gene Q (Qiagen, Германия) и рекомендованные ФБУН ЦНИИ Эпидемиологии Роспотребнадзора в методических рекомендациях по применению данного набора реагентов).

ВЗЯТИЕ, ТРАНСПОРТИРОВАНИЕ И ХРАНЕНИЕ ИССЛЕДУЕМОГО МАТЕРИАЛА

Отбор материала для исследования производится в соответствии с методическими указаниями МУК 4.2.2218-07 «Лабораторная диагностика холеры». Утверждены Главным государственным санитарным врачом Российской Федерации 31 мая 2007 г. Введены 1 августа 2007 г.

ИССЛЕДУЕМЫЙ МАТЕРИАЛ

Образцы клинического материала:

- фекалии нативные 1,0–2,0 г (или 1-2 мл при наличии диареи) или помещённые в пробирку с 5 мл 1 % пептонной воды используются после предварительной подготовки;
- рвотные массы (1-2 мл) нативные или помещённые в 5 мл 1 % пептонной воды используются после предварительной подготовки;
- мазок содержимого прямой кишки с глубины 5-6 см, взятый ректальным ватным тампоном (ректальной металлической петлей), помещается в пробирку объёмом 1,5 мл с 0,5 мл 1 % пептонной воды, тщательно взбалтывается, ватный тампон отжимается о стенки пробирки и удаляется в емкость с дезраствором. Для исследования используется 50 мкл раствора.

Образцы секционного материала:

- содержимое верхней, средней и нижней частей тонкой кишки по 0,5 мл помещают в пустые бактериологические пробирки (исследуют как нативный материал фекалий) и в пробирки с 5 мл 1 % пептонной воды (исследуют как подрощенный материал).

Образцы из окружающей среды (с целью мониторинга):

- вода (сточная, из водоема, питьевая, объемом 1 л) отбирается и подвергается обработке по МУК 4.2.2218-07. Для исследования используется первая пептонная вода (после предварительной подготовки);
- ил, гидробионты отбираются и подвергаются обработке по МУК 4.2.2218-07. Для исследования используется первая пептонная вода (после предварительной подготовки).

Образцы из окружающей среды в очаге:

- вода (сточная, из водоема, питьевая) отбирается по МУК 4.2.2218-07, подвергается предварительному фильтрованию через фильтры с диаметром пор 8 мкм (или бумажные фильтры) и окончательному фильтрованию с использованием фильтров с диаметром пор 0,45 мкм. Фильтры измельчают и помещают в стерильные пробирки объемом 10-15 мл с 5 мл 0,9 % раствора хлорида натрия, встряхивают в течение 10 минут с помощью шейкера. Для исследования методом ПЦР отбирают 1,0 мл в пробирки с завинчивающейся крышкой объемом 1,5 мл и центрифугируют при 12 тыс об/мин в течение 10 мин.

Осадок ресуспендируют в 100 мкл 0,9 % раствора хлорида натрия.

В случае получения отрицательного результата анализа необходимо провести посев смывов с фильтров по МУК 4.2.2218-07 и тестирование первой пептонной воды (после предварительной обработки);

- смывы с поверхностей предметов (площадью 10 х 10 см), взятые стерильным зондом, смоченным физиологическим раствором (рабочая часть зонда с тампоном помещается в пробирку объёмом 1,5 мл с 0,5 мл 1 % пептонной воды, остальная часть зонда отламывается и удаляется). Для исследования используется 50 мкл раствора без предварительной подготовки.

Пищевые продукты отбираются и подвергаются обработке по МУК 4.2.2218-07. Для исследования используется первая пептонная вода (после предварительной подготовки).

Культуры микроорганизмов, подозрительные на *Vibrio cholerae*:

- колонию ресуспендировать в 0,5 мл физиологического раствора или фосфатно-буферной смеси. Для исследования использовать 50 мкл суспензии.

Допускается хранение и транспортирование в лабораторию для проведения исследования вышеперечисленного материала: в течение 2 ч при температуре окружающей среды, в течение 1 сут – при температуре от 2 до 8 °С и длительно – при температуре не выше минус 16 °С. Допускается однократное замораживание – оттаивание материала.

Все работы по транспортированию проб исследуемого материала осуществляют в строгом соответствии с требованиями СП 1.2.036-95 «Порядок учета, хранения, передачи и транспортирования микроорганизмов I–IV групп патогенности».

ПОДГОТОВКА ИССЛЕДУЕМОГО МАТЕРИАЛА К ЭКСТРАКЦИИ ДНК

Нативные фекалии:

А. Приготовление 10-20 % фекальной суспензии (фекалии водянистой консистенции используются без приготовления суспензии).

1. В пробирки на 5 мл с плотно закрывающейся (завинчивающейся) крышкой, внести по 4 мл физиологического раствора или фосфатно-буферной смеси.
2. В каждую пробирку отдельными наконечниками с фильтрами (или одноразовыми лопатками) внести по 0,5–1,0 г (около 0,5–1,0 мл) фекалий и тщательно перемешать содержимое до образования гомогенной суспензии. При необходимости хранения к суспензии добавляют глицерин до концентрации 20 %, перемешивают и хранят при температуре **не выше минус 16 °С**.

Б.1. Приготовление бактериальной фракции фекалий (для фекалий плотной консистенции):

Из пробирок с фекальной суспензией перенести 1 мл суспензии в пробирки на 1,5 мл с плотно закрывающейся крышкой и центрифугировать на микроцентрифуге 5 мин при 12 тыс об/мин. Для экстракции ДНК использовать 50 мкл светлой фракции, находящейся на границе жидкой прозрачной и твердой фракций фекалий.

Б.2. Приготовление бактериального осадка фекалий (для фекалий водянистой консистенции):

Из пробирок с фекальной суспензией перенести 1 мл суспензии в пробирки на 1,5 мл с плотно закрывающейся крышкой и центрифугировать на микроцентрифуге 5 мин при 12 тыс об/мин. Удалить часть надосадочной жидкости, используя вакуумный отсасыватель и отдельный наконечник для каждой пробы, оставить 100-150 мкл жидкости над осадком. Тщательно ресуспендировать осадок в оставшемся объеме и далее использовать полученную суспензию для экстракции ДНК.

Фекалии или рвотные массы, помещённые в 1 % пептонную воду:

А. Тщательно перемешать содержимое пробирок до

образования гомогенной суспензии.

Б. Приготовление бактериальной фракции:

1 мл суспензии перенести в пробирки на 1,5 мл с плотно закрывающейся крышкой и центрифугировать на микроцентрифуге 5 мин при 12 тыс об/мин. Для экстракции ДНК использовать 50 мкл светлой фракции, находящейся на границе жидкой прозрачной и твердой тёмной фракций.

Образцы секционного материала (содержимое тонкой кишки)**:**

Тщательно перемешать содержимое пробирок до образования гомогенной суспензии. Для экстракции ДНК использовать 50 мкл суспензии.

Первичная или вторичная среда накопления (после подращивания):

С поверхности пептонной воды отобрать 1,0 мл в пробирку объёмом 1,5 мл и центрифугировать в течение 10 мин при 12 тыс об/мин. Удалить надосадочную жидкость с помощью пипетки, используя наконечники с фильтром. Осадок ресуспендируют в 300 мкл физиологического раствора или фосфатно-буферной смеси. Для исследования используют 50 мкл раствора.

ФОРМАТ FRT
СОСТАВ

Комплект реагентов «ДНК-сорб-В» вариант 50 – комплект реагентов для выделения ДНК из клинического материала **– включает:**

Реактив	*Описание*	*Объем, мл*	*Кол-во*
Лизирующий раствор	Прозрачная бесцветная жидкость[4]	15	1 флакон
Раствор для отмывки 1	Прозрачная бесцветная жидкость	15	1 флакон
Раствор для отмывки 2	Прозрачная бесцветная жидкость	50	1 флакон
Сорбент универсальный	Суспензия белого цвета	1,25	1 пробирка
ТЕ-буфер для элюции ДНК	Прозрачная бесцветная жидкость	5,0	1 пробирка

Комплект реагентов рассчитан на выделение ДНК из 50 образцов, включая контроли. Входит в состав формы комплектации 1.

Комплект реагентов «ПЦР-комплект» вариант FRT – комплект реагентов для амплификации фрагмента ДНК *Vibrio cholerae* и идентификации патогенных штаммов *Vibrio cholerae* с гибридизационно-флуоресцентной детекцией в режиме «реального времени» **– включает:**

Реактив	*Описание*	*Объем, мл*	*Кол-во*
ПЦР-смесь-1-FRT *Vibrio cholerae* скрин раскапана под воск	Прозрачная бесцветная жидкость	0,008	55 пробирок объемом 0,2 мл
ПЦР-смесь-1-FRT *Vibrio cholerae* тип раскапана под воск	Прозрачная бесцветная жидкость	0,008	55 пробирок объемом 0,2 мл
ПЦР-смесь-2-FL	Прозрачная бесцветная жидкость	0,77	1 пробирка
ПКО ДНК *Vibrio cholerae* скрин	Прозрачная бесцветная жидкость	0,1	1 пробирка
ПКО ДНК *Vibrio cholerae* тип	Прозрачная бесцветная жидкость	0,1	1 пробирка
ПКО ВК	Прозрачная бесцветная жидкость	0,1	1 пробирка
ДНК-буфер	Прозрачная бесцветная жидкость	0,5	1 пробирка

Комплект реагентов рассчитан на проведение 55 реакций амплификации, включая контроли.

[4] При хранении лизирующего раствора при температуре от 2 до 8 °С возможно образование осадка в виде кристаллов.

К комплекту реагентов прилагаются контрольные образцы этапа экстракции:

Реактив	*Описание*	*Объем, мл*	*Кол-во*
ОКО	Прозрачная бесцветная жидкость	1,6	2 пробирки
ВКО *Vibrio cholerae*	Прозрачная бесцветная жидкость	0,5	1 пробирка

ПРОВЕДЕНИЕ ПЦР-ИССЛЕДОВАНИЯ

ПЦР-исследование состоит из следующих этапов:

- Экстракция ДНК из исследуемых образцов.
- Проведение амплификации с гибридизационно-флуоресцентной детекцией в режиме «реального времени».
- Анализ и интерпретация результатов.

Детальная информация по процедуре проведения ПЦР-исследования изложена в методических рекомендациях к инструкции **«АмплиСенс® *Vibrio cholerae*-FL»**.

ОБЕЗЗАРАЖИВАНИЕ ОБРАЗЦОВ

Проводится в соответствии с МУ 1.3.2569-09 «Организация работы лабораторий, использующих методы амплификации нуклеиновых кислот при работе с материалом, содержащим микроорганизмы I–IV групп патогенности». К подготовленным образцам добавляют натрия мертиолят до концентрации 1:10000 (0,01 %) с последующим прогреванием их при (56±1) °С в течение 30 минут. Далее необходимое количество материала добавляют в лизирующий раствор, входящий в комплект реагентов «ДНК-сорб-В» (порядок работы см. в приложении 1) или в раствор для лизиса, входящий в комплект реагентов «РИБО-преп» (порядок работы см. в приложении 2). Материал считается обеззараженным после выполнения этапа инкубации при температуре 65 °С в течение 15 минут.

ЭКСТРАКЦИЯ ДНК ИЗ ИССЛЕДУЕМЫХ ОБРАЗЦОВ

Для экстракции ДНК используются наборы реагентов, рекомендованные ФБУН ЦНИИ Эпидемиологии Роспотребнадзора, в соответствии с инструкцией к используемому набору. Экстракция ДНК из каждого клинического образца проводится в присутствии внутреннего контрольного образца – ВКО *Vibrio cholerae* (ВКО).

В работе с формой комплектации набора 1 для экстракции ДНК используется входящий в набор комплект реагентов «ДНК-сорб-В» (порядок работы см. в приложении 1).

Для фекалий водянистой консистенции после их предварительной подготовки рекомендуется использовать комплект реагентов «РИБО-преп» (порядок работы см. в приложении 2).

ПРОВЕДЕНИЕ АМПЛИФИКАЦИИ С ДЕТЕКЦИЕЙ В РЕЖИМЕ «РЕАЛЬНОГО ВРЕМЕНИ»

А. Подготовка пробирок для амплификации

Для внесения в пробирки реагентов, проб ДНК и контрольных образцов используются одноразовые наконечники с фильтрами.

Общий объем реакционной смеси – 25 мкл, включая объем пробы ДНК – 10 мкл.

1. Отобрать необходимое количество пробирок с **ПЦР-смесью-1-FRT *Vibrio cholerae* скрин** и **ПЦР-смесью-1-FRT *Vibrio cholerae* тип** для амплификации ДНК исследуемых и контрольных проб. Пробирки промаркировать – «С» и «Т».
2. На поверхность воска внести по **7 мкл ПЦР-смеси-2-FL**, при этом она не должна проваливаться под воск и смешиваться с **ПЦР-смесью-1-FRT**.
3. В подготовленные пробирки внести по **10 мкл проб ДНК**, полученных в результате экстракции из исследуемых или контрольных образцов. **Необходимо избегать попадания сорбента в реакционную смесь (в случае использования сорбентной методики экстракции ДНК)**.
4. Поставить контрольные реакции:
   а) **отрицательный контроль ПЦР (К–)** – вместо ДНК-пробы внести в пробирку **10 мкл ДНК-буфера**.
   б) **положительный контроль (К+$_{скрин}$)** – в подготовленную для ПЦР пробирку с **ПЦР-смесью-1-FRT *Vibrio cholerae* скрин** внести **10 мкл ПКО ДНК *Vibrio cholerae* скрин.**
   в) **положительный контроль (К+$_{тип}$)** – в подготовленную для ПЦР пробирку с **ПЦР-смесью-1-FRT *Vibrio cholerae* тип** внести **10 мкл ПКО ДНК *Vibrio cholerae* тип**.
   г) **положительный контроль (ВК+)** – в подготовленную для ПЦР пробирку с **ПЦР-смесью-1-FRT *Vibrio cholerae* скрин** внести **10 мкл ПКО ВК**.

Б. Проведение амплификации с детекцией в режиме «реального времени»

1. Запрограммировать прибор (амплификатор с системой детекции в режиме «реального времени») для выполнения соответствующей программы амплификации и детекции флуоресцентного сигнала (см. табл. 1).

Таблица 1

	Приборы роторного типа[5]		
Цикл	Температура, °С	Время	Кол-во циклов
1	**95**	5 мин	1
2	**95**	10 с	10
	60	25 с	
	72	10 с	
3	**95**	10 с	35
	56	25 с детекция флуоресц. сигнала	
	72	10 с	

Детекция флуоресцентного сигнала назначается по каналам для флуорофоров FAM, JOE, ROX.

2. Установить пробирки в ячейки реакционного модуля прибора. **Лунка №1 обязательно должна быть заполнена какой-либо исследуемой пробиркой.**

ВНИМАНИЕ! Если проводится одновременная постановка «Скрин» и «Тип», калибровку необходимо проводить по пробирке «К-» с **ПЦР-смесью-1-FRT *Vibrio cholerae* скрин**, то есть поместить её в 1-ю позицию ротора.

3. Запустить выполнение программы амплификации с детекцией флуоресцентного сигнала.
4. По окончании выполнения программы приступить к анализу и интерпретации результатов.

АНАЛИЗ И ИНТЕРПРЕТАЦИЯ РЕЗУЛЬТАТОВ

Анализ результатов проводят с помощью программного обеспечения используемого прибора для проведения ПЦР с детекцией в режиме «реального времени». Более подробный порядок проведения обработки и интерпретации полученных результатов описан в методических рекомендациях по применению набора реагентов **«АмплиСенс® *Vibrio cholerae*-FL».**

[5] Например, Rotor-Gene 3000, Rotor-Gene 6000 (Corbett Research, Австралия); Rotor-Gene Q (Qiagen, Германия) и рекомендованные ФБУН ЦНИИ Эпидемиологии Роспотребнадзора в методических рекомендациях по применению данного набора реагентов).

ВНИМАНИЕ! Анализ данных для каждой ПЦР-смеси-1 следует проводить индивидуально, выделив область пробирок, относящихся к данной ПЦР-смеси-1.

Анализ результатов амплификации с ПЦР-смесью-1-FRT *Vibrio cholerae* скрин:

Анализируют кривые накопления флуоресцентного сигнала по трем каналам:

- По каналу для флуорофора FAM регистрируется сигнал, свидетельствующий о накоплении продукта амплификации фрагмента ДНК гена ctxA;
- По каналу для флуорофора JOE регистрируется сигнал, свидетельствующий о накоплении продукта амплификации фрагмента ДНК ВКО *Vibrio cholerae* (ВКО);
- По каналу для флуорофора ROX регистрируется сигнал, свидетельствующий о накоплении продукта амплификации фрагмента ДНК гена tcpA.

Анализ результатов амплификации с ПЦР-смесью-1-FRT *Vibrio cholerae* тип:

Анализируют кривые накопления флуоресцентного сигнала по трем каналам:

- По каналу для флуорофора FAM регистрируется сигнал, свидетельствующий о накоплении продукта амплификации фрагмента ДНК гена wbeT (принадлежность к серогруппе O1),
- По каналу для флуорофора JOE регистрируется сигнал, свидетельствующий о накоплении продукта амплификации фрагмента ДНК гена hly (холерные вибрионы всех серогрупп),
- По каналу для флуорофора ROX регистрируется сигнал, свидетельствующий о накоплении продукта амплификации фрагмента ДНК гена wbfR (принадлежность к серогруппе O139).

Результаты интерпретируются на основании наличия (или отсутствия) пересечения кривой флуоресценции с установленной на соответствующем уровне пороговой линией, что определяет наличие (или отсутствие) для данной пробы ДНК значения порогового цикла *Ct* в соответствующей графе в таблице результатов.

Принцип интерпретации результатов следующий:

1. **Образец считается положительным** по искомой мишени, если в таблице результатов пороговых циклов по соответствующему каналу для флуорофора, например, FAM («*Quant. Results – Cycling A. FAM/Green*»), для него определено значение *Ct*, не превышающее граничного значения.
2. **Образец считается отрицательным** по искомой мишени, если в таблице пороговых циклов по соответствующему каналу для него не указывается значение *Ct* (кривая флуоресценции не пересекает пороговую линию – **Threshold**).
3. Образцы с ПЦР-смесью-1-FRT *Vibrio cholerae* скрин для которых отсутствуют значения *Ct* по каналам для флуорофоров FAM и ROX, а также отсутствует значение *Ct* (или получено значение *Ct* более граничного значения) по каналу для флуорофора JOE, требуют повторного проведения этапов экстракции ДНК и ПЦР.
4. Результаты тестирования образцов, для которых получен положительный результат по любой мишени, кроме hly (отрицательный результат по каналу для флуорофора JOE с ПЦР-смесью-1-FRT *Vibrio cholerae* тип) и получено значение *Ct* менее граничного по каналу для флуорофора JOE с ПЦР-смесью-1-FRT *Vibrio cholerae* скрин, считать невалидными. Требуются повторные забор материала и исследование.
5. Результаты тестирования образцов с ПЦР-смесью-1-FRT *Vibrio cholerae* тип, для которых отсутствует значение *Ct* по каналу для флуорофора JOE, и выполняются условия пункта 3, считаются невалидными и требуют повторного проведения экстракции ДНК и ПЦР.

ВНИМАНИЕ! Граничные значения *Ct* указаны во вкладыше, **прилагаемом к набору реагентов**. См. также методические рекомендации по применению набора реагентов «АмплиСенс® *Vibrio cholerae*-FL».

Результат ПЦР-исследования считается достоверным, если получены правильные результаты для положительного и отрицательного контролей амплификации и отрицательного контроля экстракции ДНК, в соответствии с таблицами оценки результатов контрольных реакций (табл. 2, 3).

Таблица 2

Результаты для контролей различных этапов ПЦР-исследования с ПЦР-смесью-1-FRT *Vibrio cholerae* скрин

Контроль	Контролируемый этап ПЦР-исследования	Значение порогового цикла *Ct* по каналу для флуорофора		
		FAM (ctxA)	JOE (ВКО)	ROX (tcpA)
ОК	Экстракция ДНК	Нет значений	< граничного значения	Нет значений
К–	ПЦР	Нет значений	Нет значений	Нет значений
$К+_{скрин}$	ПЦР	< граничного значения	Нет значений	< граничного значения
ВК+	ПЦР	Нет значений	< граничного значения	Нет значений

Таблица 3

Результаты для контролей различных этапов ПЦР-исследования с ПЦР-смесью-1-FRT *Vibrio cholerae* тип

Контроль	Контролируемый этап ПЦР-исследования	Значение порогового цикла *Ct* по каналу для флуорофора		
		FAM (O1)	JOE (*V.cholerae*)	ROX (O139)
ОК	Экстракция ДНК	Нет значений	Нет значений	Нет значений
К-	ПЦР	Нет значений	Нет значений	Нет значений
$К+_{тип}$	ПЦР	< граничного значения	< граничного значения	< граничного значения

Результаты интерпретируются в соответствии с табл. 4, методическими рекомендациями по применению набора реагентов и вкладышем к набору реагентов **«АмплиСенс® *Vibrio cholerae*-FL».**

Таблица 4

Интерпретация результатов ПЦР-исследования

Варианты	ПЦР-смесь-1-FRT *Vibrio cholerae* скрин			ПЦР-смесь-1-FRT *Vibrio cholerae* тип		
	Значение порогового *Ct* цикла по каналу					
	FAM (ctxA)	JOE (ВКО)	ROX (tcpA)	FAM (O1)	JOE (*V.cholerae*)	ROX (O139)
V.cholerae O1 токсигенный	< граничного значения	Любое значение или отсутствие	< граничного значения	< граничного значения	< граничного значения	Нет значений
V.cholerae O139 токсигенный	< граничного значения	Любое значение или отсутствие	< граничного значения	Нет значений	< граничного значения	< граничного значения
V.cholerae O1 НЕ токсигенный, но содержащий последовательность **tcpA**	Нет значений	< граничного значения	< граничного значения	< граничного значения	< граничного значения	Нет значений
V.cholerae O139 НЕ токсигенный, но содержащий последователь-ность **tcpA**	Нет значений	< граничного значения	< граничного значения	Нет значений	< граничного значения	< граничного значения
V.cholerae O1 НЕ токсигенный	Нет значений	< граничного значения	Нет значений	< граничного значения	< граничного значения	Нет значений
V.cholerae O139 НЕ токсигенный	Нет значений	< граничного значения	Нет значений	Нет значений	< граничного значения	< граничного значения
V.cholerae НЕ O1 и НЕ O139	Нет значений	< граничного значения	Нет значений	Нет значений	< граничного значения	Нет значений
Холерные вибрионы НЕ обнаружены	Нет значений	< граничного значения	Нет значений	Нет значений	Нет значений	Нет значений

ВНИМАНИЕ!

1. Появление любого значения *Ct* в таблице результатов для отрицательного контрольного образца этапа экстракции (на каналах для флуорофоров FAM и/или ROX – для ПЦР-смеси-1-FRT *Vibrio cholerae* скрин и/или на любом из каналов – для ПЦР-смеси-1-FRT *Vibrio cholerae* тип) и для отрицательного контроля ПЦР (ДНК-буфер) (на любом из каналов) свидетельствует о наличии контаминации реактивов или образцов. В этом случае результаты анализа положительных по данному каналу проб считаются недействительными. Требуется повторить анализ всех

положительных по данному каналу проб, а также предпринять меры по выявлению и ликвидации источника контаминации.

2. Отсутствие положительного сигнала в пробах с положительными контролями ПЦР может свидетельствовать о неправильно выбранной программе амплификации и о других ошибках, допущенных на этапе постановки ПЦР. В таком случае необходимо провести ПЦР повторно для всех отрицательных проб.

СРОК ГОДНОСТИ. УСЛОВИЯ ТРАНСПОРТИРОВАНИЯ И ХРАНЕНИЯ

Срок годности. 9 мес. Набор реагентов с истекшим сроком годности применению не подлежит. Срок годности вскрытых реагентов соответствует сроку годности, указанному на этикетках для невскрытых реагентов, если в инструкции не указано иное.

Транспортирование. Набор реагентов транспортировать при температуре от 2 до 8 °С не более 5 сут.

Хранение. Комплект реагентов «ДНК-сорб-В» хранить при температуре от 2 до 25 °С. Комплект реагентов «ПЦР-комплект» вариант FRT хранить при температуре от 2 до 8 °С. ПЦР-смесь-1-FRT *Vibrio cholerae* скрин и ПЦР-смесь-1-FRT *Vibrio cholerae* тип хранить в защищенном от света месте.

Условия отпуска. Для лечебно-профилактических и санитарно-профилактических учреждений.

Рекламации на качество набора реагентов **«АмплиСенс® *Vibrio cholerae*-FL»** направлять на предприятие-изготовитель ФБУН ЦНИИ Эпидемиологии Роспотребнадзора (111123 г. Москва, ул. Новогиреевская, д. 3а) в отдел по работе с рекламациями и организации обучения (тел. (495) 974-96-46, факс (495) 916-18-18, e-mail: products@pcr.ru)[6].

Заведующий НПЛ ОМДиЭ ФБУН ЦНИИ Эпидемиологии Роспотребнадзора — Е.Н. Родионова

Главный врач ФГБУ «Поликлиника № 1» Управления делами Президента Российской Федерации — Е.Л. Никонов

[6] Отзывы и предложения о продукции «АмплиСенс» вы можете оставить, заполнив анкету потребителя на сайте: www.amplisens.ru.

ПРИЛОЖЕНИЕ 1. Экстракция ДНК из проб при использовании комплекта реагентов «ДНК-сорб-В» (проводится в ЗОНЕ 1 – помещении для обработки исследуемого материала).
Объем пробы, необходимый для экстракции ДНК – 0,05 мл.

Порядок работы.

1. **Лизирующий раствор** (если он хранился при температуре от 2 до 8 °С) прогреть при температуре 65 °С до полного растворения кристаллов.
2. Отобрать необходимое количество одноразовых пробирок (включая отрицательный контроль экстракции). Внести в каждую пробирку по **300 мкл лизирующего раствора**. Промаркировать пробирки.
3. В пробирки с **лизирующим раствором** внести по **50 мкл ОКО** и **50 мкл проб** (после обработки мертиолятом натрия в соответствии с разделом «Обеззараживание образцов»), используя наконечники с фильтром. В пробирку отрицательного контроля (ОК) экстракции внести **100 мкл ОКО**.
4. Пробы тщательно перемешать на вортексе, процентрифугировать в течение 5 с на микроцентрифуге для удаления капель с внутренней поверхности крышки пробирки и прогреть 15 мин при температуре 65 °С.
5. Внести в каждую пробирку по **10 мкл ВКО *Vibrio cholerae***, перемешать и инкубировать 5 минут при температуре 65 °С.
6. Процентрифугировать пробирку 5 мин на микроцентрифуге при 8-10 тыс g (10-13 тыс об/мин при радиусе ротора 70 мм) и использовать для экстракции ДНК надосадочную жидкость, перенеся ее в новую пробирку.
7. Тщательно ресуспендировать **сорбент универсальный** на вортексе. В каждую пробирку отдельным наконечником добавить по **25 мкл** ресуспендированного **сорбента универсального**. Перемешать на вортексе, поставить в штатив на 5 мин, еще раз перемешать и оставить в штативе на 5 мин.
8. Осадить сорбент универсальный в пробирках центрифугированием при 8-10 тыс g (10-13 тыс об/мин при радиусе ротора 70 мм) в течение 30 с. Удалить

надосадочную жидкость, используя вакуумный отсасыватель и отдельный наконечник для каждой пробы.

9. Добавить в пробы по **300 мкл раствора для отмывки 1**, перемешать на вортексе до полного ресуспендирования сорбента универсального, процентрифугировать 30 с при 8-10 тыс g (10-13 тыс об/мин при радиусе ротора 70 мм) на микроцентрифуге. Удалить надосадочную жидкость, используя вакуумный отсасыватель и отдельный наконечник для каждой пробы.
10. Добавить в пробы по **500 мкл раствора для отмывки 2**, перемешать на вортексе до полного ресуспендирования сорбента универсального, процентрифугировать 30 с при 8-10 тыс g (10-13 тыс об/мин при радиусе ротора 70 мм) на микроцентрифуге. Удалить надосадочную жидкость, используя вакуумный отсасыватель и отдельный наконечник для каждой пробы.
11. Повторить отмывку еще раз, следуя пункту **10**, удалить надосадочную жидкость полностью.
12. Поместить пробирки в термостат с температурой 65 °С на 5-10 мин для подсушивания сорбента универсального. При этом крышки пробирок должны быть открыты.
13. В пробирки добавить по **50 мкл ТЕ-буфера для элюции ДНК**. Перемешать на вортексе. Поместить в термостат с температурой 65 °С на 5 мин, периодически встряхивая на вортексе.
14. Процентрифугировать пробирки при 8-10 тыс g (10-13 тыс об/мин при радиусе ротора 70 мм) в течение 1 мин на микроцентрифуге. Надосадочная жидкость содержит очищенную ДНК. Пробы готовы к постановке ПЦР.

Очищенную ДНК можно хранить в течение 1 нед при температуре от 2 до 8 °С и в течение года – при температуре не выше минус 16 °С.

ПРИЛОЖЕНИЕ 2. Экстракция ДНК ИЗ ПРОБ. При использовании комплекта реагентов «РИБО-преп» (проводится в ЗОНЕ 1 – помещении для обработки исследуемого материала).
Объем пробы, необходимый для экстракции ДНК – 0,10 мл.

Порядок работы.

1. **Раствор для лизиса** (если он хранился при температуре от 2 до 8 °С) прогреть при температуре 65 °С до полного растворения кристаллов.
2. Отобрать необходимое количество одноразовых пробирок на 1,5 мл с плотно закрывающимися крышками (включая отрицательный контроль экстракции). Промаркировать пробирки.
3. В пробирки с **раствором для лизиса** внести по **100 мкл подготовленных проб** (после обработки мертиолятом натрия в соответствии с разделом «Обеззараживание образцов»), используя наконечники с фильтром. В пробирку отрицательного контроля (ОК) экстракции внести **100 мкл ОКО.**
4. Содержимое пробирок тщательно перемешать на вортексе, процентрифугировать в течение 5 с на микроцентрифуге для удаления капель с внутренней поверхности крышки пробирки и прогреть 1**5 мин при 65 °С** в термостате.
5. Внести в каждую пробирку по **10 мкл ВКО *Vibrio cholerae***. Содержимое пробирок тщательно перемешать на вортексе, процентрифугировать в течение 5 с на микроцентрифуге для удаления капель с внутренней поверхности крышки пробирки и прогреть **5 мин при 65 °С** в термостате. При обнаружении в пробирках взвешенных частиц (не растворившегося полностью материала) следует провести центрифугирование при 10 тыс об/мин в течение 1 мин на микроцентрифуге и перенести надосадочную жидкость в другие пробирки.
6. Добавить в пробирки по **400 мкл раствора для преципитации**, перемешать на вортексе.
7. Процентрифугировать пробирки на микроцентрифуге в течение **5 мин** при **13 тыс об/мин.**
8. Аккуратно отобрать надосадочную жидкость, не задевая

осадок, используя вакуумный отсасыватель и отдельный наконечник **на 200 мкл** для каждой пробы.

9. Добавить в пробирки по **500 мкл раствора для отмывки 3**, плотно закрыть крышки и осторожно промыть осадок, переворачивая пробирки 3-5 раз. Можно провести процедуру одновременно для всех пробирок, для этого необходимо накрыть пробирки в штативе сверху крышкой или другим штативом, прижать их и переворачивать штатив.
10. Процентрифугировать при **13 тыс об/мин в течение 1-2 мин** на микроцентрифуге.
11. Осторожно, не захватывая осадок, отобрать надосадочную жидкость, используя вакуумный отсасыватель и отдельный наконечник на **10 мкл** для каждой пробы.
12. Добавить в пробирки по **200 мкл раствора для отмывки 4**, плотно закрыть крышки и осторожно промыть осадок, переворачивая пробирки 3-5 раз.
13. Процентрифугировать при **13 тыс об/мин** в течение **1-2 мин** на микроцентрифуге.
14. Осторожно, не захватывая осадок, отобрать надосадочную жидкость, используя вакуумный отсасыватель и отдельный наконечник на **10 мкл** для каждой пробы.
15. Поместить пробирки в термостат с температурой **65 °С на 5 мин** для подсушивания осадка (при этом крышки пробирок должны быть открыты).
16. Добавить в пробирки по **50 мкл РНК-буфера**. Перемешать на вортексе. Поместить в термостат с температурой **65 °С на 5 мин**, периодически встряхивая на вортексе.
17. Процентрифугировать пробирки при **13 тыс об/мин в течение 1 мин** на микроцентрифуге.
18. Надосадочная жидкость содержит очищенные ДНК. Рекомендуется проводить реакцию обратной транскрипции сразу по окончании экстракции.

Очищенные ДНК можно хранить до 4 ч при температуре от 2 до 8 °С, в течение месяца – при температуре не выше минус 16 °С, более длительно – при температуре не выше минус 68 °С.

СИМВОЛЫ, ИСПОЛЬЗУЕМЫЕ В ПЕЧАТНОЙ ПРОДУКЦИИ

REF	Номер в каталоге		Осторожно! Обратитесь к сопроводительной документации
LOT	Код партии		Максимальное число тестов
IVD	Изделие для in vitro диагностики		Использовать до
VER	Дата изменения		Обратитесь к руководству по эксплуатации
	Ограничение температуры		Не допускать попадания солнечного света
	Верхнее ограничение температуры		Дата изготовления
	Производитель		

Приказом Росздравнадзора
от 04.07.13г. № 2291-Пр/13

УТВЕРЖДАЮ
Директор Федерального бюджетного учреждения науки «Центральный научно-исследовательский институт эпидемиологии» Федеральной службы по надзору в сфере защиты прав потребителей и благополучия человека
_____ В.И.Покровский
«04» 12 2012 г.

ИНСТРУКЦИЯ

по применению набора реагентов

для выявления генов металло-β-лактамаз групп VIM, IMP

и NDM методом полимеразной цепной реакции (ПЦР)

с гибридизационно-флуоресцентной детекцией

«АмплиСенс® MDR MBL-FL»

АмплиСенс®

ФБУН ЦНИИ Эпидемиологии
Роспотребнадзора,
Российская Федерация, 111123,
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IVD

ОГЛАВЛЕНИЕ

СПИСОК СОКРАЩЕНИЙ

В настоящей инструкции применяются следующие сокращения и обозначения:

БАЛ	- Бронхоальвеолярный лаваж
ВКО-FL	- Внутренний контрольный образец для наборов с гибридизационно-флуоресцентной детекцией
В–	- Отрицательный контроль экстракции
К+	- Положительный контроль ПЦР
К–	- Отрицательный контроль ПЦР
МБЛ, MBL	- Металло-β-лактамазы
ОКО	- Отрицательный контрольный образец
ПКО	- Положительный контрольный образец
ПЦР	- Полимеразная цепная реакция
ФБУН ЦНИИ Эпидемиологии Роспотребнадзора	- Федеральное бюджетное учреждение науки «Центральный научно-исследовательский институт эпидемиологии» Федеральной службы по надзору в сфере защиты прав потребителей и благополучия человека
MDR	- Полирезистентность (Multidrug-resistance)
FRT	- Флуоресцентная детекция в режиме «реального времени»

НАЗНАЧЕНИЕ

Набор реагентов **«АмплиСенс® MDR MBL-FL»** предназначен для выявления генов приобретенных карбапенемаз класса металло-β-лактамаз (МБЛ) групп VIM, IMP и NDM методом ПЦР с гибридизационно-флуоресцентной детекцией продуктов амплификации в режиме «реального времени». Материалом для проведения ПЦР служат пробы ДНК, полученные путем экстракции из образцов чистой бактериальной культуры, положительной гемокультуры, смеси бактериальных культур, полученной путем первичного посева клинического материала (ликвора, БАЛ, раневого отделяемого и др.) на плотные или жидкие питательные среды, а также из образцов клинического материала: мочи, мазков со слизистых оболочек ротоглотки, прямой кишки.

ВНИМАНИЕ! Результаты ПЦР-исследования учитываются в комплексной диагностике заболевания.[1]

ПРИНЦИП МЕТОДА

Выявление фрагментов ДНК генов приобретенных металло-β-лактамаз групп VIM, IMP и NDM методом полимеразной цепной реакции (ПЦР) с гибридизационно-флуоресцентной детекцией

[1] В соответствии с Директивой Европейского Союза 98/79/ЕС.

включает в себя два этапа: экстракцию ДНК из образцов биологического материала и амплификацию фрагментов выявляемых генов MBL с гибридизационно-флуоресцентной детекцией, которая производится непосредственно в ходе ПЦР. Экстракция ДНК из биологического материала проводится в присутствии внутреннего контрольного образца (ВКО-FL), который позволяет контролировать выполнение процедуры исследования для каждого образца. Затем с полученными пробами ДНК проводится реакция амплификации при помощи специфичных праймеров и фермента Taq-полимеразы. В составе реакционной смеси присутствуют флуоресцентно-меченые олигонуклеотидные зонды, которые гибридизуются с комплементарным участком амплифицируемой ДНК-мишени, в результате чего происходит нарастание интенсивности флуоресценции. Это позволяет регистрировать накопление специфического продукта амплификации путем измерения интенсивности флуоресцентного сигнала. Результаты амплификации фрагментов генов МБЛ групп VIM, IMP и NDM регистрируются по трем различным каналам флуоресцентной детекции: для группы VIM – по каналу для флуорофора FAM, для группы IMP – по каналу для флуорофора JOE, для группы NDM – по каналу для флуорофора Cy5. По каналу для флуорофора ROX детектируется продукт амплификации ДНК ВКО (внутреннего контрольного образца).

Канал для флуорофора	FAM[2]	JOE[2]	ROX[2]	Cy5[2]
ДНК-мишень	гены МБЛ группы VIM	гены МБЛ группы IMP	ВКО	гены МБЛ группы NDM

ФОРМАТЫ И ФОРМЫ ВЫПУСКА НАБОРА РЕАГЕНТОВ

Набор реагентов выпускается в 1 формате

Формат FRT

Набор реагентов выпускается в 2 формах комплектации:

Форма 1 включает комплект реагентов «ПЦР-комплект» вариант FRT-100 F.

Форма 2 включает наборы реагентов оптом, расфасованные по отдельным реагентам, с маркировкой реагентов на их оптовой фасовке.

[2] Или аналогичный канал для детекции указанного флуорофора в зависимости от используемого прибора.

Форма комплектации 1 предназначена для проведения амплификации фрагментов генов МБЛ групп VIM, IMP и NDM с гибридизационно-флуоресцентной детекцией в режиме «реального времени». Для проведения полного ПЦР-исследования необходимо использовать комплекты реагентов для экстракции ДНК, рекомендованные ФБУН ЦНИИ Эпидемиологии Роспотребнадзора.

Форма комплектации 2 предназначена для производственных целей для последующей маркировки на языке заказчика и комплектации по наборам.

ВНИМАНИЕ! Форма комплектации 2 используется только в соответствии с регламентом, утвержденным ФБУН ЦНИИ Эпидемиологии Роспотребнадзора.

АНАЛИТИЧЕСКИЕ ХАРАКТЕРИСТИКИ

Аналитическая чувствительность

Вид биологического материала	Транспортная среда	Комплект/реагент для экстракции ДНК	Аналитическая чувствительность, копий/мл[3]
Гемокультура, смесь бактериальных культур, полученная путем посева клинического материала на жидкую или плотную[4] питательную среду,	—	«ГК-экспресс»	$5x10^5$
		«ДНК-сорб-АМ»	$1x10^5$
Моча	—	«ДНК-сорб-АМ»	$5x10^2$
		«РИБО-преп»	
Мазки со слизистых оболочек ротоглотки, прямой кишки	«Транспортная среда для мазков» или «Транспортная среда с муколитиком (ТСМ)»	«ДНК-сорб-АМ»	$2x10^3$

С использованием данного набора реагентов были выявлены гены MBL соответствующих групп при анализе образцов ДНК контрольных штаммов, несущих гены известных MBL

[3] Данная чувствительность достигается при соблюдении правил предварительной обработки образцов биоматериала, изложенных ниже, и рекомендуемом исследуемом объеме образца.

[4] Для бактериальных культур, полученных путем посева на плотную питательную среду, указана чувствительность в отношении суспензии бактериальных клеток в реагенте «ГК-экспресс» или в лизирующем растворе «ДНК-сорб-АМ» соответственно.

следующих групп: VIM-1, VIM-2, VIM-4, VIM-10, IMP-1, IMP-2, IMP-12, IMP-13.

Аналитическая специфичность

Отсутствовали неспецифические реакции при тестировании образцов ДНК человека и образцов ДНК следующих микроорганизмов: *Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, Klebsiella oxytoca, Serratia marcescens, Acinetobacter baumannii, Proteus mirabilis, Enterococcus faecalis, Staphylococcus* spp*., Streptococcus* spp., *Candida* spp.

МЕРЫ ПРЕДОСТОРОЖНОСТИ

Работа должна проводиться в лаборатории, выполняющей молекулярно-биологические (ПЦР) исследования биологического материала на наличие возбудителей инфекционных болезней, с соблюдением санитарно-эпидемических правил СП 1.3.2322-08 «Безопасность работы с микроорганизмами III–IV групп патогенности (опасности) и возбудителями паразитарных болезней», СанПиН 2.1.7.2790-10 «Санитарно-эпидемиологические требования к обращению с медицинскими отходами» и методических указаний МУ 1.3.2569-09 «Организация работы лабораторий, использующих методы амплификации нуклеиновых кислот при работе с материалом, содержащим микроорганизмы I-IV групп патогенности».

При работе всегда следует выполнять следующие требования:

- Следует рассматривать исследуемые образцы как инфекционно-опасные, организовывать работу и хранение в соответствии с СП 1.3.2322-08 «Безопасность работы с микроорганизмами III–IV групп патогенности (опасности) и возбудителями паразитарных болезней».
- Убирать и дезинфицировать разлитые образцы или реактивы, используя дезинфицирующие средства в соответствии с СП 1.3.2322-08 «Безопасность работы с микроорганизмами III–IV групп патогенности (опасности) и возбудителями паразитарных болезней».
- Лабораторный процесс должен быть однонаправленным. Анализ проводится в отдельных помещениях (зонах). Работу следует начинать в Зоне Выделения, продолжать в Зоне Амплификации и Детекции. Не возвращать образцы, оборудование и реактивы в зону, в которой была проведена

предыдущая стадия процесса.
- Неиспользованные реактивы, реактивы с истекшим сроком годности, а также использованные реактивы следует удалять в соответствии с требованиями СанПиН 2.1.7.2790-10 «Санитарно-эпидемиологические требования к обращению с медицинскими отходами».

ВНИМАНИЕ! При удалении отходов после амплификации (пробирок, содержащих продукты ПЦР) недопустимо открывание пробирок и разбрызгивание содержимого, поскольку это может привести к контаминации продуктами ПЦР лабораторной зоны, оборудования и реагентов.

- Использовать и менять при каждой операции одноразовые наконечники для автоматических дозаторов с фильтром. Одноразовую пластиковую посуду необходимо сбрасывать в специальный контейнер, содержащий дезинфицирующее средство, которое может быть использовано для обеззараживания медицинских отходов.
- Поверхности столов, а также помещения, в которых проводится постановка ПЦР, до начала и после завершения работ необходимо подвергать ультрафиолетовому облучению в течение 30 мин.
- Применять набор строго по назначению, согласно данной инструкции.
- Допускать к работе с набором только специально обученный персонал.
- Не использовать набор по истечении срока годности.
- Использовать одноразовые перчатки, лабораторные халаты, защищать глаза во время работы с образцами и реактивами. Тщательно вымыть руки по окончании работы.
- Избегать контакта с кожей, глазами и слизистой оболочкой. При контакте немедленно промыть пораженное место водой и обратиться за медицинской помощью.
- Листы безопасности материалов (MSDS – material safety data sheet) доступны по запросу.

ДОПОЛНИТЕЛЬНЫЕ МАТЕРИАЛЫ И ОБОРУДОВАНИЕ

<u>Проведение предварительной подготовки исследуемого материала</u>

1. Одноразовые полипропиленовые завинчивающиеся или плотно закрывающиеся пробирки объемом 1,5 мл

(например, Axygen, США).

Проведение экстракции ДНК из исследуемых образцов

2. Комплект реагентов/реагент для выделения ДНК – «ДНК-сорб-АМ» (форма комплектации без контролей), «РИБО-преп», «ГК-экспресс» или другие комплекты, рекомендованные ФБУН ЦНИИ Эпидемиологии Роспотребнадзора.
3. Дополнительные материалы и оборудование для экстракции ДНК – согласно инструкции к комплекту реагентов/реагенту для экстракции ДНК.

Проведение амплификации с гибридизационно-флуоресцентной детекцией продуктов амплификации

4. Бокс абактериальной воздушной среды (ПЦР-бокс) (например, «БАВ-«Ламинар.-с», «Ламинарные системы», Россия).
5. Центрифуга/вортекс (например, «ТЭТА-2», «Биоком», Россия).
6. Автоматические дозаторы переменного объема (от 5 до 20 мкл и от 20 до 200 мкл) (например, «Ленпипет», Россия).
7. Одноразовые наконечники с фильтром до 100 мкл в штативах (например, Axygen, США).
8. Штативы для пробирок объемом 0,2 мл или 0,1 мл (например, «ИнтерЛабСервис», Россия).
9. Холодильник от 2 до 8 °С с морозильной камерой не выше минус 16 °С для выделенных проб ДНК.
10. Отдельный халат, шапочки, обувь и одноразовые перчатки по МУ 1.3.2569-09.
11. Емкость для сброса наконечников.
12. Программируемый амплификатор с системой детекции флуоресцентного сигнала в режиме «реального времени» (например, Rotor-Gene 6000 (Corbett Research, Австралия), Rotor-Gene Q (Qiagen, Германия), CFX96 (Bio-Rad, США) и рекомендованные ФБУН ЦНИИ Эпидемиологии Роспотребнадзора в методических рекомендациях по применению данного набора реагентов).
13. Одноразовые полипропиленовые пробирки для ПЦР объемом 0,2 мл или 0,1 мл:
    а) тонкостенные пробирки для ПЦР объемом 0,2 мл с круглой или плоской оптически прозрачной крышкой –

при использовании прибора планшетного типа;

б) тонкостенные пробирки для ПЦР объемом 0,2 мл с плоской крышкой (например, Axygen, США) или пробирки для ПЦР к Rotor-Gene, объемом 0,1 мл в стрипах по 4 шт. с крышками (например, Corbett Research, Австралия; QIAGEN, Германия) – при использовании прибора роторного типа.

ВЗЯТИЕ, ТРАНСПОРТИРОВАНИЕ И ХРАНЕНИЕ ИССЛЕДУЕМОГО МАТЕРИАЛА

Перед началом работы следует ознакомиться с методическими рекомендациями «Взятие, транспортировка, хранение клинического материала для ПЦР-диагностики», разработанными ФГУН ЦНИИЭ Роспотребнадзора, Москва, 2010 г.

Материалом для исследования служат: положительная гемокультура, смесь бактериальных культур, полученная путем первичного посева клинического материала (ликвора, БАЛ, раневого отделяемого, мочи и др.) на плотные или жидкие питательные среды, чистая бактериальная культура, а также образцы клинического материала: моча (при острых инфекциях мочевыводящих путей), мазки со слизистых оболочек ротоглотки, прямой кишки (при проведении скрининга колонизации бактериями, обладающими приобретенными карбапенемазами).

Мазки со слизистых оболочек ротоглотки или прямой кишки должны быть помещены в транспортную среду «Транспортная среда для мазков» или «Транспортная среда с муколитиком (ТСМ)» производства ФБУН ЦНИИ Эпидемиологии Роспотребнадзора.

ПОДГОТОВКА ИССЛЕДУЕМОГО МАТЕРИАЛА К ЭКСТРАКЦИИ ДНК

Гемокультура, смесь бактериальных культур, полученная путем первичного посева клинического материала на жидкую питательную среду

Перенести от 0,1 до 0,25 мл гемокультуры или посева на среду обогащения в стерильную одноразовую пробирку объемом 1,5 мл (с помощью одноразового шприца).

Центрифугировать 10 мин при 10 000 g (12 тыс об/мин на центрифуге MiniSpin, Eppendorf). Используя вакуумный

отсасыватель с колбой-ловушкой, полностью удалить надосадочную жидкость, не захватывая осадок и используя для каждого образца отдельный наконечник без фильтра.

Моча

Взболтать флакон с мочой. Перенести 1 мл мочи в стерильную одноразовую пробирку объемом 1,5 мл, используя отдельный наконечник с фильтром для каждого образца. Центрифугировать 10 мин при 10 000 g (12 тыс об/мин на центрифуге MiniSpin, Eppendorf). При наличии большого количества солей ресуспендировать только верхний слой осадка солей в объеме 1 мл и затем снова центрифугировать. Используя вакуумный отсасыватель с колбой-ловушкой, полностью удалить надосадочную жидкость, не захватывая осадок и используя для каждого образца отдельный наконечник без фильтра.

С полученными после предварительной обработки образцами (осадками) провести процедуру экстракции ДНК в соответствии с инструкцией к используемому комплекту реагентов.

Полученные после предварительной обработки образцы (осадки) можно хранить:

- при температуре не выше минус 16 °С – в течение недели,
- при температуре не выше минус 68 °С - длительно.

ФОРМАТ FRT

СОСТАВ

Комплект реагентов «ПЦР-комплект» вариант FRT-100 F – комплект реагентов для амплификации фрагментов генов металло-β-лактамаз групп VIM, IMP и NDM с гибридизационно-флуоресцентной детекцией в режиме «реального времени» – **включает**:

Реактив	*Описание*	*Объем, мл*	*Кол-во*
ПЦР-смесь-1-FRT MBL	Прозрачная бесцветная жидкость	1,2	1 пробирка
ПЦР-смесь-2-FRT	Прозрачная бесцветная жидкость	0,3	2 пробирки
Полимераза (TaqF)	Прозрачная бесцветная жидкость	0,03	2 пробирки
К–	Прозрачная бесцветная жидкость	0,2	1 пробирка
ПКО-1 MBL	Прозрачная бесцветная жидкость	0,2	1 пробирка
ПКО-2 MBL	Прозрачная бесцветная жидкость	0,2	1 пробирка

Комплект реагентов рассчитан на проведение 110 реакций амплификации, включая контроли.

К комплекту реагентов прилагаются контрольные образцы этапа экстракции:

Реактив	*Описание*	*Объем, мл*	*Кол-во*
ОКО	Прозрачная бесцветная жидкость	1,2	1 пробирка
ВКО-FL	Прозрачная бесцветная жидкость	1,0	1 пробирка

ПРОВЕДЕНИЕ ПЦР-ИССЛЕДОВАНИЯ

ПЦР-исследование состоит из следующих этапов:

- Экстракция ДНК из исследуемых образцов.
- Амплификация с гибридизационно-флуоресцентной детекцией в режиме «реального времени».
- Анализ и интерпретация результатов.

Детальная информация по процедуре проведения ПЦР-исследования в зависимости от используемого оборудования изложена в методических рекомендациях по применению наборов реагентов для выявления генов карбапенемаз методом полимеразной цепной реакции (ПЦР) с гибридизационно-флуоресцентной детекцией «АмплиСенс®

MDR MBL-FL» и «АмплиСенс® MDR KPC/OXA-48-FL», разработанных ФБУН ЦНИИ Эпидемиологии Роспотребнадзора.

Таблица 1

Схема проведения ПЦР-исследования в зависимости от вида биологического материала

Вид биологического материала	Объем для экстракции, мкл	Комплект/реагент для экстракции ДНК	Добавление ВКО-FL при экстракции	Программа амплификации	Используемый положительный контроль амплификации
Гемокультура, смесь бактериальных культур, полученная путем посева клинического материала на **жидкую питательную среду**	Осадок из 100-250 мкл, полученный после предобработки	«ГК-экспресс»	–	«АмплиСенс-B»	ПКО-1 MBL
		«ДНК-сорб-АМ»	+	«АмплиСенс-1»	ПКО-2 MBL
Смесь бактериальных культур, полученная путем посева клинического материала на **плотную питательную среду**	10^7-10^9 бактериальных клеток	«ГК-экспресс»	–	«АмплиСенс-B»	ПКО-1 MBL
		«ДНК-сорб-АМ»	+	«АмплиСенс-1»	ПКО-2 MBL
Моча	Осадок из 1000 мкл, полученный после предобработки	«ДНК-сорб-АМ»	+	«АмплиСенс-1»	ПКО-2 MBL
		«РИБО-преп»			
Мазки со слизистых оболочек ротоглотки, прямой кишки	100	«ДНК-сорб-АМ»	+	«АмплиСенс-1»	ПКО-2 MBL

ЭКСТРАКЦИЯ ДНК ИЗ ИССЛЕДУЕМЫХ ОБРАЗЦОВ

Для экстракции ДНК используются комплекты реагентов / реагент:

– «ГК-экспресс» или «ДНК-сорб-АМ» для экстракции ДНК из образцов **положительной гемокультуры, смеси бактериальных культур, полученной при посеве на жидкую питательную среду (после предварительной обработки), образцов чистой культуры или смеси бактериальных культур, полученной при посеве на**

плотную питательную среду, в соответствии с инструкцией к используемому комплекту реагентов.

– «ДНК-сорб-АМ» или «РИБО-преп» для экстракции ДНК из образцов **мочи** после предварительной обработки, в соответствии с инструкцией к используемому комплекту реагентов.
– «ДНК-сорб-АМ» для экстракции ДНК из образцов **мазков со слизистых оболочек ротоглотки, прямой кишки** в соответствии с инструкцией к используемому комплекту реагентов.

Экстракция ДНК из каждого исследуемого образца проводится в присутствии внутреннего контрольного образца (ВКО-FL). В качестве пробы В- используется реактив ОКО. В случае использования для экстракции ДНК реагента «ГК-экспресс» добавление ВКО-FL в исследуемые образцы и ОКО в пробу В- не требуется.

При проведении экстракции ДНК из образцов, после предобработки представляющих собой осадки, лизирующий раствор или реагент «ГК-экспресс» добавляют непосредственно в пробирку с осадком, используя для каждого образца отдельный наконечник с фильтром.

При проведении экстракции из образцов чистой культуры или смеси бактериальных культур, полученной при посеве на плотную питательную среду, бактериальные клетки, взятые стерильной петлей (или стерильным наконечником) в количестве 10^7-10^9 клеток, помещают непосредственно в пробирку объемом 1,5 мл, содержащую реагент «ГК-экспресс» или лизирующий раствор набора «ДНК-сорб-АМ».

ВНИМАНИЕ! Не рекомендуется одновременно проводить экстракцию ДНК из образцов гемокультуры, чистой культуры или смеси бактериальных культур, полученной путем посева на питательную среду, и из образцов биологического материала других видов, т.к. при этом существует высокий риск контаминации от образцов положительной гемокультуры или бактериальных культур, содержащих высокие концентрации ДНК возбудителя.

ПРОВЕДЕНИЕ АМПЛИФИКАЦИИ С ДЕТЕКЦИЕЙ В РЕЖИМЕ «РЕАЛЬНОГО ВРЕМЕНИ»

Выбор пробирок для амплификации зависит от используемого амплификатора с системой детекции в режиме «реального времени».

Для внесения в пробирки реагентов, проб ДНК и контрольных образцов используются одноразовые наконечники с фильтрами.

А. Подготовка пробирок для амплификации

Общий объем реакционной смеси – 25 мкл, включая объем пробы ДНК – 10 мкл.

Компоненты реакционной смеси следует смешивать непосредственно перед проведением эксперимента. Смешивать реагенты из расчета расходования на одну реакцию:

- **10 мкл ПЦР-смеси-1-FRT MBL,**
- **5 мкл** смеси **ПЦР-смеси-2-FRT,**
- **0,5 мкл полимеразы (TaqF).**

1. Предварительно необходимо подготовить смесь **ПЦР-смеси-2-FRT** и **полимеразы (TaqF)**. Содержимое одной пробирки с **полимеразой (TaqF) (30 мкл)** необходимо полностью перенести в пробирку с **ПЦР-смесью-2-FRT (300 мкл)** и аккуратно перемешать на центрифуге/вортексе, не допуская образования пены. Промаркировать пробирку, указав дату приготовления смеси.

ВНИМАНИЕ! Приготовленная смесь рассчитана на 60 реакций. Смесь хранить при температуре от 2 до 8 °С в течение 3 мес и использовать по мере необходимости.

В случае если данная смесь не может быть израсходована в течение трех месяцев, необходимо готовить смесь на меньшее количество реакций, например, смешать 150 мкл ПЦР-смеси-2-FRT и 15 мкл полимеразы (TaqF) (полученная смесь рассчитана на 30 реакций).

2. Перемешать содержимое пробирки с реагентом **ПЦР-смесь-1-FRT MBL** и осадить капли кратковременным центрифугированием с помощью центрифуги/вортекса.

Сделать расчет на необходимое число реакций, включающее тестирование исследуемых и контрольных образцов, можно согласно **расчетной таблице, приведенной в приложении 1.**

Следует учитывать, что для тестирования даже одного исследуемого образца ДНК необходимо проводить постановку еще **3-х контрольных реакций: К+, К– и В–**.

Необходимо брать реагенты с запасом: для тестирования N образцов приготовить реагенты для (N+1) реакций.

3. В отдельной пробирке подготовить реакционную смесь. Смешать необходимое количество **ПЦР-смеси-1-FRT MBL, ПЦР-смеси-2-FRT** с **полимеразой (TaqF)**, приготовленной согласно п.1.
4. Отобрать необходимое количество пробирок или стрипов для амплификации ДНК исследуемых и контрольных проб.
5. Внести в пробирки по **15 мкл** готовой реакционной смеси.
6. В подготовленные пробирки внести по **10 мкл проб ДНК**, полученных в результате экстракции из исследуемых образцов.
7. Поставить контрольные реакции:
   а) **отрицательный контроль ПЦР (К–)** – внести в пробирку **10 мкл К–.**
   б) **положительный контроль ПЦР (К+)** – в одну пробирку внести **10 мкл ПКО-1 MBL** (при анализе проб ДНК, полученных из образцов гемокультуры, чистой культуры или смеси бактериальных культур, при использовании программы амплификации «АмплиСенс-В») или **10 мкл ПКО-2 MBL** (при анализе проб ДНК, полученных из образцов исходного клинического материала или из образцов гемокультуры, чистой культуры или смеси бактериальных культур, при использовании программы амплификации «АмплиСенс-1»).
   в) **отрицательный контроль экстракции ДНК (В–) –** внести в пробирку **10 мкл** пробы, выделенной из ОКО.

Б. Проведение амплификации с детекцией в режиме «реального времени»

1. Запрограммировать прибор (амплификатор с системой детекции в режиме «реального времени») для выполнения соответствующей программы амплификации и детекции флуоресцентного сигнала. При анализе проб ДНК, полученных при экстракции с помощью реагента «ГК-экспресс» из образцов гемокультуры, чистой культуры или смеси бактериальных культур, полученной путем посева на

питательную среду, используется программа **«АмплиСенс-В»** (см. табл. 2). При анализе проб ДНК, полученных из образцов исходного клинического материала, или проб ДНК, полученных при экстракции с помощью комплекта реагентов «ДНК-сорб-АМ» из образцов гемокультуры, чистой культуры или смеси бактериальных культур, используется программа **«АмплиСенс-1»** (см. табл. 3).

Таблица 2

Программа «АмплиСенс-В»

Цикл	**Приборы роторного типа[5]**			**Приборы планшетного типа[6]**		
	Температура, °С	Время	Кол-во циклов	Температура, °С	Время	Кол-во циклов
1	**95**	15 мин	1	**95**	15 мин	1
2	**95**	5 с	35	**95**	5 с	35
	60	20 с детекция флуоресц. сигнала		**60**	30 с детекция флуоресц. сигнала	
	72	15 с		**72**	15 с	

Таблица 3

Программа «АмплиСенс-1»

Цикл	**Приборы роторного типа[5]**			**Приборы планшетного типа[6]**		
	Температура, °С	Время	Кол-во циклов	Температура, °С	Время	Кол-во циклов
1	**95**	15 мин	1	**95**	15 мин	1
2	**95**	5 с	5	**95**	5 с	5
	60	20 с		**60**	20 с	
	72	15 с		**72**	15 с	
3	**95**	5 с	40	**95**	5 с	40
	60	20 с детекция флуоресц. сигнала		**60**	30 с детекция флуоресц. сигнала	
	72	15 с		**72**	15 с	

Детекция флуоресцентного сигнала назначается по четырем каналам – для флуорофоров FAM[7], JOE[7], ROX[7] и Cy5[7].

[5] Например, Rotor-Gene 6000 (Corbett Research, Австралия), Rotor-Gene Q (QIAGEN, Германия) и рекомендованные ФБУН ЦНИИ Эпидемиологии Роспотребнадзора в методических рекомендациях по применению данного набора реагентов.

[6] Например, CFX, iQ5 (Bio-Rad, США) и рекомендованные ФБУН ЦНИИ Эпидемиологии Роспотребнадзора в методических рекомендациях по применению данного набора реагентов.

[7] Название каналов детекции для соответствующего прибора см. в методических рекомендациях к набору реагентов.

2. Установить пробирки в ячейки реакционного модуля прибора.
3. Запустить выполнение программы амплификации с детекцией флуоресцентного сигнала.
4. По окончании выполнения программы приступить к анализу и интерпретации результатов.

АНАЛИЗ И ИНТЕРПРЕТАЦИЯ РЕЗУЛЬТАТОВ

Анализ результатов проводят с помощью программного обеспечения прибора используемого для проведения ПЦР с детекцией в режиме «реального времени». Анализируют графики накопления флуоресцентного сигнала по четырем каналам**:**

- по каналу для флуорофора **FAM** регистрируется сигнал, свидетельствующий о накоплении продукта амплификации фрагментов **генов МБЛ группы VIM;**
- по каналу для флуорофора **JOE** регистрируется сигнал, свидетельствующий о накоплении продукта амплификации фрагментов **генов МБЛ группы IMP;**
- по каналу для флуорофора **ROX** регистрируется сигнал, свидетельствующий о накоплении продукта амплификации ДНК **внутреннего контроля;**
- по каналу для флуорофора **Cy5** регистрируется сигнал, свидетельствующий о накоплении продукта амплификации фрагментов **генов МБЛ группы NDM.**

Результаты интерпретируются на основании наличия (или отсутствия) пересечения графика флуоресценции с пороговой линией, установленной на уровне экспоненциального подъема сигнала, что определяет наличие (или отсутствие) для данной ДНК-мишени значения порогового цикла *Ct* в соответствующей графе таблицы результатов.

Принцип интерпретации результатов следующий:

- **Гены МБЛ** соответствующей группы **обнаружены**, если для данной пробы в таблице результатов по каналу для флуорофора FAM или/и JOE, или/и Cy5 определено значение порогового цикла *Ct,* не превышающее указанного граничного значения. При этом кривая флуоресценции данной пробы должна пересекать пороговую линию на

участке характерного экспоненциального подъема флуоресценции.

- **Гены МБЛ** соответствующей группы **не обнаружены,** если для данной пробы в таблице результатов по каналам для флуорофоров FAM, JOE и Cy5 не определено (отсутствует) значение порогового цикла *Ct* (кривая флуоресценции не пересекает пороговую линию), а в таблице результатов по каналу для флуорофора ROX определено значение порогового цикла *Ct*, не превышающее указанное (граничное) значение.
- Результат анализа **невалидный**, если для исследуемого образца отсутствуют значения пороговых циклов Ct по каналам для флуорофоров FAM, JOE и Cy5, и по каналу для флуорофора ROX значение Ct также отсутствует или превышает указанное граничное значение. В этом случае необходимо повторно провести ПЦР-исследование соответствующего образца, начиная с этапа экстракции ДНК.

ВНИМАНИЕ! Граничные значения *Ct* указаны во вкладыше, прилагаемом к набору реагентов. См. также методические рекомендации по применению наборов реагентов для выявления генов карбапенемаз методом полимеразной цепной реакции (ПЦР) с гибридизационно-флуоресцентной детекцией «АмплиСенс® MDR MBL-FL» и «АмплиСенс® MDR KPC/OXA-48-FL», разработанные ФБУН ЦНИИ Эпидемиологии Роспотребнадзора.

Результат ПЦР-исследования считается достоверным, если получены правильные результаты для положительного и отрицательного контролей амплификации и отрицательного контроля экстракции ДНК в соответствии с таблицей 4 и вкладышем к набору реагентов.

Таблица 4

Результаты для контролей различных этапов ПЦР-исследования

Контроль	Контролируемый этап ПЦР-исследования	Значение порогового цикла *Ct*	
		по каналам для флуорофоров FAM, JOE, Cy5	**по каналу для флуорофора ROX**
В–	Экстракция ДНК	Значение отсутствует	Определено значение **меньше** граничного
К–	ПЦР	Значение отсутствует	Значение отсутствует
К+	ПЦР	Определено значение **меньше** граничного	Не оценивается

ВНИМАНИЕ!

1. Если для положительного контроля ПЦР (К+) значения порогового цикла по каналам для флуорофоров FAM, JOE, Cy5 отсутствуют или превышают указанное граничное значение, необходимо повторить амплификацию для всех образцов.
2. Если для отрицательного контроля экстракции ДНК (В–) и/или отрицательного контроля ПЦР (К–) регистрируется значение порогового цикла *Ct* по каналам для флуорофоров FAM или/и JOE, или/и Cy5, необходимо повторить ПЦР-исследование для всех образцов, для которых определено значение порогового цикла, соответственно, по каналам для флуорофоров FAM или/и JOE, или/и Cy5.

Клиническая интерпретация результатов теста должна проводиться врачом только при условии комплексного обследования пациента, с учетом данных анамнеза, клинического и эпидемиологического статуса, в соответствии с существующими клиническими и методическими рекомендациями.

СРОК ГОДНОСТИ. УСЛОВИЯ ТРАНСПОРТИРОВАНИЯ И ХРАНЕНИЯ

Срок годности. 9 мес. Набор реагентов с истекшим сроком годности применению не подлежит. Срок годности вскрытых реагентов соответствует сроку годности, указанному на этикетках для невскрытых реагентов, если в инструкции не указано иное.

Транспортирование. Набор реагентов транспортировать при температуре от 2 до 8 °С не более 5 сут. «ПЦР-комплект» вариант FRT-100 F при получении разукомплектовать в соответствии с указанными температурами хранения.

Хранение. Комплект реагентов «ПЦР-комплект» хранить при температуре от 2 до 8 °С. ПЦР-смесь-1-FRT MBL хранить в защищенном от света месте. ПЦР-смесь-2-FRT и полимеразу (TaqF) хранить при температуре от минус 24 до минус 16 °С.

Рекламации на качество набора реагентов **«АмплиСенс® MDR MBL-FL»** направлять на предприятие-изготовитель ФБУН ЦНИИ Эпидемиологии Роспотребнадзора (111123 г. Москва, ул. Новогиреевская, д. 3а) в отдел по работе с рекламациями и организации обучения (тел. (495) 974-96-46, факс (495) 916-18-18, e-mail: products@pcr.ru)[8].

Заведующий НПЛ ОМДиЭ
ФБУН ЦНИИ Эпидемиологии Роспотребнадзора — Е.Н. Родионова

Директор НИИАХ ГБОУ ВПО СГМА
Минздрава России — Р.С.Козлов

[8] Отзывы и предложения о продукции «АмплиСенс» вы можете оставить, заполнив анкету потребителя на сайте: www.amplisens.ru.

ПРИЛОЖЕНИЕ 1.

Схема приготовления реакционных смесей

	Объем реагентов на указанное количество реакций (мкл)	
Объем реагентов на одну реакцию (мкл)	**10 мкл**	**5 мкл**
Количество исследуемых образцов*	**ПЦР-смесь-1-FRT ***	**Смесь ПЦР-смеси-2-FRT и полимеразы (TaqF)***
2	**60**	**30**
3	**70**	**35**
4	**80**	**40**
5	**90**	**45**
6	**100**	**50**
7	**110**	**55**
8	**120**	**60**
9	**130**	**65**
10	**140**	**70**
11	**150**	**75**
12	**160**	**80**
13	**170**	**85**
14	**180**	**90**
15	**190**	**95**
16	**200**	**100**
17	**210**	**105**
18	**220**	**110**
19	**230**	**115**
20	**240**	**120**
21	**250**	**125**
22	**260**	**130**
23	**270**	**135**
24	**280**	**140**
25	**290**	**145**

*Приведены значения с учетом запаса (расчет на одну реакцию больше) и с учетом необходимости постановки 3 контрольных реакций: К+, В– и К–.

СИМВОЛЫ, ИСПОЛЬЗУЕМЫЕ В ПЕЧАТНОЙ ПРОДУКЦИИ

REF	Номер в каталоге	Σ	Максимальное число тестов
LOT	Код партии		Использовать до
IVD	Изделие для in vitro диагностики		Обратитесь к руководству по эксплуатации
VER	Дата изменения		Не допускать попадания солнечного света
	Ограничение температуры		Дата изготовления
	Производитель		

УТВЕРЖДЕНА
Приказом Росздравнадзора
от 11.06.2010г. № 5463-Пр/10

УТВЕРЖДАЮ
Директор Федерального государственного учреждения науки «Центральный научно-исследовательский институт эпидемиологии» Федеральной службы по надзору в сфере защиты прав потребителей и благополучия человека
_____ В.И.Покровский
«17» 03 2010 г.

ИНСТРУКЦИЯ

по применению набора реагентов
для выявления и дифференциации ДНК диарогенных *E.coli*
в объектах окружающей среды и клиническом материале
методом полимеразной цепной реакции (ПЦР)
с гибридизационно-флуоресцентной детекцией

«АмплиСенс® Эшерихиозы-FL»

СОДЕРЖАНИЕ

СПИСОК СОКРАЩЕНИЙ

В настоящей инструкции применяются следующие сокращения и обозначения:

ВКО	- внутренний контрольный образец
ОКО	- отрицательный контрольный образец
ОК	- отрицательный контроль этапа экстракции ДНК/РНК
ПКО	- положительный контрольный образец
ПЦР	- полимеразная цепная реакция
ФГУН ЦНИИЭ Роспотребнадзора	- федеральное государственное учреждение науки «Центральный научно-исследовательский институт эпидемиологии» Федеральной службы по надзору в сфере защиты прав потребителей и благополучия человека
FEP	- детекция по «конечной точке»
FRT	- детекция в режиме «реального времени»
EPEC	- энтеропатогенные *E.coli*
ETEC	- энтеротоксигенные *E.coli*
EIEC	- энтероинвазивные *E.coli*
EHEC	- энтерогеморрагические *E.coli*
EAgEC	- энтероаггрегативные *E.coli*

НАЗНАЧЕНИЕ

Набор реагентов **«АмплиСенс® Эшерихиозы-FL»** предназначен для выявления и дифференциации ДНК различных групп диарогенных эшерихий (*EPEC*, *ETEC*, *EIEC*, *EHEC*, *EAgEC*) в объектах окружающей среды и клиническом материале методом полимеразной цепной реакции (ПЦР) с гибридизационно-флуоресцентной детекцией.

Для экстракции ДНК и проведения реакции обратной транскрипции используются наборы реагентов, рекомендованные ФГУН ЦНИИЭ Роспотребнадзора («ДНК-сорб- В», «РИБО-сорб» или «РИБО-преп»).

ВНИМАНИЕ! Результаты ПЦР-исследования учитываются в комплексной диагностике заболевания.

ПРИНЦИП МЕТОДА

Выявление ДНК различных групп диарогенных эшерихий (*EPEC*, *ETEC*, *EIEC*, *EHEC*, *EAgEC*) методом полимеразной цепной реакции (ПЦР) с гибридизационно-флуоресцентной детекцией включает в себя три этапа: экстракцию (выделение) ДНК из образцов клинического материала, ПЦР-амплификацию участка ДНК данного микроорганизма и гибридизационно-флуоресцентную детекцию, которая производится либо непосредственно в ходе ПЦР (вариант FRT), либо после ее

завершения (вариант FEP). Экстракция ДНК из клинического материала проводится в присутствии внутреннего контрольного образца (ВКО-FL), который позволяет контролировать выполнение процедуры исследования для каждого образца. Пробы ДНК используются для амплификации участка ДНК перечисленных выше возбудителей при помощи специфичных к этому участку ДНК праймеров и фермента Taq-полимеразы. В составе реакционной смеси присутствуют флуоресцентно-меченые олигонуклеотидные зонды, которые гибридизуются с комплементарным участком амплифицируемой ДНК-мишени, в результате чего происходит нарастание интенсивности флуоресценции. Это позволяет регистрировать накопление специфического продукта амплификации путем измерения интенсивности флуоресцентного сигнала. Детекция флуоресцентного сигнала при использовании варианта FEP осуществляется после окончания ПЦР с помощью флуоресцентного ПЦР-детектора, а при использовании варианта FRT – непосредственно в ходе ПЦР с помощью амплификатора с системой детекции флуоресцентного сигнала в режиме «реального времени».

ВАРИАНТЫ И ФОРМЫ ВЫПУСКА НАБОРА РЕАГЕНТОВ

Набор реагентов выпускается в 1 варианте

Вариант FEP/FRT

Набор реагентов выпускается в 1 форме комплектации:

Форма 1 включает комплект реагентов «ПЦР-комплект» вариант FEP/FRT-50 F.

Форма комплектации 1 предназначена для проведения реакции амплификации и дифференциации ДНК различных групп диарогенных эшерихий (*EPEC*, *ETEC*, *EIEC*, *EHEC*, *EAgEC*) с гибридизационно-флуоресцентной детекцией по «конечной точке» и в режиме «реального времени». Для проведения полного ПЦР-исследования необходимо использовать комплекты реагентов для экстракции ДНК, рекомендованные ФГУН ЦНИИЭ Роспотребнадзора.

АНАЛИТИЧЕСКИЕ ХАРАКТЕРИСТИКИ

Аналитическая чувствительность

Патоген	Вид клинического материала	Комплект для экстракции ДНК	Комплект для амплификации и детекции	Аналитическая чувствительность
EPEC	Фекалии	«РИБО-преп»	«ПЦР-комплект» вариант FEP/FRT-50 F	$1x10^3$ ГЭ/мл
ETEC				
EIEC				
EHEC				
EAgEC				

Аналитическая специфичность

Специфичность набора реагентов проверялась на следующих штаммах микроорганизмов:

Штаммы *E.coli* из коллекции ГИСК им. Л.А. Тарасевича: O157H7 № 4, O157H7 № 23, O157H7 № 212, O157H7 № 214, O157H7 № 1330, O143, O124 № 227, O144, O86 № 990, O125 Carioni, O85, O61 № 10167в/41, O59 № 9095/41, № 409 (O34), K12, 3912/41, Крым № 56, O148H28 B7a, O6 № 3091, 113/3, 675, O111 № 153, O62 10524/41, O126 № 611, M17, Крым № 1274, 168/59, O57 8198/41, Крым № 14169, O48, NCTC 9001.

Штаммы *E. coli* из коллекции ФГУ ВГНКИ: *Salmonella enteritidis* S-6, *Salmonella choleraesuis* 370, *Salmonella typhimurium* 371, *Salmonella dublin* 373, *Salmonella typhi* C1, *Salmonella abortusovis* 372, *Salmonella gallinarum-pullorum*, *Shigella flexneri* 851b, *Campylobacter fetus subsp. fetus* 25936, *Campylobacter jejuni subsp. jejuni* 43435, *Klebsiella K* 65 SW4, *Listeria monocytogenes* УСХЧ 19, *Listeria monocytogenes* УСХЧ 52, *Proteus vulgaris* 115/98, *Pseudomonas aeruginosa* ДН с1, *Staphylococcus aureus* 653, *Staphylococcus aureus* 29112, *Morganella morganii* 619 с 01, *Enterococcus faecalis* 356.

Штаммы *Yersinia enterocolitica* (12 штаммов) и *Yersinia pseudotuberculosis* (6 штаммов) из собрания ФГУН ЦНИИЭ Роспотребнадзора.

Специфичность тестирования диарогенных штаммов *E. coli* подтверждалась методом секвенирования детектируемых участков генома.

При проведении тестирования данных панелей, а также образцов ДНК человека неспецифических реакций выявлено не было.

МЕРЫ ПРЕДОСТОРОЖНОСТИ

Работа должна проводиться в лаборатории, выполняющей молекулярно-биологические (ПЦР) исследования клинического материала на наличие возбудителей инфекционных болезней, с соблюдением санитарно-эпидемических правил СП 1.3.2322-08 «Безопасность работы с микроорганизмами III – IV групп патогенности (опасности) и возбудителями паразитарных болезней», СП 2.1.7.728-99 «Правила сбора, хранения и удаления отходов лечебно-профилактических учреждений» и методических указаний МУ 1.3.1888-04 «Организация работы при исследованиях методом ПЦР материала, инфицированного патогенными биологическими агентами III – IV групп патогенности».

При работе всегда следует выполнять следующие требования:

- Следует рассматривать исследуемые образцы как инфекционно-опасные, организовывать работу и хранение в соответствии с СП 1.3.2322-08 «Безопасность работы с микроорганизмами III – IV групп патогенности (опасности) и возбудителями паразитарных болезней».
- Убирать и дезинфицировать разлитые образцы или реактивы, используя дезинфицирующие средства в соответствии с СП 1.3.2322-08 «Безопасность работы с микроорганизмами III – IV групп патогенности (опасности) и возбудителями паразитарных болезней».
- Удалять неиспользованные реактивы в соответствии с СП 2.1.7.728-99 «Правила сбора, хранения и удаления отходов лечебно-профилактических учреждений».

ВНИМАНИЕ! При утилизации пробирок, содержащих продукты ПЦР после амплификации, недопустимо их открывание и разбрызгивание содержимого, поскольку это может привести к контаминации продуктами амплификации лабораторной зоны, оборудования и реагентов.

- Применять набор строго по назначению, согласно данной инструкции.
- Допускать к работе с набором только специально обученный персонал.
- Не использовать набор по истечении срока годности.
- Избегать контакта с кожей, глазами и слизистой оболочкой. При контакте немедленно промыть пораженное место водой и обратиться за медицинской помощью.
- Лист безопасности материалов (MSDS – material safety data sheet) доступен по запросу.

ДОПОЛНИТЕЛЬНЫЕ МАТЕРИАЛЫ И ОБОРУДОВАНИЕ

1. Комплект реагентов для выделения ДНК – «ДНК-сорб-В» (ТУ 9398-003-01897593-2009), «РИБО-преп» (ТУ 9398-071-01897593-2008) или другие рекомендованные ФГУН ЦНИИЭ Роспотребнадзора.
2. Дополнительные материалы и оборудование для экстракции ДНК – согласно инструкции к комплекту реагентов для выделения ДНК.
3. Бокс абактериальной воздушной среды (ПЦР-бокс).
4. Центрифуга/вортекс.
5. Автоматические дозаторы переменного объема (от 5 до 20 мкл, от 20 до 200 мкл).
6. Одноразовые наконечники с фильтром до 100 мкл в штативах.
7. Штативы для микропробирок объемом 0,2 мл или 0,5 мл (в соответствии с используемыми комплектами реагентов).
8. Холодильник от 2 до 8 °С с морозильной камерой не выше минус 16 °С для выделенных проб ДНК.
9. Отдельный халат, шапочки, обувь и одноразовые перчатки по МУ 1.3.1888-04.
10. Емкость для сброса наконечников.

<u>При детекции по «конечной точке»:</u>

11. Программируемый амплификатор (например, «Терцик» («ДНК-Технология», Россия), Gradient Palm Cycler (Corbett Research, Австралия), MaxyGene (Axygen, США), GeneAmp PCR System 2700 (Applied Biosystems, США) или аналогичные).
12. Флуоресцентный ПЦР-детектор (например, ALA-1/4 (BioSan,

Латвия), «Джин» («ДНК-Технология», Россия) или аналогичные).

13. Одноразовые полипропиленовые пробирки для ПЦР (с плоской крышкой, нестрипованные):
    а) объемом 0,2 мл (например, Axygen, США) – для амплификаторов, адаптированных для ПЦР-пробирок 0,2 мл (Gradient Palm Cycler, GeneAmp PCR System 2700, MaxyGene и др.);
    б) объемом 0,5 мл (например, Axygen, США) – для амплификаторов, адаптированных для ПЦР-пробирок 0,5 мл («Терцик» и др.).

<u>При детекции в режиме «реального времени»:</u>

14. Программируемый амплификатор с системой детекции флуоресцентного сигнала в режиме «реального времени» (например, Rotor-Gene 3000/6000 (Corbett Research, Австралия), Rotor-Gene Q (Qiagen, Германия), iQ5 (Bio-Rad, США), Mx3000P (Stratagene, США), «ДТ-96» («ДНК-Технология», Россия) или аналогичные).
15. Одноразовые полипропиленовые пробирки для ПЦР:
    а) на 0,2 мл (с плоской крышкой, нестрипованные; например, Axygen, США) для постановки в ротор на 36 пробирок – к приборам для ПЦР в реальном времени с детекцией через дно пробирки (например, «Rotor-Gene»).
    б) на 0,2 мл (с куполообразной крышкой; например, Axygen, США) – к приборам для ПЦР в реальном времени с детекцией через крышку (например, iQ5, Mx3000P).

ВЗЯТИЕ, ТРАНСПОРТИРОВАНИЕ И ХРАНЕНИЕ ИССЛЕДУЕМОГО МАТЕРИАЛА

Перед началом работы следует ознакомиться с методическими рекомендациями «Взятие, транспортировка, хранение клинического материала для ПЦР-диагностики», разработанными ФГУН ЦНИИЭ Роспотребнадзора, Москва, 2008 г.

Материалом для исследования служат образцы фекалий, концентраты образцов воды.

ПОДГОТОВКА ИССЛЕДУЕМОГО МАТЕРИАЛА К ЭКСТРАКЦИИ ДНК

Концентраты образцов воды не требуют специальной подготовки для экстракции ДНК. Подготовка образцов фекалий проводится в соответствии с методическими рекомендациями «Взятие, транспортировка, хранение клинического материала для ПЦР-диагностики», разработанными ФГУН ЦНИИЭ Роспотребнадзора, Москва, 2008 г.

ВАРИАНТ FEP

СОСТАВ

Комплект реагентов «ПЦР-комплект» вариант FEP/FRT-50 F – комплект реагентов для проведения реакции амплификации и дифференциации ДНК различных групп диарогенных эшерихий (*EPEC*, *ETEC*, *EIEC*, *EHEC*, *EAgEC*) – **включает:**

Реактив	*Описание*	*Объем, мл*	*Кол-во*
ПЦР-смесь-1-FEP/FRT *EIEC* / *EHEC* / STI	Прозрачная бесцветная жидкость	0,6	1 пробирка
ПЦР-смесь-1-FEP/FRT *EPEC* / *ETEC* / *EAgEC*	Прозрачная бесцветная жидкость	0,6	1 пробирка
ПЦР-смесь-2-FRT	Прозрачная бесцветная жидкость	0,3	2 пробирки
Полимераза (TaqF)	Прозрачная бесцветная жидкость	0,03	2 пробирки
ПКО ДНК *EIEC* / *EHEC* / STI	Прозрачная бесцветная жидкость	0,1	1 пробирка
ПКО ДНК *EPEC* / *ETEC* / *EAgEC*	Прозрачная бесцветная жидкость	0,1	1 пробирка
ДНК-буфер	Прозрачная бесцветная жидкость	0,5	1 пробирка
Минеральное масло для ПЦР	Бесцветная вязкая жидкость	8,0	1 флакон

Комплект реагентов рассчитан на проведение 55 реакций амплификации, включая контроли.

К комплекту реагентов прилагаются контрольные образцы этапа выделения:

Реактив	*Описание*	*Объем, мл*	*Кол-во*
ВКО-FL	Прозрачная бесцветная жидкость	1,0	1 пробирка
ОКО	Прозрачная бесцветная жидкость	1,2	1 пробирка

ПРОВЕДЕНИЕ ПЦР-ИССЛЕДОВАНИЯ

ПЦР-исследование состоит из следующих этапов:

- Экстракция (выделение) ДНК из исследуемых образцов.
- Амплификация ДНК/кДНК.
- Флуоресцентная детекция продуктов амплификации по «конечной точке».
- Интерпретация результатов.

ЭКСТРАКЦИЯ ДНК ИЗ ИССЛЕДУЕМЫХ ОБРАЗЦОВ

Экстракцию ДНК провести в соответствии с инструкцией к используемому комплекту реагентов для экстракции ДНК из клинического материала («ДНК-сорб-В», «РИБО-преп» или другие комплекты реагентов, рекомендованные ФГУН ЦНИИЭ Роспотребнадзора). Экстракция ДНК из каждого клинического образца проводится в присутствии внутреннего контрольного образца (ВКО-FL).

В качестве отрицательного контроля экстракции (ОК) используют **ОКО**.

ПРОВЕДЕНИЕ АМПЛИФИКАЦИИ И ДЕТЕКЦИИ ПО «КОНЕЧНОЙ ТОЧКЕ»

Общий объем реакционной смеси – 25 мкл, включая объем пробы ДНК – 10 мкл.

Выбор пробирок для амплификации зависит от используемого амплификатора.

Для внесения в пробирки реагентов, проб ДНК и контрольных образцов используются одноразовые наконечники с фильтрами.

В комплекте реагентов применяется «горячий старт», который обеспечивается использованием химически модифицированной Taq-полимеразы (TaqF-ДНК-полимераза), которая активируется при прогреве реакционной смеси при температуре 95 °С в течение 15 мин.

ВНИМАНИЕ! Компоненты реакционной смеси следует смешивать непосредственно перед проведением анализа. Смешивать реагенты из расчета на необходимое число реакций, включающее тестирование исследуемых и контрольных образцов, необходимо согласно расчетной таблице (см. табл. 1). Следует учитывать, что для тестирования даже одного исследуемого или контрольного образца ДНК необходимо проводить постановку всех контролей этапа ПЦР (положительного контроля (К+), отрицательного контроля (К-) и двух пробирок **«Фон»** для каждого типа смеси). Рекомендуется смешивать реагенты для четного числа реакций с целью более точного дозирования.

1. До начала работы все реагенты набора разморозить,

тщательно перемешать на вортексе и осадить капли с крышек пробирок.

2. Отобрать необходимое количество пробирок с учетом количества исследуемых, контрольных образцов ДНК реагентов и пробирок **«Фон»**. Тип пробирок, стрипов или плашек выбрать в зависимости от используемого прибора.
3. Для приготовления реакционных смесей и смесей для пробирок **«Фон»** необходимо в отдельной стерильной пробирке смешать одну из **ПЦР-смесей-1 (ПЦР-смесь-1-FEP/FRT *EIEC / EHEC* / STI** или **ПЦР-смесь-1-FEP/FRT *EPEC / ETEC / EAgEC*)** и **ПЦР-смесь-2-FRT** согласно табл. 1. Тщательно перемешать смеси на вортексе и осадить капли с крышек пробирок.
4. Приготовить 4 пробирки **«Фон»** (по две для каждого типа реакционной смеси). Для этого внести по **15 мкл** приготовленных смесей (без **полимеразы (TaqF)**) в две пробирки **«Фон»**, добавить по **10 мкл ДНК-буфера**, перемешать пипетированием. Сверху раскапать по **1 капле минерального масла для ПЦР** (примерно **25 мкл**).

Таблица 1

Схема приготовления реакционных смесей для ПЦР с детекцией по «конечной точке»

Объем реагента на одну реакцию (мкл)	10.00	5.00	0.50
Число реакций[1]	ПЦР-смесь-1-FEP/FRT	ПЦР-смесь-2-FRT	Полимераза (TaqF)
8	80	40	3.0
10	100	50	4.0
12	120	60	5.0
14	140	70	6.0
16	160	80	7.0
18	180	90	8.0
20	200	100	9.0
22	220	110	10.0
24	240	120	11.0
26	260	130	12.0
28	280	140	13.0
30	300	150	14.0
32	320	160	15.0
34	340	170	16.0

[1] Число клинических образцов, контроли этапа выделения ДНК (N), контроли этапа ПЦР и пробирки «Фон» с запасом на один образец (N+5+1).

5. В оставшиеся части реакционных смесей добавить **полимеразу (TaqF)** (во все смеси) в количестве, указанном в табл. 1. Тщательно перемешать смесь на вортексе и осадить капли с крышки пробирки.

ВНИМАНИЕ! Количество добавляемого в реакционную смесь фермента полимеразы (TaqF), указанное в табл. 1, приведено с учетом уже отобранных 30 мкл реакционной смеси для двух пробирок «Фон» (с вычетом двух пробирок «Фон»).

6. Внести в оставшиеся пробирки по **15 мкл** готовых реакционных смесей. Сверху раскапать по **1 капле минерального масла для ПЦР** (примерно **25 мкл**).
7. Используя наконечники с аэрозольными барьерами, в пробирки с реакционной смесью добавить по **10 мкл ДНК-проб**, выделенных из исследуемых или контрольных проб этапа выделения нуклеиновых кислот. Неиспользованные остатки реакционной смеси выбросить.

ВНИМАНИЕ! При добавлении ДНК-проб, выделенных с помощью комплектов реагентов «ДНК-сорб-В» и «РИБО-сорб», необходимо избегать попадания сорбента в реакционную смесь для ПЦР.

8. Поставить контрольные реакции амплификации:
   а) отрицательный контроль (К-) – внести в пробирки с реакционной смесью **10 мкл ДНК-буфера**;
   б) положительный контроль (К+$_1$) – для **ПЦР-смеси-1-FEP/FRT *EIEC* / *EHEC* / STI** внести в пробирки по **10 мкл ПКО ДНК *EIEC* / *EHEC* / STI**;
   в) положительный контроль (К+$_2$) – для **ПЦР-смеси-1-FEP/FRT *EPEC* / *ETEC* / *EAgEC*** внести в пробирки по **10 мкл ПКО ДНК *EPEC* / *ETEC* / *EAgEC***.

Рекомендуется перед постановкой в амплификатор осадить капли со стенок пробирок кратким центрифугированием на центрифуге/вортексе (1-3 с).

ВНИМАНИЕ! Пробы амплифицировать сразу после соединения реакционной смеси и ДНК-пробы и контролей. Запуск реакции на приборе должен произойти не позже, чем через 10-15 минут с момента внесения проб в реакционную смесь.

9. Запустить на амплификаторе программу амплификации (см. табл.).

Таблица 2

Программа амплификации ДНК

	Амплификаторы с активным регулированием температуры (по раствору в пробирке):						Амплификаторы с матричным регулированием температуры: **Uno-2 (Biometra), MiniCycler, PTC-100 (MJ Research)**		
	GeneAmp PCR System 2400 (Perkin Elmer), «Терцик» («ДНК-Технология»)			**GeneAmp PCR System 2700 (Applied Biosystems), Gradient Palm Cycler (Corbett Research)**					
цикл	темпе-ратура	время	кол-во циклов	темпе-ратура	время	кол-во циклов	темпе-ратура	время	кол-во циклов
0	**95 °C**	пауза		**95 °C**	Пауза		**95 °C**	пауза	
1	**95 °C**	15 мин	1	**95 °C**	15 мин	1	**95 °C**	15 мин	1
2	**95 °C**	10 с	42	**95 °C**	10 с	42	**95 °C**	1 мин	42
	60 °C	10 с		**60 °C**	25 с		**60 °C**	1 мин	
	72 °C	10 с		**72 °C**	25 с		**72 °C**	1 мин	
3	**72 °C**	1 мин	1	**72 °C**	1 мин	1	**72 °C**	1 мин	1
4	**10 °C**	хранение		**10 °C**	хранение		**10 °C**	хранение	

10. По окончании выполнения программы приступить к флуоресцентной детекции.

ФЛУОРЕСЦЕНТНАЯ ДЕТЕКЦИЯ ПРОДУКТОВ АМПЛИФИКАЦИИ ПО «КОНЕЧНОЙ ТОЧКЕ»

Детекция проводится с помощью флуоресцентного ПЦР-детектора (согласно инструкции к используемому прибору) путем измерения интенсивности флуоресцентного сигнала по трем каналам.

Таблица 3

Схема соответствия тестируемых патогенов и каналов флуоресцентной детекции

Канал детекции	**ПЦР-смесь-1-FEP/FRT *EIEC* / *EHEC* / STI**	**ПЦР-смесь-1-FEP/FRT *EPEC* / *ETEC* / *EAgEC***
FAM	ДНК ВКО-FL	ДНК *EAgEC*
HEX	ДНК *EHEC*	ДНК *EPEC*
ROX	ДНК *EIEC*	ДНК *ETEC*

ВНИМАНИЕ! До проведения детекции в программном обеспечении ПЦР-детектора должны быть внесены и сохранены соответствующие настройки – см. вкладыш, прилагаемый к ПЦР-комплекту, а также методические рекомендации по применению набора реагентов для выявления и дифференциации ДНК диарогенных *E.coli* в объектах окружающей среды и клиническом материале

методом полимеразной цепной реакции (ПЦР) с гибридизационно-флуоресцентной детекцией «АмплиСенс® Эшерихиозы-FL», разработанные ФГУН ЦНИИЭ Роспотребнадзора.

ИНТЕРПРЕТАЦИЯ РЕЗУЛЬТАТОВ

Полученные результаты интерпретируют на основании данных об уровне флуоресцентного сигнала относительно фона по соответствующим каналам для контрольных образцов и проб ДНК, выделенных из клинических образцов. Интерпретация производится автоматически с помощью программного обеспечения используемого прибора (см. табл. 4 и методические рекомендации к инструкции).

Таблица 4

Интерпретация результатов ПЦР-исследования

ПЦР-смесь-1	**Уровень флуоресценции**			**Результат**
	Канал FAM	**Канал HEX**	**Канал ROX**	
ПЦР-смесь-1-FEP/FRT *EIEC / EHEC* / STI	**Выше** порогового значения	Ниже порогового значения отрицательного результата	Ниже порогового значения отрицательного результата	В пробе не выявлена ДНК *EIEC* и *EHEC*
	Выше или Ниже порогового значения	**Выше** порогового значения положительного результата	Выше или Ниже порогового значения отрицательного результата	В пробе выявлена ДНК *EHEC*
	Выше или Ниже порогового значения	Выше или Ниже порогового значения отрицательного результата	**Выше** порогового значения положительного результата	В пробе выявлена ДНК *EIEC*
	Ниже порогового значения	Ниже порогового значения отрицательного результата	Ниже порогового значения отрицательного результата	Результат **невалидный** - проба требует повторной экстракции и амплификации

ВАРИАНТ FEP

ПЦР-смесь-1	Уровень флуоресценции			Результат
	Канал FAM	Канал HEX	Канал ROX	
ПЦР-смесь-1-FEP/FRT *EPEC / ETEC / EAgEC*	**Выше** порогового значения положительного результата	Выше или Ниже порогового значения отрицательного результата	Выше или Ниже порогового значения отрицательного результата	В пробе выявлена ДНК *EAgEC*
	Выше или Ниже порогового значения отрицательного результата	**Выше** порогового значения положительного результата	Выше или Ниже порогового значения отрицательного результата	В пробе выявлена ДНК *EPEC**
	Выше или Ниже порогового значения отрицательного результата	Выше или Ниже порогового значения отрицательного результата	**Выше** порогового значения положительного результата	В пробе выявлена ДНК *ETEC*
	Ниже порогового значения отрицательного результата	Ниже порогового значения отрицательного результата	Ниже порогового значения отрицательного результата	В пробе не выявлены *EPEC / ETEC / EAgEC*[2]

***** Если для данного образца выявлен уровень флуоресценции выше порогового значения положительного результата по каналу HEX при использовании **ПЦР-смеси-1-FEP/FRT *EIEC / EHEC /* STI**, то результат интерпретируется как «В пробе выявлена ДНК *EHEC*».

Если значение уровня флуоресценции для пробы находится между пороговыми значениями положительного и отрицательного результата, он расценивается как **сомнительный** и требует повторения ПЦР-исследования соответствующего исследуемого образца.

Результат ПЦР-исследования считается достоверным, если получены правильные результаты для положительного и отрицательного контролей амплификации и отрицательного контроля выделения ДНК в соответствии с табл. 5.

[2] При уровне флуоресценции выше порогового значения по каналу FAM на ПЦР-смеси-1-FEP/FRT *EIEC* / *EHEC* / STI.

Таблица 5

Результаты для контролей различных этапов ПЦР-исследования

ПЦР-смесь-1	Контроль	Контроли-руемый этап	Канал FAM	Канал HEX	Канал ROX
ПЦР-смесь-1-FEP/FRT *EIEC / EHEC* / STI	ОК	Экстракция ДНК	Выше порогового значения	Ниже порогового значения отрицательного результата	Ниже порогового значения отрицательного результата
	К-	ПЦР	Ниже порогового значения	Ниже порогового значения отрицательного результата	Ниже порогового значения отрицательного результата
	К+$_1$	ПЦР	Выше порогового значения	Выше порогового значения положительного результата	Выше порогового значения положительного результата
ПЦР-смесь-1-FEP/FRT *EPEC / ETEC / EAgEC*	ОК	Экстракция ДНК	Ниже порогового значения отрицательного результата	Ниже порогового значения отрицательного результата	Ниже порогового значения отрицательного результата
	К-	ПЦР	Ниже порогового значения отрицательного результата	Ниже порогового значения отрицательного результата	Ниже порогового значения отрицательного результата
	К+$_2$	ПЦР	Выше порогового значения положительного результата	Выше порогового значения положительного результата	Выше порогового значения положительного результата

ВНИМАНИЕ!

1. Если для положительного контроля амплификации (К+) сигнал по каналам HEX, FAM или ROX ниже порогового значения положительного результата, необходимо повторить амплификацию и детекцию для всех образцов, в которых сигнал по каналам HEX, FAM или ROX был ниже порогового значения положительного результата на соответствующем типе ПЦР-смеси-1.
2. Если для отрицательного контроля выделения ДНК (ОК) (кроме **ПЦР-смеси-1-FEP/FRT *EIEC / EHEC* / STI** по каналу

FAM) и/или отрицательного контроля амплификации (К-) (по всем каналам) сигнал выше порогового значения положительного результата, необходимо повторить ПЦР-исследование для всех образцов, в которых обнаружена ДНК соответствующих патогенов, начиная с этапа выделения (экстракции) ДНК.

ВАРИАНТ FRT

СОСТАВ

Комплект реагентов «ПЦР-комплект» вариант FEP/FRT-50 F – комплект реагентов для проведения реакции амплификации и дифференциации ДНК различных групп диарогенных эшерихий (*EPEC*, *ETEC*, *EIEC*, *EHEC*, *EAgEC*) – **включает:**

Реактив	*Описание*	*Объем, мл*	*Кол-во*
ПЦР-смесь-1-FEP/FRT *EIEC* / *EHEC* / STI	Прозрачная бесцветная жидкость	0,6	1 пробирка
ПЦР-смесь-1-FEP/FRT *EPEC* / *ETEC* / *EAgEC*	Прозрачная бесцветная жидкость	0,6	1 пробирка
ПЦР-смесь-2-FRT	Прозрачная бесцветная жидкость	0,3	2 пробирки
Полимераза (TaqF)	Прозрачная бесцветная жидкость	0,03	2 пробирки
ПКО ДНК *EIEC* / *EHEC* / STI	Прозрачная бесцветная жидкость	0,1	1 пробирка
ПКО ДНК *EPEC* / *ETEC* / *EAgEC*	Прозрачная бесцветная жидкость	0,1	1 пробирка
ДНК-буфер	Прозрачная бесцветная жидкость	0,5	1 пробирка
Минеральное масло для ПЦР	Бесцветная вязкая жидкость	8,0	1 флакон

Комплект реагентов рассчитан на проведение 55 реакций амплификации, включая контроли.

К комплекту реагентов прилагаются контрольные образцы выделения:

Реактив	*Описание*	*Объем, мл*	*Кол-во*
ВКО-FL	Прозрачная бесцветная жидкость	1,0	1 пробирка
ОКО	Прозрачная бесцветная жидкость	1,2	1 пробирка

ПРОВЕДЕНИЕ ПЦР-ИССЛЕДОВАНИЯ

ПЦР-исследование состоит из следующих этапов:

- Экстракция (выделение) ДНК из исследуемых образцов.
- Проведение амплификации с детекцией в режиме «реального времени».
- Анализ и интерпретация результатов.

ЭКСТРАКЦИЯ (ВЫДЕЛЕНИЕ) ДНК ИЗ ИССЛЕДУЕМЫХ ОБРАЗЦОВ

Экстракцию ДНК провести в соответствии с инструкцией к используемому комплекту реагентов для экстракции ДНК из клинического материала («ДНК-сорб-В», «РИБО-сорб», «РИБО-преп» или другие комплекты реагентов, рекомендованные ФГУН ЦНИИЭ Роспотребнадзора). Экстракция ДНК из каждого клинического образца проводится в присутствии внутреннего контрольного образца (ВКО-FL).

В качестве отрицательного контроля выделения (ОК) используют ОКО.

ПРОВЕДЕНИЕ АМПЛИФИКАЦИИ С ДЕТЕКЦИЕЙ В РЕЖИМЕ «РЕАЛЬНОГО ВРЕМЕНИ»

Общий объем реакционной смеси – 25 мкл, включая объем пробы ДНК – 10 мкл.

Выбор пробирок для амплификации зависит от используемого амплификатора с системой детекции в режиме «реального времени».

Для внесения в пробирки реагентов, проб ДНК и контрольных образцов используются одноразовые наконечники с фильтрами.

ВНИМАНИЕ! Компоненты реакционных смесей следует смешивать непосредственно перед проведением анализа. Смешивать реагенты из расчета на необходимое число реакций, включающее тестирование исследуемых и контрольных образцов, необходимо согласно **расчетной таблице** (см. таблицу 6). Следует учитывать, что **для тестирования даже одного исследуемого образца ДНК необходимо проводить постановку всех контролей этапа ПЦР (положительного контроля (К+) и отрицательного контроля (К-) для каждого типа смеси).** Рекомендуется смешивать реагенты для четного числа реакций с целью более точного дозирования.

1. До начала работы все реагенты набора разморозить, тщательно перемешать на вортексе и осадить капли с крышек пробирок.
2. Отобрать необходимое количество пробирок с учетом количества исследуемых, контрольных образцов ДНК. Тип пробирок, стрипов или плашек выбрать в зависимости от

используемого прибора.

3. Для приготовления реакционных смесей необходимо в отдельной стерильной пробирке смешать одну из **ПЦР-смесей-1 (ПЦР-смесь-1-FEP/FRT *EIEC / EHEC* / STI** или **ПЦР-смесь-1-FEP/FRT *EPEC / ETEC / EAgEC*)**, **ПЦР-смесь-2-FRT** и **полимеразу (TaqF)** в количестве, указанном в таблице 6. Тщательно перемешать смеси на вортексе и осадить капли с крышек пробирок.
4. Внести в отобранные пробирки по **15 мкл** готовых реакционных смесей.

Таблица 6

Схема приготовления реакционных смесей для ПЦР с детекцией в режиме «реального времени»

Объем реагента на одну реакцию (мкл)	10.00	5.00	0.50
Число реакций[3]	ПЦР-смесь-1-FEP/FRT	ПЦР-смесь-2-FRT	Полимераза (TaqF)
6	60	30	3.0
8	80	40	4.0
10	100	50	5.0
12	120	60	6.0
14	140	70	7.0
16	160	80	8.0
18	180	90	9.0
20	200	100	10.0
22	220	110	11.0
24	240	120	12.0
26	260	130	13.0
28	280	140	14.0
30	300	150	15.0
32	320	160	16.0

5. Используя наконечники с аэрозольными барьерами, в пробирки с реакционной смесью добавить по **10 мкл ДНК-проб**, выделенных из исследуемых или контрольных проб этапа выделения нуклеиновых кислот. Неиспользованные остатки реакционной смеси выбросить.

ВНИМАНИЕ! При добавлении ДНК-проб, выделенных с помощью комплектов реагентов «ДНК-сорб-В» и «РИБО-сорб», необходимо избегать попадания сорбента в реакционную смесь для ПЦР.

[3] Число клинических образцов, контроли этапа выделения ДНК (N), контроли этапа ПЦР с запасом на один образец (N+3+1).

6. Поставить контрольные реакции амплификации:
   а) отрицательный контроль (К-) – внести в пробирки с реакционной смесью **10 мкл ДНК-буфера**;
   б) положительный контроль (К+$_1$) – для **ПЦР-смеси-1-FEP/FRT *EIEC* / *EHEC* / STI** внести в пробирки по **10 мкл ПКО ДНК *EIEC* / *EHEC* / STI**;
   в) положительный контроль (К+$_2$) – для **ПЦР-смеси-1-FEP/FRT *EPEC* / *ETEC* / *EAgEC*** внести в пробирки по **10 мкл ПКО ДНК *EPEC* / *ETEC* / *EAgEC***.
7. Запрограммировать прибор (амплификатор с системой детекции в режиме «реального времени») для выполнения соответствующей программы амплификации и детекции флуоресцентного сигнала (см. табл. 7 и методические рекомендации к инструкции).

Таблица 7

Программа амплификации

	Приборы роторного типа[4]			**Приборы планшетного типа[5]**		
Цикл	Температура, °С	Время	Кол-во циклов	Температура, °С	Время	Кол-во циклов
1	**95**	15 мин	1	**95**	15 мин	1
2	**95**	10 с	45	**95**	10 с	45
	60	25 с детекция флуоресц. сигнала		**60**	25 с детекция флуоресц. сигнала	
	72	10 с		**72**	10 с	

Детекция флуоресцентного сигнала назначается по каналам для флуорофоров FAM, JOE и ROX (при одновременном проведении нескольких тестов назначается детекция и по другим используемым каналам).

8. Установить пробирки в ячейки реакционного модуля прибора.
9. Запустить выполнение программы амплификации с детекцией флуоресцентного сигнала.
10. По окончании выполнения программы приступить к анализу и учету результатов.

[4] Например, Rotor-Gene 3000, Rotor-Gene 6000, Rotor-Gene Q или аналогичные.
[5] Например, iCycler, iQ5, Mx3000P, Mx3000, «ДТ-96» или аналогичные.

АНАЛИЗ И ИНТЕРПРЕТАЦИЯ РЕЗУЛЬТАТОВ

Анализ результатов проводят с помощью программного обеспечения используемого прибора для проведения ПЦР с детекцией в режиме «реального времени». Анализируют кривые накопления флуоресцентного сигнала по трем каналам: FAM/Green, JOE/Yellow/HEX и ROX/Orange.

Результаты интерпретируются на основании наличия (или отсутствия) пересечения кривой флуоресценции с установленной на соответствующем уровне пороговой линией, что определяет наличие (или отсутствие) для данной пробы ДНК значения порогового цикла «Ct» в соответствующей графе в таблице результатов.

Результаты интерпретируются в соответствии с табл. 8 и вкладышем к набору реагентов **«АмплиСенс® Эшерихиозы-FL»**.

Таблица 8

Интерпретация результатов ПЦР-исследования

ПЦР-смесь-1	Значение порогового цикла			Результат
	Канал FAM	Канал HEX	Канал ROX	
ПЦР-смесь-1-FEP/FRT *EIEC / EHEC / STI*	**Меньше** граничного значения	Значение отсутствует или больше граничного значения	Значение отсутствует или больше граничного значения	В пробе не выявлена ДНК *EIEC* и *EHEC*
	Больше или меньше граничного значения	**Меньше** граничного значения	Больше или меньше граничного значения	В пробе выявлена ДНК *EHEC*
	Больше или меньше граничного значения	Больше или меньше граничного значения	**Меньше** граничного значения	В пробе выявлена ДНК *EIEC*
	Больше граничного значения	Больше граничного значения	Больше граничного значения	Результат **невалидный** - проба требует повторной экстракции и амплификации

ВАРИАНТ FRT

ПЦР-смесь-1	Значение порогового цикла			Результат
	Канал FAM	Канал HEX	Канал ROX	
ПЦР-смесь-1-FEP/FRT *EPEC / ETEC / EAgEC*	**Меньше** граничного значения	Больше или меньше граничного значения	Больше или меньше граничного значения	В пробе выявлена ДНК *EAgEC*
	Больше или меньше граничного значения	**Меньше** граничного значения	Больше или меньше граничного значения	В пробе выявлена ДНК *EPEC**
	Больше или меньше граничного значения	Больше или меньше граничного значения	**Меньше** граничного значения	В пробе выявлена ДНК *ETEC*
	Значение отсутствует или больше граничного значения	Значение отсутствует или больше граничного значения	Значение отсутствует или больше граничного значения	В пробе не выявлены *EPEC / ETEC /EAgEC*[6]

***** Если для данного образца выявлено *Ct* меньше граничного значения по каналу HEX при использовании **ПЦР-смеси-1-FEP/FRT *EIEC / EHEC* / STI**, то результат интерпретируется как «В пробе выявлена ДНК *EHEC*».

ВНИМАНИЕ! Граничные значения Ct указаны во вкладыше, прилагаемом к ПЦР-комплекту. См. также методические рекомендации по применению набора реагентов для выявления и дифференциации ДНК диарогенных *E.coli* в объектах окружающей среды и клиническом материале методом полимеразной цепной реакции (ПЦР) с гибридизационно-флуоресцентной детекцией «АмплиСенс® Эшерихиозы-FL», разработанные ФГУН ЦНИИЭ Роспотребнадзора.

Результат ПЦР-исследования считается достоверным, если получены правильные результаты для положительного и отрицательного контролей амплификации и отрицательного контроля выделения ДНК, в соответствии с таблицей оценки результатов контрольных реакций (табл. 9).

[6] При значении порогового цикла меньше граничного по каналу FAM на ПЦР-смеси-1-FEP/FRT *EIEC*. / *EHEC* / STI.

Таблица 9

Результаты контролей различных этапов ПЦР-исследования

ПЦР-смесь-1	Контроль	Контроли-руемый этап	Канал FAM	Канал HEX	Канал ROX
ПЦР-смесь-1-FEP/FRT *EIEC / EHEC / STI*	ОК	Экстракция ДНК	**Меньше** граничного значения	значение отсутствует или больше граничного значения	значение отсутствует или больше граничного значения
	К-	ПЦР	значение отсутствует или больше граничного значения	значение отсутствует или больше граничного значения	значение отсутствует или больше граничного значения
	$К+_1$	ПЦР	**Меньше** граничного значения	**Меньше** граничного значения	**Меньше** граничного значения
ПЦР-смесь-1-FEP/FRT *EPEC / ETEC / EAgEC*	ОК	Экстракция ДНК	значение отсутствует или больше граничного значения	значение отсутствует или больше граничного значения	значение отсутствует или больше граничного значения
	К-	ПЦР	значение отсутствует или больше граничного значения	значение отсутствует или больше граничного значения	значение отсутствует или больше граничного значения
	$К+_2$	ПЦР	**Меньше** граничного значения	**Меньше** граничного значения	**Меньше** граничного значения

ВНИМАНИЕ!

1. Если для положительного контроля этапа ПЦР (К+) сигнал по каналам HEX, FAM или ROX отсутствует или больше граничного значения, необходимо повторить амплификацию и детекцию для всех образцов, в которых сигнал по каналам HEX, FAM или ROX не была выявлена ДНК различных групп диарогенных эшерихий на соответствующем типе ПЦР-смеси-1*.*
2. Если для отрицательного контроля этапа экстракции ДНК (ОК) (кроме **ПЦР-смеси-1-FEP/FRT *EIEC / EHEC / STI*** по каналу FAM) и/или отрицательного контроля этапа ПЦР (К-) (по всем каналам) сигнал меньше граничного значения, необходимо повторить ПЦР-исследование для всех образцов, в которых обнаружена ДНК

соответствующих патогенов, начиная с этапа выделения (экстракции) ДНК.

СРОК ГОДНОСТИ. УСЛОВИЯ ТРАНСПОРТИРОВАНИЯ И ХРАНЕНИЯ

Срок годности. 9 мес. Набор реагентов с истекшим сроком годности применению не подлежит.

Транспортирование. Набор реагентов транспортировать при температуре от 2 до 8 °С не более 5 сут. «ПЦР-комплект» вариант FEP/FRT-50 F при получении разукомплектовать в соответствии с указанными температурами хранения.

Хранение. Набор реагентов хранить при температуре от 2 до 8 °С (кроме ПЦР-смеси-1-FEP/FRT *EIEC / EHEC /* STI, ПЦР-смеси-1-FEP/FRT *EPEC / ETEC / EAgEC*, ПЦР-смеси-2-FRT, полимеразы (TaqF)). ПЦР-смесь-1-FEP/FRT *EIEC / EHEC /* STI, ПЦР-смесь-1-FEP/FRT *EPEC / ETEC / EAgEC*, ПЦР-смесь-2-FRT и полимеразу (TaqF) хранить при температуре не выше минус 16 °С.

Условия отпуска. Для лечебно-профилактических и санитарно-профилактических учреждений.

Рекламации на качество набора реагентов **«АмплиСенс® Эшерихиозы-FL»** направлять в адрес ФГУН Государственный научно-исследовательский институт стандартизации и контроля медицинских биологических препаратов им. Л.А. Тарасевича Роспотребнадзора (119002 г. Москва, пер. Сивцев Вражек, д. 41), тел./факс (499) 241-39-22, а также на предприятие-изготовитель ФГУН ЦНИИЭ Роспотребнадзора (111123 г. Москва, ул. Новогиреевская, д. 3а), тел. (495) 974-96-42, факс (495) 305-54-23, e-mail: obtk@pcr.ru, и в отдел по работе с рекламациями и организации обучения (тел. (495) 925-05-54, факс (495) 916-18-18, e-mail: products@pcr.ru).

Отзывы и предложения о продукции **«АмплиСенс®»** можно оставить, заполнив анкету потребителя на сайте www.amplisens.ru.

Заведующий НПЛ
ФГУН ЦНИИЭ Роспотребнадзора — Е.Н. Родионова

Руководитель Государственных испытаний — Г.М.Игнатьев

Зав. лабораторией вирусных кишечных инфекций
и молекулярной биологии ФГУН ГИСК им.Тарасевича Роспотребнадзора

Приказом Росздравнадзора
от 12.07.2013 № 3154-Пр/13

УТВЕРЖДАЮ
Директор Федерального бюджетного учреждения науки «Центральный научно-исследовательский институт эпидемиологии» Федеральной службы по надзору в сфере защиты прав потребителей и благополучия человека
_____ В.И.Покровский
«03» 12 2012 г.

ИНСТРУКЦИЯ

по применению набора реагентов
для выявления генов карбапенемаз групп KPC и OXA-48
в биологическом материале методом полимеразной цепной реакции (ПЦР) с гибридизационно-флуоресцентной детекцией

«АмплиСенс® MDR KPC/OXA-48-FL»

АмплиСенс®

ФБУН ЦНИИ Эпидемиологии
Роспотребнадзора,
Российская Федерация, 111123,
город Москва, улица Новогиреевская, дом 3а

IVD

ОГЛАВЛЕНИЕ

СПИСОК СОКРАЩЕНИЙ

В настоящей инструкции применяются следующие сокращения и обозначения:

БАЛ	- Бронхоальвеолярный лаваж
ВКО-FL	- Внутренний контрольный образец для наборов с гибридизационно-флуоресцентной детекцией
В–	- Отрицательный контроль экстракции
К+	- Положительный контроль ПЦР
К–	- Отрицательный контроль ПЦР
ОКО	- Отрицательный контрольный образец
ПКО	- Положительный контрольный образец
ПЦР	- Полимеразная цепная реакция
ФБУН ЦНИИ Эпидемиологии Роспотребнадзора	- Федеральное бюджетное учреждение науки «Центральный научно-исследовательский институт эпидемиологии» Федеральной службы по надзору в сфере защиты прав потребителей и благополучия человека
MDR	- Полирезистентность (Multidrug-resistance)
FRT	- Флуоресцентная детекция в режиме «реального времени»

НАЗНАЧЕНИЕ

Набор реагентов **«АмплиСенс® MDR KPC/OXA-48-FL»** предназначен для выявления генов приобретенных карбапенемаз групп KPC и OXA-48-подобных (типы OXA-48 и OXA-162) методом ПЦР с гибридизационно-флуоресцентной детекцией продуктов амплификации в режиме «реального времени». Материалом для проведения ПЦР служат пробы ДНК, полученные путем экстракции из образцов чистой бактериальной культуры, положительной гемокультуры, смеси бактериальных культур, полученной путем первичного посева клинического материала (ликвора, БАЛ, раневого отделяемого и др.) на плотные или жидкие питательные среды, а также из образцов клинического материала: мочи, мазков со слизистых оболочек ротоглотки, прямой кишки.

ВНИМАНИЕ! Результаты ПЦР-исследования учитываются в комплексной диагностике заболевания.[1]

ПРИНЦИП МЕТОДА

Выявление фрагментов ДНК генов приобретенных карбапенемаз групп KPC и OXA-48-подобных методом полимеразной цепной реакции (ПЦР) с гибридизационно-флуоресцентной детекцией включает в себя два этапа:

[1] В соответствии с Директивой Европейского Союза 98/79/EC.

экстракцию ДНК из образцов биологического материала, амплификацию фрагментов выявляемых генов с гибридизационно-флуоресцентной детекцией, которая производится непосредственно в ходе ПЦР. Экстракция ДНК из биологического материала проводится в присутствии внутреннего контрольного образца (ВКО-FL), который позволяет контролировать выполнение процедуры исследования для каждого образца. Затем с полученными пробами ДНК проводится реакция амплификации при помощи специфичных праймеров и фермента Taq-полимеразы. В составе реакционной смеси присутствуют флуоресцентно-меченые олигонуклеотидные зонды, которые гибридизуются с комплементарным участком амплифицируемой ДНК-мишени, в результате чего происходит нарастание интенсивности флуоресценции. Это позволяет регистрировать накопление специфического продукта амплификации путем измерения интенсивности флуоресцентного сигнала. Результаты амплификации фрагментов генов карбапенемаз групп KPC и OXA-48-подобных регистрируются по двум различным каналам флуоресцентной детекции: для группы KPC – по каналу для флуорофора FAM, для группы OXA-48-подобных – по каналу для флуорофора JOE. По каналу для флуорофора ROX детектируется продукт амплификации ДНК ВКО (внутреннего контрольного образца).

Канал для флуорофора	FAM[2]	JOE[2]	ROX[2]
ДНК-мишень	Гены карбапенемаз группы KPC	Гены карбапенемаз группы OXA-48-подобных	ВКО

ФОРМАТЫ И ФОРМЫ ВЫПУСКА НАБОРА РЕАГЕНТОВ

Набор реагентов выпускается в 1 формате.

Формат FRT

Набор реагентов выпускается в 2 формах комплектации:

Форма 1 включает комплект реагентов «ПЦР-комплект» вариант FRT-100 F.

Форма 2 включает наборы реагентов оптом, расфасованные по отдельным реагентам, с маркировкой реагентов на их оптовой фасовке.

[2] Или аналогичный канал для детекции указанного флуорофора в зависимости от используемого прибора.

Форма комплектации 1 предназначена для проведения амплификации фрагментов генов карбапенемаз групп KPC и OXA-48-подобных с гибридизационно-флуоресцентной детекцией в режиме «реального времени». Для проведения полного ПЦР-исследования необходимо использовать комплекты реагентов для экстракции ДНК, рекомендованные ФБУН ЦНИИ Эпидемиологии Роспотребнадзора.

Форма комплектации 2 предназначена для производственных целей для последующей маркировки на языке заказчика и комплектации по наборам.

ВНИМАНИЕ! Форма комплектации 2 используется только в соответствии с регламентом, утвержденным ФБУН ЦНИИ Эпидемиологии Роспотребнадзора.

АНАЛИТИЧЕСКИЕ ХАРАКТЕРИСТИКИ

Аналитическая чувствительность

Вид биологического материала	Транспортная среда	Комплект/реагент для экстракции ДНК	Аналитическая чувствительность, копий/мл[3]
Гемокультура, смесь бактериальных культур, полученная путем посева клинического материала на жидкую или плотную[4] питательную среду	-	«ГК-экспресс»	$5x10^5$
		«ДНК-сорб-АМ»	$1x10^5$
Моча	-	«ДНК-сорб-АМ»	$5x10^2$
		«РИБО-преп»	
Мазки со слизистых оболочек ротоглотки, прямой кишки	«Транспортная среда для мазков» или «Транспортная среда с муколитиком (ТСМ)»	«ДНК-сорб-АМ»	$2x10^3$

[3] Данная чувствительность достигается при соблюдении правил предварительной обработки образцов биоматериала, изложенных ниже, и рекомендуемом объеме исследуемого образца.

[4] Для бактериальных культур, полученных путем посева на плотную питательную среду, указана чувствительность в отношении суспензии бактериальных клеток в реагенте «ГК-экспресс» или в лизирующем растворе «ДНК-сорб-АМ», соответственно.

С использованием данного набора реагентов были выявлены гены карбапенемаз соответствующих групп при анализе образцов ДНК контрольных штаммов, несущих гены известных карбапенемаз типов KPC-3 и OXA-48.

Аналитическая специфичность

Отсутствовали неспецифические реакции при тестировании образцов ДНК человека и образцов ДНК следующих микроорганизмов: *Escherichia coli, Klebsiella pneumoniae, Klebsiella oxytoca, Serratia marcescens, Pseudomonas aeruginosa, Acinetobacter baumannii, Proteus mirabilis, Enterococcus faecalis, Staphylococcus* spp., *Streptococcus* spp., *Candida* spp.

МЕРЫ ПРЕДОСТОРОЖНОСТИ

Работа должна проводиться в лаборатории, выполняющей молекулярно-биологические (ПЦР) исследования биологического материала на наличие возбудителей инфекционных болезней, с соблюдением санитарно-эпидемических правил СП 1.3.2322-08 «Безопасность работы с микроорганизмами III–IV групп патогенности (опасности) и возбудителями паразитарных болезней», СанПиН 2.1.7.2790-10 «Санитарно-эпидемиологические требования к обращению с медицинскими отходами» и методических указаний МУ 1.3.2569-09 «Организация работы лабораторий, использующих методы амплификации нуклеиновых кислот при работе с материалом, содержащим микроорганизмы I-IV групп патогенности».

При работе всегда следует выполнять следующие требования:

- Следует рассматривать исследуемые образцы как инфекционно-опасные, организовывать работу и хранение в соответствии с СП 1.3.2322-08 «Безопасность работы с микроорганизмами III–IV групп патогенности (опасности) и возбудителями паразитарных болезней».
- Убирать и дезинфицировать разлитые образцы или реактивы, используя дезинфицирующие средства в соответствии с СП 1.3.2322-08 «Безопасность работы с микроорганизмами III–IV групп патогенности (опасности) и возбудителями паразитарных болезней».
- Лабораторный процесс должен быть однонаправленным. Анализ проводится в отдельных помещениях (зонах). Работу

следует начинать в Зоне Выделения, продолжать в Зоне Амплификации и Детекции. Не возвращать образцы, оборудование и реактивы в зону, в которой была проведена предыдущая стадия процесса.

- Неиспользованные реактивы, реактивы с истекшим сроком годности, а также использованные реактивы следует удалять в соответствии с требованиями СанПиН 2.1.7.2790-10 «Санитарно-эпидемиологические требования к обращению с медицинскими отходами».

ВНИМАНИЕ! При удалении отходов после амплификации (пробирок, содержащих продукты ПЦР) недопустимо открывание пробирок и разбрызгивание содержимого, поскольку это может привести к контаминации продуктами ПЦР лабораторной зоны, оборудования и реагентов.

- Использовать и менять при каждой операции одноразовые наконечники для автоматических дозаторов с фильтром. Одноразовую пластиковую посуду необходимо сбрасывать в специальный контейнер, содержащий дезинфицирующее средство, которое может быть использовано для обеззараживания медицинских отходов.
- Поверхности столов, а также помещения, в которых проводится постановка ПЦР, до начала и после завершения работ необходимо подвергать ультрафиолетовому облучению в течение 30 мин.
- Применять набор строго по назначению, согласно данной инструкции.
- Допускать к работе с набором только специально обученный персонал.
- Не использовать набор по истечении срока годности.
- Использовать одноразовые перчатки, лабораторные халаты, защищать глаза во время работы с образцами и реактивами. Тщательно вымыть руки по окончании работы.
- Избегать контакта с кожей, глазами и слизистой оболочкой. При контакте немедленно промыть пораженное место водой и обратиться за медицинской помощью.
- Листы безопасности материалов (MSDS – material safety data sheet) доступны по запросу.

ДОПОЛНИТЕЛЬНЫЕ МАТЕРИАЛЫ И ОБОРУДОВАНИЕ

Проведение предварительной подготовки исследуемого материала

1. Одноразовые полипропиленовые завинчивающиеся или плотно закрывающиеся пробирки объемом 1,5 мл (например, Axygen, США).

Проведение экстракции ДНК из исследуемых образцов

2. Комплект реагентов для выделения ДНК – «ДНК-сорб-АМ», «РИБО-преп» или «ГК-экспресс» или другие комплекты, рекомендованные ФБУН ЦНИИ Эпидемиологии Роспотребнадзора. Дополнительные материалы и оборудование для экстракции ДНК – согласно инструкции к комплекту реагентов/реагенту для экстракции ДНК.

Проведение амплификации с гибридизационно-флуоресцентной детекцией продуктов амплификации

3. Бокс абактериальной воздушной среды (ПЦР-бокс) (например, «БАВ-«Ламинар.-с», «Ламинарные системы», Россия).
4. Центрифуга/вортекс (например, «ТЭТА-2», «Биоком», Россия).
5. Автоматические дозаторы переменного объема (от 5 до 20 мкл и от 20 до 200 мкл) (например, «Ленпипет», Россия).
6. Одноразовые наконечники с фильтром до 100 мкл в штативах (например, Axygen, США).
7. Штативы для пробирок объемом 0,2 мл или 0,1 мл (например, «ИнтерЛабСервис», Россия).
8. Холодильник от 2 до 8 °С с морозильной камерой не выше минус 16 °С для выделенных проб ДНК.
9. Отдельный халат, шапочки, обувь и одноразовые перчатки по МУ 1.3.2569-09.
10. Емкость для сброса наконечников.
11. Программируемый амплификатор с системой детекции флуоресцентного сигнала в режиме «реального времени» (например, Rotor-Gene 6000 (Corbett Research, Австралия), Rotor-Gene Q (QIAGEN, Германия), CFX96 (Bio-Rad, США) и рекомендованные ФБУН ЦНИИ Эпидемиологии Роспотребнадзора в методических рекомендациях по применению данного набора реагентов).
12. Одноразовые полипропиленовые пробирки для ПЦР объемом 0,2 мл или 0,1 мл:

а) тонкостенные пробирки для ПЦР объемом 0,2 мл с круглой или плоской оптически прозрачной крышкой (например, Axygen, США) – при использовании прибора планшетного типа;
б) тонкостенные пробирки для ПЦР объемом 0,2 мл с плоской крышкой (например, Axygen, США) или пробирки для ПЦР к Rotor-Gene, объемом 0,1 мл в стрипах по 4 шт. с крышками (например, Corbett Research, Австралия; QIAGEN, Германия) – при использовании прибора роторного типа.

ВЗЯТИЕ, ТРАНСПОРТИРОВАНИЕ И ХРАНЕНИЕ ИССЛЕДУЕМОГО МАТЕРИАЛА

Перед началом работы следует ознакомиться с методическими рекомендациями «Взятие, транспортировка, хранение клинического материала для ПЦР-диагностики», разработанными ФГУН ЦНИИЭ Роспотребнадзора, Москва, 2010 г.

Материалом для исследования служат положительная гемокультура, смесь бактериальных культур, полученная путем первичного посева клинического материала (ликвора, БАЛ, раневого отделяемого, мочи и др.) на плотные или жидкие питательные среды, чистая бактериальная культура, а также образцы клинического материала: моча (при острых инфекциях мочевыводящих путей), мазки со слизистых оболочек ротоглотки, прямой кишки (при проведении скрининга колонизации бактериями, обладающими приобретенными карбапенемазами).

Мазки со слизистых оболочек ротоглотки или прямой кишки должны быть помещены в транспортную среду «Транспортная среда для мазков» или «Транспортная среда с муколитиком (ТСМ)» производства ФБУН ЦНИИ Эпидемиологии Роспотребнадзора.

ПОДГОТОВКА ИССЛЕДУЕМОГО МАТЕРИАЛА К ЭКСТРАКЦИИ ДНК

Гемокультура, смесь бактериальных культур, полученная путем первичного посева клинического материала на жидкую питательную среду

Перенести от 0,1 до 0,25 мл гемокультуры или посева на среду обогащения в стерильную одноразовую пробирку объемом 1,5 мл (с помощью одноразового шприца).

Центрифугировать 10 мин при 10000 g (12 тыс об/мин на центрифуге MiniSpin, Eppendorf). Используя вакуумный отсасыватель с колбой-ловушкой, полностью удалить надосадочную жидкость, не захватывая осадок и используя для каждого образца отдельный наконечник без фильтра.

Моча

Взболтать флакон с мочой. Перенести 1 мл мочи в стерильную одноразовую пробирку объемом 1,5 мл, используя отдельный наконечник с фильтром для каждого образца. Центрифугировать 10 мин при 10000 g (12 тыс об/мин на центрифуге MiniSpin, Eppendorf). При наличии большого количества солей ресуспендировать только верхний слой осадка солей в объеме 1 мл и затем снова центрифугировать. Используя вакуумный отсасыватель с колбой-ловушкой, полностью удалить надосадочную жидкость, не захватывая осадок и используя для каждого образца отдельный наконечник без фильтра.

С полученными после предварительной обработки образцами (осадками) провести процедуру экстракции ДНК в соответствии с инструкцией к используемому комплекту реагентов.

Полученные после предварительной обработки образцы (осадки) можно хранить:

– при температуре не выше минус 16 °С – в течение недели,
– при температуре не выше минус 68 °С – длительно.

ФОРМАТ FRT

СОСТАВ

Комплект реагентов «ПЦР-комплект» вариант FRT-100 F – комплект реагентов для амплификации фрагментов генов карбапенемаз групп KPC и OXA-48-подобных с гибридизационно-флуоресцентной детекцией в режиме «реального времени» – **включает**:

Реактив	*Описание*	*Объем, мл*	*Кол-во*
ПЦР-смесь-1-FRT KPC/OXA-48	Прозрачная бесцветная жидкость	1,2	1 пробирка
ПЦР-смесь-2-FRT	Прозрачная бесцветная жидкость	0,3	2 пробирки
Полимераза (TaqF)	Прозрачная бесцветная жидкость	0,03	2 пробирки
К–	Прозрачная бесцветная жидкость	0,2	1 пробирка
ПКО-1 KPC/OXA-48	Прозрачная бесцветная жидкость	0,2	1 пробирка
ПКО-2 KPC/OXA-48	Прозрачная бесцветная жидкость	0,2	1 пробирка

Комплект реагентов рассчитан на проведение 110 реакций амплификации, включая контроли.

К комплекту реагентов прилагаются контрольные образцы этапа экстракции:

Реактив	*Описание*	*Объем, мл*	*Кол-во*
ОКО	Прозрачная бесцветная жидкость	1,2	1 пробирка
ВКО-FL	Прозрачная бесцветная жидкость	1,0	1 пробирка

ПРОВЕДЕНИЕ ПЦР-ИССЛЕДОВАНИЯ

ПЦР-исследование состоит из следующих этапов:

- Экстракция ДНК из из исследуемых образцов.
- Амплификация с гибридизационно-флуоресцентной детекцией в режиме «реального времени».
- Анализ и интерпретация результатов.

Детальная информация по процедуре проведения ПЦР-исследования в зависимости от используемого оборудования изложена в методических рекомендациях по применению наборов реагентов для выявления генов карбапенемаз методом полимеразной цепной реакции (ПЦР) с

гибридизационно-флуоресцентной детекцией «АмплиСенс® MDR MBL-FL» и «АмплиСенс® MDR KPC/OXA-48-FL», разработанных ФБУН ЦНИИ Эпидемиологии Роспотребнадзора.

Таблица 1

Схема проведения ПЦР-исследования в зависимости от вида биологического материала

Вид биологического материала	Объем для экстракции, мкл	Комплект/реагент для экстракции ДНК	Добавление ВКО-FL при экстракции	Программа амплификации	Используемый положительный контроль амплификации
Гемокультура, смесь бактериальных культур, полученная путем посева клинического материала на **жидкую питательную среду**	Осадок из 100-250 мкл, полученный после предобработки	«ГК-экспресс»	–	«АмплиСенс-B»	ПКО-1 KPC/OXA-48
		«ДНК-сорб-АМ»	+	«АмплиСенс-1»	ПКО-2 KPC/OXA-48
Смесь бактериальных культур, полученная путем посева клинического материала на **плотную питательную среду**	10^7-10^9 бактериальных клеток	«ГК-экспресс»	–	«АмплиСенс-B»	ПКО-1 KPC/OXA-48
		«ДНК-сорб-АМ»	+	«АмплиСенс-1»	ПКО-2 KPC/OXA-48
Моча	Осадок из 1000 мкл, полученный после предобработки	«ДНК-сорб-АМ»	+	«АмплиСенс-1»	ПКО-2 KPC/OXA-48
		«РИБО-преп»			
Мазки со слизистых оболочек ротоглотки, прямой кишки	100	«ДНК-сорб-АМ»	+	«АмплиСенс-1»	ПКО-2 KPC/OXA-48

ЭКСТРАКЦИЯ ДНК ИЗ ИССЛЕДУЕМЫХ ОБРАЗЦОВ

Для экстракции ДНК используются комплекты реагентов / реагент:

– «ГК-экспресс» или «ДНК-сорб-АМ» для экстракции ДНК из образцов **положительной гемокультуры, смеси бактериальных культур, полученной при посеве на жидкую питательную среду,** после предварительной

обработки, образцов **чистой культуры или смеси бактериальных культур, полученной при посеве на плотную питательную среду** в соответствии с инструкцией к используемому комплекту реагентов/реагенту.

– «ДНК-сорб-АМ» или «РИБО-преп» для экстракции ДНК из образцов **мочи** после предварительной обработки в соответствии с инструкцией к используемому комплекту реагентов.
– «ДНК-сорб-АМ» для экстракции ДНК из образцов **мазков со слизистых оболочек ротоглотки, прямой кишки** в соответствии с инструкцией к используемому комплекту реагентов.

Экстракция ДНК из каждого исследуемого образца проводится в присутствии внутреннего контрольного образца (ВКО-FL). В качестве пробы В– используется реактив ОКО. В случае использования для экстракции ДНК реагента «ГК-экспресс» добавления ВКО-FL в исследуемые образцы и ОКО в пробу В– не требуется.

При проведении экстракции ДНК из образцов, после предобработки представляющих собой осадки, лизирующий раствор или реагент «ГК-экспресс» добавляют непосредственно в пробирку с осадком, используя для каждого образца отдельный наконечник с фильтром.

При проведении экстракции из образцов чистой культуры или смеси бактериальных культур, полученной при посеве на плотную питательную среду, бактериальные клетки, взятые стерильной петлей (или стерильным наконечником) в количестве 10^7-10^9 клеток, помещают непосредственно в пробирку объемом 1,5 мл, содержащую реагент «ГК-экспресс» или лизирующий раствор набора «ДНК-сорб-АМ».

ВНИМАНИЕ! Не рекомендуется одновременно проводить экстракцию ДНК из образцов гемокультуры, чистой культуры или смеси бактериальных культур, полученной путем посева на питательную среду, и из образцов биологического материала других видов, т.к. при этом существует высокий риск контаминации от образцов положительной гемокультуры или бактериальных культур, содержащих высокие концентрации ДНК возбудителя.

ПРОВЕДЕНИЕ АМПЛИФИКАЦИИ С ДЕТЕКЦИЕЙ В РЕЖИМЕ «РЕАЛЬНОГО ВРЕМЕНИ»

Выбор пробирок для амплификации зависит от используемого амплификатора с системой детекции в режиме «реального времени».

Для внесения в пробирки реагентов, проб ДНК и контрольных образцов используются одноразовые наконечники с фильтрами.

А. Подготовка пробирок для амплификации

Общий объем реакционной смеси – 25 мкл, включая объем пробы ДНК – 10 мкл.

Компоненты реакционной смеси следует смешивать непосредственно перед проведением эксперимента. Смешивать реагенты из расчета расходования на одну реакцию:

- **10 мкл ПЦР-смеси-1-FRT KPC/OXA-48,**
- **5 мкл** смеси **ПЦР-смеси-2-FRT,**
- **0,5 мкл полимеразы (TaqF).**

1. Предварительно необходимо подготовить **смесь ПЦР-смеси-2-FRT** и **полимеразы (TaqF)**. Содержимое одной пробирки с **полимеразой (TaqF) (30 мкл)** необходимо полностью перенести в пробирку с **ПЦР-смесью-2-FRT (300 мкл)** и аккуратно перемешать на центрифуге/вортексе, не допуская образования пены. Промаркировать пробирку, указав дату приготовления смеси.

ВНИМАНИЕ! Приготовленная смесь рассчитана на 60 реакций. Смесь хранить при температуре от 2 до 8 °С в течение 3 мес и использовать по мере необходимости.

В случае если данная смесь не может быть израсходована в течение трех месяцев, необходимо готовить смесь на меньшее количество реакций, например, смешать 150 мкл ПЦР-смеси-2-FRT и 15 мкл полимеразы (TaqF) (полученная смесь рассчитана на 30 реакций).

2. Перемешать содержимое пробирки с реагентом **ПЦР-смесь-1-FRT KPC/OXA-48** и осадить капли кратковременным центрифугированием с помощью центрифуги/вортекса.

Сделать расчет на необходимое число реакций, включающее тестирование исследуемых и контрольных образцов, можно согласно **расчетной таблице, приведенной в приложении 1.**

Следует учитывать, что для тестирования даже одного исследуемого образца ДНК необходимо проводить постановку еще **3-х контрольных реакций: К+, К– и В–**.

Необходимо брать реагенты с запасом: для тестирования N образцов приготовить реагенты для (N+1) реакций.

3. В отдельной пробирке подготовить реакционную смесь. Смешать необходимое количество **ПЦР-смеси-1-FRT KPC/OXA-48, ПЦР-смеси-2-FRT** с **полимеразой (TaqF)**, приготовленной согласно п.1.
4. Отобрать необходимое количество пробирок или стрипов для амплификации ДНК исследуемых и контрольных проб.
5. Внести в пробирки по **15 мкл** готовой реакционной смеси.
6. В подготовленные пробирки внести по **10 мкл проб ДНК**, полученных в результате экстракции из исследуемых образцов.
7. Поставить контрольные реакции:
   а) **отрицательный контроль экстракции ДНК (В–) –** внести в пробирку **10 мкл** пробы, выделенной из ОКО.
   б) **отрицательный контроль ПЦР (К–)** – внести в пробирку **10 мкл К–.**
   в) **положительный контроль ПЦР (К+)** – в одну пробирку внести **10 мкл ПКО-1 KPC/OXA-48** (при анализе проб ДНК, полученных из образцов гемокультуры, чистой культуры или смеси бактериальных культур, полученной путем посева на питательную среду, при использовании программы амплификации «АмплиСенс-В») или **10 мкл ПКО-2 KPC/OXA-48** (при анализе проб ДНК, полученных из образцов исходного клинического материала или из образцов гемокультуры, чистой культуры или смеси бактериальных культур, при использовании программы амплификации «АмплиСенс-1»).

Б. Проведение амплификации с детекцией в режиме «реального времени»

1. Запрограммировать прибор (амплификатор с системой детекции в режиме «реального времени») для выполнения соответствующей программы амплификации и детекции флуоресцентного сигнала. При анализе проб ДНК, полученных при экстракции с помощью реагента «ГК-экспресс» из образцов гемокультуры, чистой культуры или

смеси бактериальных культур, полученной путем посева на питательную среду, используется программа **«АмплиСенс-В»** (см. табл. 2). При анализе проб ДНК, полученных из образцов исходного клинического материала, или проб ДНК, полученных при экстракции с помощью комплекта реагентов «ДНК-сорб-АМ» из образцов гемокультуры, чистой культуры или смеси бактериальных культур, используется программа **«АмплиСенс-1»** (см. табл. 3).

Таблица 2

Программа «АмплиСенс-В»

Цикл	**Приборы роторного типа[5]**			**Приборы планшетного типа[6]**		
	Температура, °С	Время	Кол-во циклов	Температура, °С	Время	Кол-во циклов
1	**95**	15 мин	1	**95**	15 мин	1
2	**95**	5 с	35	**95**	5 с	35
	60	20 с детекция флуоресц. сигнала		**60**	30 с детекция флуоресц. сигнала	
	72	15 с		**72**	15 с	

Таблица 3

Программа «АмплиСенс-1»

Цикл	**Приборы роторного типа[5]**			**Приборы планшетного типа[6]**		
	Температура, °С	Время	Кол-во циклов	Температура, °С	Время	Кол-во циклов
1	**95**	15 мин	1	**95**	15 мин	1
2	**95**	5 с	5	**95**	5 с	5
	60	20 с		**60**	20 с	
	72	15 с		**72**	15 с	
3	**95**	5 с	40	**95**	5 с	40
	60	20 с детекция флуоресц. сигнала		**60**	30 с детекция флуоресц. сигнала	
	72	15 с		**72**	15 с	

Детекция флуоресцентного сигнала назначается по трем

[5] Например, Rotor-Gene 6000 (Corbett Research, Австралия), Rotor-Gene Q (QIAGEN, Германия) и рекомендованные ФБУН ЦНИИ Эпидемиологии Роспотребнадзора в методических рекомендациях по применению данного набора реагентов.

[6] Например, CFX, iQ5 (Bio-Rad, США) и рекомендованные ФБУН ЦНИИ Эпидемиологии Роспотребнадзора в методических рекомендациях по применению данного набора реагентов.

каналам – для флуорофоров FAM[7], JOE[7] и ROX[7].

2. Установить пробирки в ячейки реакционного модуля прибора.
3. Запустить выполнение программы амплификации с детекцией флуоресцентного сигнала.
4. По окончании выполнения программы приступить к анализу и интерпретации результатов.

АНАЛИЗ И ИНТЕРПРЕТАЦИЯ РЕЗУЛЬТАТОВ

Анализ результатов проводят с помощью программного обеспечения прибора, используемого для проведения ПЦР с детекцией в режиме «реального времени». Анализируют графики накопления флуоресцентного сигнала по трем каналам:

- по каналу для флуорофора **FAM** регистрируется сигнал, свидетельствующий о накоплении продукта амплификации фрагментов **генов карбапенемаз группы KPC**;
- по каналу для флуорофора **JOE** регистрируется сигнал, свидетельствующий о накоплении продукта амплификации фрагментов **генов карбапенемаз группы OXA-48-подобных**;
- по каналу для флуорофора **ROX** регистрируется сигнал, свидетельствующий о накоплении продукта амплификации ДНК **внутреннего контроля.**

Результаты интерпретируются на основании наличия (или отсутствия) пересечения графика флуоресценции с пороговой линией, установленной на уровне экспоненциального подъема сигнала, что определяет наличие (или отсутствие) для данной ДНК-мишени значения порогового цикла *Ct* в соответствующей графе таблицы результатов.

Принцип интерпретации результатов следующий:

- **Гены карбапенемаз** соответствующей группы **обнаружены**, если для данной пробы в таблице результатов по каналу для флуорофора FAM или/и JOE определено значение порогового цикла *Ct,* не превышающее указанное граничное значение. При этом кривая флуоресценции данной пробы должна пересекать

[7] Название каналов детекции для соответствующего прибора см. в методических рекомендациях по применению данного набора реагентов.

пороговую линию на участке характерного экспоненциального подъема флуоресценции.

- **Гены карбапенемаз групп KPC и OXA-48-подобных не обнаружены,** если для данной пробы в таблице результатов по каналам для флуорофоров FAM и JOE не определено (отсутствует) значение порогового цикла *Ct* (кривая флуоресценции не пересекает пороговую линию), а в таблице результатов по каналу для флуорофора ROX определено значение порогового цикла *Ct*, не превышающее указанное (граничное) значение.
- Результат анализа **невалидный**, если для исследуемого образца отсутствуют значения пороговых циклов *Ct* по каналам для флуорофоров FAM и JOE, и по каналу для флуорофора ROX значение *Ct* также отсутствует или превышает указанное граничное значение. В этом случае необходимо повторно провести ПЦР-исследование соответствующего образца, начиная с этапа экстракции ДНК.

ВНИМАНИЕ! Граничные значения *Ct* указаны во вкладыше, прилагаемом к набору реагентов. См. также методические рекомендации по применению наборов реагентов для выявления генов карбапенемаз методом полимеразной цепной реакции (ПЦР) с гибридизационно-флуоресцентной детекцией «АмплиСенс® MDR MBL-FL» и «АмплиСенс® MDR KPC/OXA-48-FL», разработанные ФБУН ЦНИИ Эпидемиологии Роспотребнадзора.

Результат ПЦР-исследования считается достоверным, если получены правильные результаты для положительного и отрицательного контролей амплификации и отрицательного контроля экстракции ДНК в соответствии с таблицей 4 и вкладышем к набору реагентов.

Таблица 4

Результаты для контролей различных этапов ПЦР-анализа

Контроль	Контролируемый этап ПЦР-исследования	Значение порогового цикла *Ct*	
		по каналам для флуорофоров FAM, JOE	по каналу для флуорофора ROX
В–	Экстракция ДНК	Значение отсутствует	Определено значение **меньше** граничного
К–	ПЦР	Значение отсутствует	Значение отсутствует
К+	ПЦР	Определено значение **меньше** граничного	Не оценивается

Результат ПЦР-исследования считается недостоверным в следующих случаях:

1. Если для положительного контроля ПЦР (К+) значения порогового цикла по каналам для флуорофоров FAM и/или JOE отсутствуют или превышают указанное граничное значение, необходимо повторить амплификацию для всех образцов.
2. Если для отрицательного контроля экстракции ДНК (В-) и/или отрицательного контроля ПЦР (К-) регистрируется значение порогового цикла *Ct* по каналам для флуорофоров FAM или/и JOE, необходимо повторить ПЦР-исследование для всех образцов, для которых определено значение порогового цикла, соответственно, по каналам для флуорофоров FAM или/и JOE.

Клиническая интерпретация результатов теста должна проводиться врачом только при условии комплексного обследования пациента, с учетом данных анамнеза, клинического и эпидемиологического статуса, в соответствии с существующими клиническими и методическими рекомендациями.

СРОК ГОДНОСТИ. УСЛОВИЯ ТРАНСПОРТИРОВАНИЯ И ХРАНЕНИЯ

Срок годности. 9 мес. Набор реагентов с истекшим сроком годности применению не подлежит. Срок годности вскрытых реагентов соответствует сроку годности, указанному на этикетках для невскрытых реагентов, если в инструкции не указано иное.

Транспортирование. Набор реагентов транспортировать при температуре от 2 до 8 °С не более 5 сут. «ПЦР-комплект» вариант FRT-100 F при получении разукомплектовать в соответствии с указанными температурами хранения.

Хранение. Комплект реагентов «ПЦР-комплект» хранить при температуре от 2 до 8 °С. ПЦР-смесь-1-FRT KPC/OXA-48 хранить в защищенном от света месте. ПЦР-смесь-2-FRT и полимеразу (TaqF) хранить при температуре от минус 24 до минус 16 °С.

Рекламации на качество набора реагентов **«АмплиСенс® MDR KPC/OXA-48-FL»** направлять на предприятие-изготовитель ФБУН ЦНИИ Эпидемиологии Роспотребнадзора (111123 г. Москва, ул. Новогиреевская, д. 3а) в отдел по работе с рекламациями и организации обучения (тел. (495) 974-96-46, факс (495) 916-18-18, e-mail: products@pcr.ru)[8].

Заведующий НПЛ ОМДиЭ
ФБУН ЦНИИ Эпидемиологии Роспотребнадзора — Е.Н. Родионова

Директор НИИ антимикробной химиотерапии
ГБОУ ВПО СГМА Минздрава РФ — Р.С. Козлов

[8] Отзывы и предложения о продукции «АмплиСенс» вы можете оставить, заполнив анкету потребителя на сайте: www.amplisens.ru.

ПРИЛОЖЕНИЕ 1.

Схема приготовления реакционных смесей

	Объем реагентов на указанное количество реакций (мкл)	
Объем реагентов на одну реакцию (мкл)	**10 мкл**	**5 мкл**
Количество исследуемых образцов*	**ПЦР-смесь-1-FRT ***	**Смесь ПЦР-смеси-2-FRT и полимеразы (TaqF)***
2	**60**	**30**
3	**70**	**35**
4	**80**	**40**
5	**90**	**45**
6	**100**	**50**
7	**110**	**55**
8	**120**	**60**
9	**130**	**65**
10	**140**	**70**
11	**150**	**75**
12	**160**	**80**
13	**170**	**85**
14	**180**	**90**
15	**190**	**95**
16	**200**	**100**
17	**210**	**105**
18	**220**	**110**
19	**230**	**115**
20	**240**	**120**
21	**250**	**125**
22	**260**	**130**
23	**270**	**135**
24	**280**	**140**
25	**290**	**145**

*Приведены значения с учетом запаса (расчет на одну реакцию больше) и с учетом необходимости постановки 3 контрольных реакций: К+, В– и К–.

СИМВОЛЫ, ИСПОЛЬЗУЕМЫЕ В ПЕЧАТНОЙ ПРОДУКЦИИ

REF	Номер в каталоге	Σ	Максимальное число тестов
LOT	Код партии		Использовать до
IVD	Изделие для in vitro диагностики		Обратитесь к руководству по эксплуатации
VER	Дата изменения		Не допускать попадания солнечного света
	Ограничение температуры		Дата изготовления
	Производитель		

УТВЕРЖДЕНА
Приказом Росздравнадзора
от 26.09.2012 № 1619-Пр/12

УТВЕРЖДАЮ
Директор Федерального бюджетного учреждения науки «Центральный научно-исследовательский институт эпидемиологии» Федеральной службы по надзору в сфере защиты прав потребителей и благополучия человека
_____ В.И.Покровский
«28» декабря 2011 г.

ИНСТРУКЦИЯ

по применению набора реагентов

для выявления ДНК *Coxiella burnetii* в биологическом материале методом полимеразной цепной реакции (ПЦР) с гибридизационно-флуоресцентной детекцией

«АмплиСенс® *Coxiella burnetii*-FL»

АмплиСенс®

Федеральное бюджетное учреждение науки
«Центральный научно-исследовательский институт эпидемиологии»,
Российская Федерация, 111123,
город Москва, улица Новогиреевская, дом 3а

IVD

ОГЛАВЛЕНИЕ

СПИСОК СОКРАЩЕНИЙ

В настоящей инструкции применяются следующие сокращения и обозначения:

ВКО	- внутренний контрольный образец
В–	- отрицательный контроль экстракции
К+	- положительный контроль ПЦР
К–	- отрицательный контроль ПЦР
НК	- нуклеиновые кислоты (РНК/ДНК)
ПКО	- положительный контрольный образец
ПЦР	- полимеразная цепная реакция
ФБУН ЦНИИ Эпидемиологии Роспотребнадзора	- федеральное бюджетное учреждение науки «Центральный научно-исследовательский институт эпидемиологии» Федеральной службы по надзору в сфере защиты прав потребителей и благополучия человека
FRT	- флуоресцентная детекция в режиме «реального времени»

НАЗНАЧЕНИЕ

Набор реагентов **«АмплиСенс® *Coxiella burnetii*-FL»** предназначен для выявления ДНК *Coxiella burnetii* в клещах, биологическом материале от людей (кровь, мокрота, промывные воды бронхов, ликвор, секционный материал) и материале от животных (кровь, секционный материал, плацента и абортивный материал) методом ПЦР с гибридизационно-флуоресцентной детекцией продуктов амплификации.

ВНИМАНИЕ! Результаты ПЦР-исследования учитываются в комплексной диагностике заболевания.[1]

ПРИНЦИП МЕТОДА

Выявление *Coxiella burnetii* методом полимеразной цепной реакции (ПЦР) с гибридизационно-флуоресцентной детекцией включает в себя три этапа: экстракцию ДНК из образцов биологического материала, ПЦР-амплификацию участка ДНК данного микроорганизма и гибридизационно-флуоресцентную детекцию, которая производится непосредственно в ходе ПЦР (формат FRT). Экстракция ДНК из биологического материала проводится в присутствии внутреннего контрольного образца (ВКО STI-87), который позволяет контролировать выполнение процедуры исследования для каждого образца. Затем с полученными пробами проводится реакция амплификации участка ДНК *Coxiella burnetii* при помощи специфичных к этому

[1] В соответствии с директивой Европейского Союза 98/79/ЕС

участку ДНК праймеров и фермента TaqF-полимеразы. В составе реакционной смеси присутствует флуоресцентно-меченый олигонуклеотидный зонд, который гибридизуется с комплементарным участком амплифицируемой ДНК-мишени, в результате чего происходит нарастание интенсивности флуоресценции. Это позволяет регистрировать накопление специфических продуктов амплификации путем измерения интенсивности флуоресцентных сигналов. Детекция флуоресцентных сигналов при использовании формата FRT происходит непосредственно в ходе ПЦР с помощью амплификатора с системой детекции флуоресцентного сигнала в режиме «реального времени».

ФОРМАТЫ И ФОРМЫ ВЫПУСКА НАБОРА РЕАГЕНТОВ

Набор реагентов выпускается в 1 формате.

Формат FRT

Набор реагентов выпускается в 3 формах комплектации:

Форма 1 включает комплект реагентов «ПЦР-комплект» вариант FRT-50 F.

Форма 2 включает комплекты реагентов «РИБО-преп» вариант 50, «ПЦР-комплект» вариант FRT-50 F.

Форма 3 включает наборы реагентов оптом, расфасованные по отдельным реагентам, с маркировкой реагентов на их оптовой фасовке.

Форма комплектации 1 предназначена для проведения амплификации ДНК *Coxiella burnetii* с гибридизационно-флуоресцентной детекцией в режиме «реального времени». Для проведения полного ПЦР-исследования необходимо использовать комплекты реагентов для экстракции РНК/ДНК, рекомендованные ФБУН ЦНИИ Эпидемиологии Роспотребнадзора.

Форма комплектации 2 предназначена для проведения полного ПЦР-исследования, включающего экстракцию ДНК из биологического материала и амплификацию ДНК *Coxiella burnetii* с гибридизационно-флуоресцентной детекцией в режиме «реального времени».

Форма комплектации 3 предназначена для производственных целей для последующей маркировки на языке заказчика и комплектации по наборам.

ВНИМАНИЕ! Форма комплектации 3 используется только в соответствии с регламентом, утвержденным ФБУН ЦНИИ Эпидемиологии Роспотребнадзора.

АНАЛИТИЧЕСКИЕ ХАРАКТЕРИСТИКИ

Аналитическая чувствительность

Вид биологического материала (объем исследуемой пробы)	Комплект для выделения РНК/ДНК	Комплект для амплификации и детекции	Аналитическая чувствитель-ность[2], ГЭ/мл	Пробоподготовка материала
-клещи рода *Dermacentor* (50 мкл клещевой суспензии); -кровь (лейкоцитарная фракция крови, 200 мкл); -10 % суспензия тканей селезенки и печени (50 мкл)	**«РИБО-преп»**	«ПЦР-комплект» вариант FRT-50 F	$5x10^3$	Данная чувствительность достигается при соблюдении нижеизложенных правил пробоподготовки биоматериала и рекомендуемом исследуемом объеме пробы

Аналитическая специфичность

Аналитическая специфичность изучена на бактериях *Rickettsia conorii* ssp. *Caspia, Ehrlichia muris* и *Francisella tularensis,* а также вирусах – вирусе Западного Нила, вирусе Крымской-Конго геморрагической лихорадки и *Herpesvirus*.

При работе с РНК/ДНК вышеперечисленных микроорганизмов, а также ДНК человека, ДНК клещей и ДНК грызунов не выявлено ложноположительных результатов.

МЕРЫ ПРЕДОСТОРОЖНОСТИ

Взятие, хранение материала, транспортирование на исследование и работу с ним проводят в соответствии с инструктивно-методическими документами, регламентирующими выполнение исследований: СП 1.3. 1285-3 «Безопасность работы с микроорганизмами I–II групп патогенности (опасности)», МУ 1.3.2569-09 «Организация

[2] Количество геномных эквивалентов микроорганизма (ГЭ) в 1 мл образца исследуемого материала.

работы лабораторий, использующих методы амплификации нуклеиновых кислот при работе с материалом, содержащим микроорганизмы I–IV групп патогенности» и СП 1.2.036-95 «Порядок учета, хранения, передачи и транспортирования микроорганизмов I–IV групп патогенности».

При работе необходимо выполнять следующие требования:

- Следует рассматривать исследуемые образцы как потенциально инфекционные и работать с ними в биологическом кабинете в соответствии СП 1.3. 1285-3 «Безопасность работы с микроорганизмами I–II групп патогенности (опасности)»
- Убирать и дезинфицировать разлитые образцы или реактивы, используя дезинфицирующие средства в соответствии с СП 1.3. 1285-3 «Безопасность работы с микроорганизмами I–II групп патогенности (опасности)».
- Лабораторный процесс должен быть однонаправленным. Анализ проводится в отдельных помещениях (зонах). Работу следует начинать в Зоне Выделения, продолжать в Зоне Амплификации и Детекции. Не возвращать образцы, оборудование и реактивы в Зону, в которой была проведена предыдущая стадия процесса.
- Уничтожать образцы в соответствии с СП 1.3. 1285-3 «Безопасность работы с микроорганизмами I–II групп патогенности (опасности)»

ВНИМАНИЕ! При удалении отходов после амплификации (пробирок, содержащих продукты ПЦР) недопустимо открывание пробирок и разбрызгивание содержимого, поскольку это может привести к контаминации продуктами ПЦР лабораторной зоны, оборудования и реагентов.

- Применять набор строго по назначению, согласно данной инструкции.
- Допускать к работе с набором только специально обученный персонал.
- Не использовать набор по истечении срока годности.
- Использовать одноразовые перчатки, лабораторные халаты, защищать глаза во время работы с образцами и реактивами. Тщательно вымыть руки по окончании работы.
- Избегать контакта с кожей, глазами и слизистой оболочкой. При контакте немедленно промыть пораженное место водой

и обратиться за медицинской помощью.
- Листы безопасности материалов (MSDS – material safety data sheet) доступны по запросу.

ДОПОЛНИТЕЛЬНЫЕ МАТЕРИАЛЫ И ОБОРУДОВАНИЕ

1. 0,15 M NaCl (физиологический раствор) или фосфатный буферный раствор (PBS) (натрия хлорид, 137 мМ; калия хлорид, 2,7 мМ; натрия монофосфат, 10 мМ; калия дифосфат, 2 мМ; pH=7,5±0,2) для проведения пробоподготовки клещей, тканей внутренних органов, секционного материала.
2. 96 % раствор этанола для проведения пробоподготовки клещей, обработанных маслом.
3. Глицерин для проведения пробоподготовки клещей.
4. Гомогенизатор TissueLyser LT (Qiagen, Германия) и металлические шарики из нержавеющей стали диаметром 5 мм и 7 мм рекомендуются для гомогенизации тканей органов и клещей.
5. Стерильные фарфоровая ступка и пестик для пробоподготовки внутренних органов и секционного материала.
6. Реагент «МУКОЛИЗИН» производства ФБУН ЦНИИ Эпидемиологии Роспотребнадзора для предварительной обработки мокроты.

ЗОНА 1. Экстракция ДНК из биологического материала.

7. Комплект реагентов для выделения РНК/ДНК «РИБО-преп» (ТУ 9398-071-01897593-2008) – при работе с формой комплектации 1.
8. Ламинарный бокс (например, «БАВп-01-«Ламинар-С»-1,2», «Ламинарные системы», Россия, класс биологической безопасности II тип А).
9. Термостат для пробирок типа «Эппендорф» от 25 до 100 °С.
10. Автоматические дозаторы переменного объема (от 20 до 200 мкл, от 100 до 1000 мкл).
11. Одноразовые полипропиленовые завинчивающиеся или плотно закрывающиеся пробирки объемом 1,5 мл.
12. Штатив для пробирок объемом 1,5 мл.
13. Одноразовые наконечники с фильтром до 100 и 1000 мкл.
14. Штативы для наконечников.

15. Микроцентрифуга для пробирок типа «Эппендорф» до 16 тыс g.
16. Вортекс.
17. Вакуумный отсасыватель медицинский с колбой-ловушкой для удаления надосадочной жидкости.
18. Холодильник от 2 до 8 °С с морозильной камерой не выше минус 16 °С.
19. Отдельный халат, шапочки, обувь и одноразовые перчатки в соответствии с МУ 1.3.2569-09.
20. Емкость для сброса наконечников.

ЗОНА 2. Проведение ПЦР и гибридизационно-флуоресцентной детекции продуктов амплификации

21. Бокс абактериальной воздушной среды (ПЦР-бокс).
22. Центрифуга/вортекс.
23. Автоматические дозаторы переменного объема (от 5 до 50 мкл, от 20 до 200 мкл).
24. Одноразовые наконечники с фильтром до 100 мкл в штативах.
25. Штативы для пробирок объемом 0,2 мл.
26. Холодильник от 2 до 8 °С с морозильной камерой не выше минус 16 °С.
27. Отдельный халат, шапочки, обувь и одноразовые перчатки в соответствии с МУ 1.3.2569-09.
28. Емкость для сброса наконечников.
29. Программируемый амплификатор с системой детекции флуоресцентного сигнала в режиме «реального времени» (например, Rotor-Gene 3000/6000 (Corbett Research, Австралия), Rotor-Gene Q (Qiagen, Германия), iCycler iQ5 (Bio-Rad, США), «ДТ-96» («ДНК-Технология», Россия) и рекомендованные ФБУН ЦНИИ Эпидемиологии Роспотребнадзора в методических рекомендациях по применению данного набора реагентов).
30. Одноразовые полипропиленовые пробирки для ПЦР объемом 0,2 мл или 0,1 мл:
    а) тонкостенные пробирки для ПЦР объемом 0,2 мл с выпуклой крышкой (например, Axygen, США) – при использовании прибора планшетного типа;
    б) тонкостенные пробирки для ПЦР объемом 0,2 мл с плоской крышкой (например, Axygen, США) или пробирки

для ПЦР к Rotor-Gene, объемом 0,1 мл в стрипах по 4 шт. с крышками (например, Corbett Research, Австралия; Qiagen, Германия) – при использовании прибора роторного типа.

ВЗЯТИЕ, ТРАНСПОРТИРОВАНИЕ И ХРАНЕНИЕ ИССЛЕДУЕМОГО МАТЕРИАЛА

Осуществляется в соответствии с методическими рекомендациями «Взятие, транспортировка, хранение клинического материала для ПЦР-диагностики», разработанными ФГУН ЦНИИЭ Роспотребнадзора, Москва, 2008 г, а также СП 1.2.036-95 «Порядок учета, хранения, передачи и транспортирования микроорганизмов I–IV групп патогенности».

Материалом для исследования служат:

- Иксодовые клещи: *Rhipicephalus, Haemaphysalis, Dermacentor, Ixodes*.

Материал от людей:

- Цельная периферическая кровь, мокрота, промывные воды бронхов, ликвор, секционный материал (ткани мозга, сердца, легких, селезенка).

Материал от животных:

- кровь, плацента, абортивный материал, секционный материал (селезенка).

Кровь, мокроту, промывные воды бронхов, ликвор, секционный материал доставляют в лабораторию в емкости со льдом в течение 1 сут.

При поступлении в лабораторию проводят пробоподготовку крови, ликвора, промывных вод бронхов с получением бактериального осадка, после чего либо сразу приступают к экстракции нуклеиновых кислот либо замораживают пробу для длительного хранения. Клещей хранят или живыми (до 1 мес), или 1 нед при температуре не выше минус 16 °С, далее – при температуре не выше минус 68 °С. Секционный и абортивный материал, а также плаценту хранят 1 нед при температуре не выше минус 16 °С, далее – при температуре не выше минус 68 °С. Допускается однократное замораживание-оттаивание материала.

ПОДГОТОВКА ИССЛЕДУЕМОГО МАТЕРИАЛА К ЭКСТРАКЦИИ ДНК

1. Клещи

Клещей предпочтительнее исследовать индивидуально. В том случае, если клещи были обработаны маслом, их следует поместить в пробирки типа «Эппендорф», добавить 500 мкл 96 % этанола и встряхнуть на вортексе. Пробирку центрифугировать в течение 3-5 с на микроцентрифуге типа вортекс для удаления капель с внутренней поверхности крышки пробирки, после чего жидкость аккуратно отобрать с помощью вакуумного отсасывателя. Затем в пробирку с клещами добавить 500 мкл стерильного 0,9 % раствора натрия хлорида или PBS-буфера, встряхнуть на вортексе, центрифугировать в течение 3-5 с на микроцентрифуге для удаления капель с внутренней поверхности крышки пробирки, после чего жидкость аккуратно отобрать с помощью вакуумного отсасывателя. Для приготовления суспензий клещей использовать стерильную фарфоровую чашку и стерильный пестик. При наличии автоматического гомогенизатора TissueLyser LT применять следующие параметры гомогенизации: 1) для клещей родов *Rhipicephalus, Haemaphysalis* и *Dermacentor* диаметр шариков 7 мм, частота 50 Гц/с, время гомогенизации 10–12 мин, объем буфера 700 мкл (ненапитавшийся клещ) или 1000-1500 мкл (напитавшийся клещ и пулы клещей); 2) для клещей рода *Ixodes* диаметр шариков 5 мм, частота 50 Гц/с, время гомогенизации 5–10 мин, объем буфера 300 мкл (ненапитавшийся клещ) или 700-1000 мкл (напитавшийся клещ и пулы клещей).

В случае гомогенизации напитавшихся клещей в ступке их предварительно следует проколоть стерильной одноразовой иглой в нескольких местах для выхода крови. Клещей растирать в 700 мкл (если проба состоит из одного ненапитавшегося клеща родов *Rhipicephalus, Haemaphysalis, Dermacentor)* или в 300 мкл (если проба состоит из одного ненапитавшегося клеща рода *Ixodes)*, в 1-1,5 мл (если гомогенизируют пул клещей или напитавшегося клеща родов *Rhipicephalus, Haemaphysalis, Dermacentor*), или в 1000 мкл (если гомогенизируют пул клещей или напитавшегося клеща рода *Ixodes)* 0,15 М раствора хлорида натрия, смешивая

раствор с клещами небольшими объемами, затем полученную суспензию центрифугировать при 5 тыс об/мин в течение 2 мин и отобрать 50 мкл надосадочной жидкости для экстракции ДНК. Оставшийся объем суспензии без осадка перенести в новую пробирку типа «Эппендорф» и внести глицерин (10 % по объему), пробу перемешать и заморозить при температуре не выше минус 16 °С для последующего исследования.

2. Кровь

Взятие цельной периферической крови у людей проводится утром натощак в пробирку с 6 % раствором ЭДТА в соотношении 1:20. Закрытую пробирку несколько раз переворачивают. В пробирку типа «Эппендорф» внести 1,5 мл цельной крови, взятой с ЭДТА, и центрифугировать при 800 об/мин (380 g при диаметре ротора 50 мм) в течение 10 мин; затем верхний слой плазмы (500-600 мкл) с лейкоцитами перенести во вторую пробирку типа «Эппендорф» и центрифугировать при 9 000 g в течение 5 мин. Надосадочную жидкость (за исключением 200 мкл жидкости над осадком клеток) перенести в контейнер с дезинфицирующим раствором, а **осадок клеток и 200 мкл надосадочной жидкости** использовать для экстракции ДНК.

3. Внутренние органы, плацента и абортивный материал от животных, секционный материал, полученный от человека

Кусочки объемом не менее 0,5 $см^3$ тщательно растереть в гомогенизаторах или с использованием стерильных фарфоровых ступок и пестиков, добавить стерильный 0,9 % раствор натрия хлорида или PBS-буфер не менее 500 мкл и тщательно перемешать. При подготовке плаценты гомогенизаторы использовать не рекомендуется. Готовую 10 % суспензию отстаивать при комнатной температуре в течение 2–3 мин, затем верхнюю фазу перенести в пробирки вместимостью 1,5 мл. ДНК выделяют из **50 мкл суспензии.**

4. Мокрота

Предобработку материала выполнять по инструкции к реагенту «МУКОЛИЗИН». Для экстракции ДНК использовать **50 мкл пробы**.

5. Ликвор и промывные воды бронхов

1,0 мл клинического образца перенести в пробирку типа

«Эппендорф» и центрифугировать при 9 000 g в течение 5 мин. Надосадочную жидкость (за исключением 200 мкл жидкости над осадком клеток) перенести в контейнер с дезинфицирующим раствором, а **осадок клеток и 200 мкл надосадочной жидкости** использовать для экстракции ДНК.

Материал после пробоподготовки до экстракции ДНК можно хранить при температуре не выше минус 20 °С в течение 1 мес или длительно при температуре не выше минус 68 °С.

ФОРМАТ FRT

СОСТАВ

Комплект реагентов «РИБО-преп» вариант 50 – комплект реагентов для выделения РНК/ДНК из клинического материала – **включает**:

Реактив	*Описание*	*Объем, мл*	*Кол-во*
Раствор для лизиса	Прозрачная жидкость голубого цвета[3]	15	1 флакон
Раствор для преципитации	Прозрачная бесцветная жидкость	20	1 флакон
Раствор для отмывки 3	Прозрачная бесцветная жидкость	25	1 флакон
Раствор для отмывки 4	Прозрачная бесцветная жидкость	10	1 флакон
РНК-буфер	Прозрачная бесцветная жидкость	1,2	4 пробирки

Комплект реагентов рассчитан на выделение РНК/ДНК из 50 проб, включая контроли. Входит в состав формы комплектации 2.

Комплект реагентов «ПЦР-комплект» вариант FRT-50 F – комплект реагентов для амплификации фрагмента ДНК *Coxiella burnetii* с гибридизационно-флуоресцентной детекцией в режиме «реального времени» – **включает**:

Реактив	*Описание*	*Объем, мл*	*Кол-во*
ПЦР-смесь-1-FRT *Coxiella burnetii*	Прозрачная бесцветная жидкость	0,6	1 пробирка
ОТ-ПЦР-смесь-2-FEP/FRT	Прозрачная бесцветная жидкость	0,3	1 пробирка
Полимераза (TaqF)	Прозрачная бесцветная жидкость	0,03	1 пробирка
ПКО ДНК *Coxiella burnetii* / STI	Прозрачная бесцветная жидкость	0,2	1 пробирка
ДНК-буфер	Прозрачная бесцветная жидкость	0,5	1 пробирка

Комплект реагентов рассчитан на проведение 60 реакций амплификации, включая контроли.

К комплекту реагентов прилагается контрольный образец этапа экстракции:

Реактив	*Описание*	*Объем, мл*	*Кол-во*
ВКО STI-87	Прозрачная бесцветная жидкость	0,6	1 пробирка

[3] При хранении раствора для лизиса при температуре от 2 до 8 °С возможно образование осадка в виде кристаллов.

ПРОВЕДЕНИЕ ПЦР-ИССЛЕДОВАНИЯ

ПЦР-исследование состоит из следующих этапов:

- Экстракция ДНК из исследуемых образцов.
- Проведение амплификации с гибридизационно-флуоресцентной детекцией в режиме «реального времени».
- Анализ и интерпретация результатов.

Детальная информация по процедуре проведения ПЦР-исследования в зависимости от типа используемого оборудования изложена в методических рекомендациях по применению набора реагентов для выявления ДНК *Coxiella burnetii* в биологическом материале методом полимеразной цепной реакции (ПЦР) с гибридизационно-флуоресцентной детекцией «АмплиСенс® *Coxiella burnetii*-FL», разработанных ФБУН ЦНИИ Эпидемиологии Роспотребнадзора.

ЭКСТРАКЦИЯ ДНК ИЗ ИССЛЕДУЕМЫХ ОБРАЗЦОВ

Для экстракции ДНК используются комплекты реагентов, рекомендованные ФБУН ЦНИИ Эпидемиологии Роспотребнадзора. Экстракция ДНК из каждого клинического образца проводится в присутствии внутреннего контрольного образца (ВКО STI-87).

При использовании комплекта реагентов **«РИБО-преп»** порядок работы см. в **приложении 1** «Экстракция ДНК с использованием комплекта реагентов «РИБО-преп».

ПРОВЕДЕНИЕ АМПЛИФИКАЦИИ С ДЕТЕКЦИЕЙ В РЕЖИМЕ «РЕАЛЬНОГО ВРЕМЕНИ»

А. Подготовка пробирок для амплификации

Выбор пробирок для амплификации зависит от используемого амплификатора с системой детекции в режиме «реального времени».

Для внесения в пробирки реагентов, проб ДНК и контрольных образцов используются одноразовые наконечники с фильтрами.

Общий объем реакционной смеси – 25 мкл, включая объем пробы ДНК – 10 мкл.

1. Приготовить реакционную смесь на необходимое количество реакций. При расчете следует учитывать, что постановка сопровождается амплификацией как минимум

трех контрольных образцов: отрицательного контроля экстракции (В–), положительного и отрицательного контролей ПЦР (К+ и К–). Кроме того, необходимо брать реагенты **с запасом**: рассчитывать на одну реакцию больше.

2. В отдельной пробирке необходимо смешать **ПЦР-смесь-1-FRT *Coxiella burnetii***, **ОТ-ПЦР-смесь-2-FEP/FRT, полимеразу (TaqF)** из расчета на каждую реакцию:
   - **10 мкл ПЦР-смеси-1-FRT *Coxiella burnetii*;**
   - **5 мкл ОТ-ПЦР-смеси-2-FEP/FRT;**
   - **0,5 мкл полимеразы (TaqF).**
3. Отобрать необходимое количество пробирок или стрипов для амплификации ДНК исследуемых и контрольных образцов.
4. Внести в каждую пробирку по **15 мкл** приготовленной реакционной смеси.

ВНИМАНИЕ! Приготовленную смесь не хранить!

5. В подготовленные пробирки внести по **10 мкл проб ДНК**, полученных в результате экстракции из исследуемых или контрольных образцов. Осторожно перемешать пипетированием.
6. Поставить контрольные реакции:
   а) **отрицательный контроль ПЦР (К–)** – внести в пробирку **10 мкл ДНК-буфера.**
   б) **положительный контроль ПЦР (К+)** – внести в пробирку **10 мкл ПКО ДНК *Coxiella burnetii* / STI.**
   в) **отрицательный контроль экстракции (В–)** – внести в пробирку **10 мкл** пробы, выделенной из (В–).

ВНИМАНИЕ! Пробы амплифицировать сразу после соединения реакционной смеси с пробами ДНК и контролями!

Б. Проведение амплификации с детекцией в режиме «реального времени»

1. Запрограммировать прибор (амплификатор с системой детекции в режиме «реального времени») для выполнения соответствующей программы амплификации и детекции флуоресцентного сигнала

Таблица 1

	Приборы роторного типа[4]			Приборы планшетного типа[5]		
Цикл	***Температура***	***Время***	***Кол-во циклов***	***Температура***	***Время***	***Кол-во циклов***
1	**95 °С**	15 мин	1	**95 °С**	15 мин	1
2	**95 °С**	5 с	5	**95 °С**	5 с	5
	60 °С	20 с		**60 °С**	25 с	
	72 °С	15 с		**72 °С**	15 с	
3	**95 °С**	5 с	40	**95 °С**	5 с	40
	56 °С	20 с детекция флуоресц. Сигнала		**56 °С**	25 с детекция флуоресц. Сигнала	
	72 °С	15 с		**72 °С**	15 с	

Детекция флуоресцентного сигнала назначается по каналам для флуорофоров FAM и JOE.

2. Установить пробирки в ячейки реакционного модуля прибора. **Лунка №1 обязательно должна быть заполнена какой-либо исследуемой пробиркой.**
3. Запустить выполнение программы амплификации с детекцией флуоресцентного сигнала.
4. По окончании выполнения программы приступить к анализу и интерпретации результатов.

АНАЛИЗ И ИНТЕРПРЕТАЦИЯ РЕЗУЛЬТАТОВ

Анализ результатов поводят с помощью программного обеспечения используемого прибора для проведения ПЦР с детекцией в режиме «реального времени». Анализируют кривые накопления флуоресцентного сигнала по двум каналам:

- по каналу для флуорофора FAM регистрируется сигнал, свидетельствующий о накоплении продукта амплификации фрагмента ДНК ВКО STI-87,
- по каналу для флуорофора JOE регистрируется сигнал, свидетельствующий о накоплении продукта амплификации фрагмента ДНК *Coxiella burnetii*.

[4] Например, Rotor-Gene 3000, Rotor-Gene 6000 и рекомендованные ФБУН ЦНИИ Эпидемиологии Роспотребнадзора в методических рекомендациях по применению данного набора реагентов.

[5] Например, iCycler iQ5, Mx3000P, Mx3000, «ДТ-96» и рекомендованные ФБУН ЦНИИ Эпидемиологии Роспотребнадзора в методических рекомендациях по применению данного набора реагентов.

Результаты интерпретируются на основании наличия (или отсутствия) пересечения кривой флуоресценции с установленной на соответствующем уровне пороговой линией, что определяет наличие (или отсутствие) для данной пробы ДНК значения порогового цикла *Ct* в соответствующей графе в таблице результатов.

Принцип интерпретации результатов следующий:

- ДНК *Coxiella burnetii* **обнаружена***,* если для данной пробы в таблице результатов по каналу для флуорофора JOE определено значение порогового цикла *Ct,* не превышающее указанное (граничное) значение. При этом кривая флуоресценции каждой исследуемой пробы должна пересекать пороговую линию на участке характерного экспоненциального подъема флуоресценции.
- ДНК *Coxiella burnetii* **не обнаружена,** если для данной пробы в таблице результатов по каналу для флуорофора FAM определено значение порогового цикла *Ct,* не превышающее указанное (граничное) значение, а по каналу JOE не определено значение порогового цикла *Ct*.
- Результат анализа **невалидный,** если для данной пробы не определено (отсутствует) значение порогового цикла *Ct* по каналу JOE и по каналу FAM значение *Ct* также не определено (отсутствует) или превышает указанное граничное значение. В этом случае требуется повторно провести ПЦР-исследование соответствующего клинического образца, начиная с этапа экстракции.

ВНИМАНИЕ! Граничные значения *Ct* указаны во вкладыше, прилагаемом к набору реагентов. См. также методические рекомендации по применению набора реагентов для выявления ДНК *Coxiella burnetii* в биологическом материале методом полимеразной цепной реакции (ПЦР) с гибридизационно-флуоресцентной детекцией «АмплиСенс® *Coxiella burnetii*-FL».

Результат ПЦР-исследования считается достоверным, если получены правильные результаты для положительного и отрицательного контролей амплификации и отрицательного контроля экстракции ДНК

в соответствии с таблицей оценки результатов контрольных реакций (табл. 2).

Таблица 2

Результаты для контролей различных этапов ПЦР-исследования

Контроль	Контролируемый этап ПЦР-исследования	Значение порогового цикла, *Ct*	
		по каналу для флуорофора JOE	по каналу для флуорофора FAM
В–	Экстракция ДНК	Значение отсутствует	Определено значение меньше граничного
К–	ПЦР	Значение отсутствует	Значение отсутствует
К+	ПЦР	Определено значение меньше граничного	Определено значение меньше граничного

ВНИМАНИЕ!

1. Если для положительного контроля ПЦР (К+) значение порогового цикла по каналу для флуорофора JOE отсутствует или превышает граничное значение, необходимо повторить амплификацию для всех образцов, в которых не обнаружена специфическая ДНК.
2. Если для отрицательного контроля экстракции ДНК (В–) по каналу для флуорофора JOE определено значение порогового цикла *Ct*, необходимо повторить ПЦР-исследование для всех образцов, в которых обнаружена ДНК *Coxiella burnetii.*
3. Если для отрицательного контроля ПЦР (К–) по каналам FAM и/или JOE определено значение порогового цикла *Ct,* необходимо повторить амплификацию для всех образцов, в которых обнаружена ДНК *Coxiella burnetii*, с постановкой «К–» не менее чем в трех повторах.

СРОК ГОДНОСТИ. УСЛОВИЯ ТРАНСПОРТИРОВАНИЯ И ХРАНЕНИЯ

Срок годности. 9 мес. Набор реагентов с истекшим сроком годности применению не подлежит. Срок годности вскрытых реагентов соответствует сроку годности, указанному на этикетках для невскрытых реагентов, если в инструкции не указано иное.

Транспортирование. Набор реагентов транспортировать при температуре от 2 до 8 °С не более 5 сут. «ПЦР-комплект» вариант FRT-50 F при получении разукомплектовать в соответствии с указанными температурами хранения.

Хранение. Комплекты реагентов «РИБО-преп» и «ПЦР-комплект» хранить при температуре от 2 до 8 °С. ПЦР-смесь-1-FRT *Coxiella burnetii,* полимеразу (TaqF) и ОТ-ПЦР-смесь-2-FEP/FRT (из комплекта реагентов «ПЦР-комплект») хранить при температуре не выше минус 16 °С. ПЦР-смесь-1-FRT *Coxiella burnetii* хранить в защищенном от света месте.

Условия отпуска. Для лечебно-профилактических и санитарно-профилактических учреждений.

Рекламации на качество набора реагентов «**АмплиСенс**® ***Coxiella burnetii*-FL**» направлять на предприятие-изготовитель ФБУН ЦНИИ Эпидемиологии Роспотребнадзора (111123, г. Москва, ул. Новогиреевская, д. 3а) в отдел по работе с рекламациями и организации обучения (тел. (495) 974-96-46, факс (495) 916-18-18, e-mail: products@pcr.ru)[6].

Заведующий НПЛ ОМДиЭ Е.Н. Родионова

ФБУН ЦНИИ Эпидемиологии Роспотребнадзора

Главный врач Областной инфекционной А.В.Буркин

клинической больницы им.А.М.Ничоги

г.Астрахань

[6] Отзывы и предложения о продукции «АмплиСенс» вы можете оставить, заполнив анкету потребителя на сайте: www.amplisens.ru.

ПРИЛОЖЕНИЕ 1. Экстракция ДНК с использованием комплекта реагентов «РИБО-преп»

Экстракция ДНК из всех видов биологического материала проводится с применением комплекта реагентов «РИБО-преп»

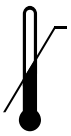
Порядок работы.

1. **Раствор для лизиса** (если он хранился при температуре от 2 до 8 °С) прогреть при температуре 65 °С до полного растворения кристаллов.
2. **В случае экстракции ДНК из суспензий клещей и тканей, мокроты** отобрать необходимое количество одноразовых пробирок на 1,5 мл с плотно закрывающимися крышками (включая отрицательный контроль экстракции). Внести в каждую пробирку, предназначенную для экстракции исследуемых проб, по **10 мкл ВКО STI-87** и по **300 мкл раствора для лизиса**. Промаркировать пробирки.
2. В пробирки с **раствором для лизиса** и **ВКО STI-87** внести по **50 мкл** суспензий клещей, суспензий тканей и обработанной муколизином мокроты.
3. **В случае экстракции ДНК из осадков клеток крови, ликвора, промывных вод бронхов** в пробирки с пробоподготовленным материалом внести по **300 мкл раствора для лизиса.** Содержимое пробирок тщательно перемешать на вортексе и процентрифугировать на вортексе для удаления капель с крышки пробирки. В пробирки с исследуемыми пробами внести по **10 мкл ВКО STI-87.** Промаркировать пробирки.
4. В пробирку отрицательного контроля (В–) экстракции внести **только 10 мкл ВКО STI-87** и **300 мкл раствора для лизиса**.
5. Содержимое пробирок тщательно перемешать на вортексе и прогреть **5 мин при 65 °С** в термостате. Добавить в пробирки по **400 мкл раствора для преципитации**, перемешать на вортексе.
6. Процентрифугировать пробирки на микроцентрифуге в течение **5 мин** при **10 000 g.**
7. Аккуратно отобрать надосадочную жидкость, не задевая осадок, используя вакуумный отсасыватель и отдельный наконечник для каждой пробы.

8. Добавить в пробирки по **500 мкл раствора для отмывки 3**, плотно закрыть крышки, осторожно промыть осадок, переворачивая пробирки 3-5 раз. Можно провести процедуру одновременно для всех пробирок, для этого необходимо накрыть пробирки в штативе сверху крышкой или другим штативом, прижать их и переворачивать штатив.
9. Процентрифугировать при **10 000 g в течение 2 мин** на микроцентрифуге.
10. Осторожно, не захватывая осадок, отобрать надосадочную жидкость, используя вакуумный отсасыватель и отдельный наконечник для каждой пробы.
11. Добавить в пробирки по **200 мкл раствора для отмывки 4**, плотно закрыть крышки и осторожно промыть осадок, переворачивая пробирки 3-5 раз.
12. Процентрифугировать при **10 000 g** в течение **2 мин** на микроцентрифуге.
13. Осторожно, не захватывая осадок, отобрать надосадочную жидкость, используя вакуумный отсасыватель и отдельный наконечник для каждой пробы.
14. Поместить пробирки в термостат при температуре **65 °С на 5 мин** для подсушивания осадка (при этом крышки пробирок должны быть открыты).
15. Добавить в пробирки по **50 мкл РНК-буфера**. Перемешать на вортексе. Поместить в термостат при температуре **65 °С на 5 мин**, периодически встряхивая на вортексе.
16. Процентрифугировать пробирки при **10 000 g в течение 1 мин** на микроцентрифуге. Надосадочная жидкость содержит очищенную ДНК. Пробы готовы к постановке ПЦР.

Очищенная ДНК может храниться до 24 ч при температуре от 2 до 8 °С и до года при температуре не выше минус 16 °С.

СИМВОЛЫ, ИСПОЛЬЗУЕМЫЕ В ПЕЧАТНОЙ ПРОДУКЦИИ

REF	Номер в каталоге		Осторожно! Обратитесь к сопроводительной документации
LOT	Код партии		Максимальное число тестов
IVD	Изделие для in vitro диагностики		Использовать до
VER	Дата изменения		Обратитесь к руководству по эксплуатации
	Ограничение температуры		Не допускать попадания солнечного света
	Верхнее ограничение температуры		Дата изготовления
	Производитель		

УТВЕРЖДЕНА

Приказом Росздравнадзора
от 09.04.08 № 2617-Пр/08

«УТВЕРЖДАЮ»

Директор Федерального государственного учреждения науки «Центральный научно-исследовательский институт эпидемиологии» Федеральной службы по надзору в сфере защиты прав потребителей и благополучия человека

_____ В.И.Покровский
«20» декабря 2007 г.

ИНСТРУКЦИЯ

по применению набора реагентов
для выявления ДНК *Bacillus anthracis* в биологическом материале и объектах окружающей среды методом полимеразной цепной реакции (ПЦР) с гибридизационно-флуоресцентной детекцией в режиме «реального времени»

«АмплиСенс® *Bacillus anthracis*-FRT»

Набор реагентов состоит из 2 комплектов реагентов:

- **«ДНК-сорб-В» вариант 50 –** комплект реагентов для выделения ДНК из клинического материала;
- **«ПЦР-комплект» вариант FRT –** комплект реагентов для ПЦР-амплификации ДНК *Bacillus anthracis* с гибридизационно-флуоресцентной детекцией в режиме «реального времени».

Допускается комплектация без комплекта реагентов «ДНК-сорб-В».

ФОРМА ВЫПУСКА.

Комплект реагентов «ДНК-сорб-В» вариант 50 включает:

Реактив	*Описание*	*Объем (мл)*	*Кол-во*
Лизирующий раствор	Прозрачная бесцветная жидкость	15	1 флакон
Раствор для отмывки 1	Прозрачная бесцветная жидкость	15	1 флакон
Раствор для отмывки 2	Прозрачная бесцветная жидкость	50	1 флакон
Сорбент универсальный	Суспензия белого цвета	1,25	1 пробирка
ТЕ-буфер для элюции ДНК	Прозрачная бесцветная жидкость	5,0	1 пробирка

Комплект реагентов рассчитан на выделение ДНК из 50 проб, включая контроли.

Комплект реагентов «ПЦР-комплект» вариант FRT включает:

Реактив	*Описание*	*Объем (мл)*	*Кол-во*
ПЦР-смесь-1-FRT *Bacillus anthracis*	Прозрачная бесцветная жидкость	0,008	55 пробирок
ПЦР-смесь-2-FL	Прозрачная бесцветная жидкость	0,77	1 пробирка
ПКО ДНК *Bacillus anthracis* pXO1	Прозрачная бесцветная жидкость	0,1	1 пробирка
ПКО ДНК *Bacillus anthracis* pXO2	Прозрачная бесцветная жидкость	0,1	1 пробирка
ПКО STI	Прозрачная бесцветная жидкость	0,1	1 пробирка
ОКО	Прозрачная жидкость от соломенно-желтого до бесцветного	1,6	1 пробирка
ВКО STI-704	Прозрачная бесцветная жидкость	0,5	1 пробирка
ДНК-буфер	Прозрачная бесцветная жидкость	0,5	1 пробирка

Комплект реагентов рассчитан на проведение 55 реакций амплификации, включая контроли.

НАЗНАЧЕНИЕ.

Набор реагентов **«АмплиСенс® *Bacillus anthracis*-FRT»** предназначен для выявления ДНК вегетативных и споровых форм *Bacillus anthracis* в биологическом материале и объектах окружающей среды, а также для определения плазмидного состава *Bacillus anthracis* путем выявления гена *pagA* (плазмида pXO1) и гена *capA* (плазмида pXO2) методом полимеразной цепной реакции (ПЦР) с гибридизационно-флуоресцентной детекцией в режиме «реального времени».

Один набор рассчитан на 50 тестов, включая контрольные образцы.

МЕРЫ ПРЕДОСТОРОЖНОСТИ.

Взятие, транспортирование, хранение материала на исследование и работу с ним проводят в соответствии с инструктивно-методическими документами, регламентирующими выполнение исследований: СП 1.3. 1285-3 «Безопасность работы с микроорганизмами I-II групп патогенности (опасности)», МУ 1.3.1794-03 «Организация работы при исследованиях методом ПЦР материала, инфицированного микроорганизмами I-II групп патогенности» и СП 1.2.036-95 «Порядок учета, хранения, передачи и транспортирования микроорганизмов I-IV групп патогенности».

ВЗЯТИЕ И ХРАНЕНИЕ МАТЕРИАЛА НА ИССЛЕДОВАНИЕ.

Для проведения анализа используются следующие материалы:

- Вода (сточная, из водоема, питьевая) – 10-20 мл.
- Почва.
- Смывы с воздушных фильтров.
- Порошкообразные вещества (корма для крупного рогатого скота (КРС), мука и т.д.)

Материал от людей:

- Цельная кровь – 5 мл. Забор крови проводится утром натощак в пробирку типа Vacuette®, с 6 % раствором ЭДТА из расчета 50 мкл ЭДТА на 1 мл крови. Закрытую пробирку с кровью несколько раз тщательно перемешивают путем

переворачивания.

- Экссудат из очагов поражения (при кожной форме), помещенный в 200 мкл стерильного раствора натрия хлорида 0,9 % (используют без предварительной обработки).
- Мокрота – в емкость с мокротой, для ее разжижения, добавляют коммерческий реагент «Муколизин» производства ФГУН «ЦНИИ эпидемиологии» Роспотребнадзора. Предобработка мокроты проводится по инструкции к реагенту «Муколизин». При необходимости повторного проведения анализа остаток обработанной мокроты замораживают.

Материал от животных:

- Цельная кровь – 5 мл. Забор крови проводят в пробирку типа Vacuette®, с 6 % раствором ЭДТА из расчета 50 мкл ЭДТА на 1 мл крови. Закрытую пробирку с кровью несколько раз переворачивают, чтобы перемешать консервант.
- Молоко КРС – без предварительной обработки.
- Паренхиматозные органы и лимфоузлы.

Биологический материал доставляют в лабораторию в емкости со льдом в течение 1 сут.

Допускается хранение вышеперечисленного материала до проведения исследования в течение 1 сут при температуре от 2 до 8 °С и в течение 6 мес при температуре не выше минус 16 °С. Допускается однократное замораживание-оттаивание материала.

Предварительная обработка материала:

Вода и смывы с воздушных фильтров.

10-20 мл воды центрифугировать 15 мин на центрифуге при 8000 g (10 000 об/мин при радиусе ротора 70 мм или 3 000 об/мин при радиусе ротора 150 мм). Надосадочную жидкость следует осторожно удалить, оставив 100 мкл. Осадок ресуспендировать в объеме 100 мкл и перенести в пробирки на 1,5 мл.

Почва:

В пробирки объемом 5 мл с плотно закрывающейся (завинчивающейся) крышкой отдельным шпателем (или одноразовыми лопатками) внести по 0,4-1,0 г (около 1,0 мл)

земли, залить 3 мл раствора натрия хлорида 0,9 %, тщательно перемешать и отстаивать 5 мин. Из пробирок с отстоявшейся землей перенести 1 мл раствора в пробирки объемом 1,5 мл с плотно закрывающейся крышкой и осадить грубодисперсную фракцию центрифугированием на микроцентрифуге 2-3 мин при 300 g (2000 об/мин при радиусе ротора 70 мм). Далее использовать осветленную надосадочную жидкость.

Порошкообразные вещества.

Порошкообразные вещества (объем около 0,05 $см^3$) растворить в 150 мкл стерильного раствора натрия хлорида 0,9 % и использовать полученный раствор в работе.

Нерастворимые в воде вещества следует обрабатывать аналогично пробам земли.

Паренхиматозные органы.

Кусочки размером не менее 1 $см^3$ и лимфоузлы (целиком) тщательно растереть в гомогенизаторах или с использованием стерильных фарфоровых ступок и пестиков, добавить равный объем (не менее 100 мкл) стерильного 0,9 % раствора натрия хлорида и тщательно перемешать. Суспензию отстаивать при комнатной температуре в течение 2-3 мин, затем верхнюю фазу перенести в пробирки вместимостью 1,5 мл и использовать далее на стадии обеззараживания.

Обеззараживание материала:

Проводят согласно МУ 3.5.5.1034-01 «Обеззараживание исследуемого материала, инфицированного бактериями I-IV групп патогенности, при работе методом ПЦР».

1. Герминация спор.
   Предварительно подготовленный исследуемый материал в количестве 0,1 мл мерной пипеткой емкостью 1-2 мл 2 класса точности засеять в пробирки (ГОСТ 1770-74) с 0,9 мл бульона Хоттингера pH 7,2±0,1 и инкубировать с интенсивной аэрацией на шуттель-аппарате при температуре (37±1) °С в течение 2,5 ч.
2. Обработка пенициллином.
   В пробирки добавить свежеприготовленный раствор пенициллина (до конечной концентрации 1000 ед/мл) и

инкубировать еще 15 мин при температуре (37±1) °С.

3. 1 мл суспензии перенести автоматической пипеткой с наконечниками с аэрозольным барьером в пробирки объемом 1,5 мл (с застегивающимися или завинчивающимися крышками, снабженными резиновыми прокладками) и подвергнуть центрифугированию при 12 тыс об/мин в течение 10 мин. Надосадочную жидкость отобрать, к осадку добавить 100 мкл 0,9 % раствора натрия хлорида, ресуспендировать. Пробирки прогреть в твердотельном термостате при температуре (110±5) °С в течение 10 мин.
4. Лизирующий раствор из комплекта реагентов «ДНК-сорб-В» (если он хранился при температуре от 2 до 8 °С) прогреть при температуре от 60 до 65 °С до полного растворения кристаллов. В каждую пробирку с исследуемыми пробами внести по 300 мкл лизирующего раствора и инкубировать в течение 15 мин при температуре 65 °С.

Дальнейшие исследования проб проводить как с обеззараженным материалом по порядку процедур, описанных в разделе «ВЫДЕЛЕНИЕ ДНК».

ДОПОЛНИТЕЛЬНЫЕ МАТЕРИАЛЫ И ОБОРУДОВАНИЕ, ТРЕБУЕМЫЕ ДЛЯ ПРОВЕДЕНИЯ ПЦР-АНАЛИЗА. (с указанием фирм-производителей/поставщиков):

ЗОНА 1.

Для выделения ДНК из исследуемого материала требуются:

1. Стерильный ламинарный шкаф (например, «БАВп-01-«Ламинар-С»-1,2», «Ламинарные системы», Россия).
2. Термостат для пробирок типа «Эппендорф» от 25 до 100 °С (например, «ТЕРМО 24-15», «Биоком», Россия).
3. Вакуумный отсасыватель медицинский с колбой-ловушкой для удаления надосадочной жидкости (например, «ОМ-1», г. Ульяновск, Россия).
4. Микроцентрифуга для пробирок типа «Эппендорф» до 16 тыс об/мин (например, «MiniSpin», «Eppendorf», Германия).
5. Вортекс (например «ТЭТА-2», «Биоком», Россия).
6. Отдельный набор автоматических пипеток переменного объема (например, «Ленпипет», Россия).
7. Одноразовые полипропиленовые завинчивающиеся или

плотно закрывающиеся микропробирки объемом 1,5 мл (например, «Axygen», США).

8. Штативы для микропробирок объемом 1,5 мл (например, «ИнтерЛабСервис», Россия) и наконечников (например, «Axygen», США).
9. Одноразовые наконечники для пипеток переменного объема с аэрозольным барьером до 200 мкл и до 1000 мкл (например, «Axygen», США).
10. Одноразовые наконечники для пипеток переменного объема до 200 мкл и до 1000 мкл (например, «Axygen», США).
11. Холодильник от 2 до 8 °С с морозильной камерой не выше минус 16 °С.
12. Отдельный халат и одноразовые перчатки.
13. Емкость с дезинфицирующим раствором.
14. Комплект средств для обработки рабочего места.

ЗОНА 2.

Для проведения ПЦР-амплификации и детекции продуктов амплификации требуются:

1. Амплификатор «Rotor-Gene» 3000 или 6000 («Corbett Research», Австралия) или эквивалентный.
2. ПЦР-бокс (например, «БАВ-ПЦР-«Ламинар-С», «Ламинарные системы», Россия).
3. Вортекс (например, «ТЭТА-2», «Биоком», Россия).
4. Отдельный набор автоматических пипеток переменного объема (например, «Ленпипет», Россия).
5. Одноразовые наконечники с аэрозольным барьером до 200 мкл (например, «Axygen», США).
6. Штативы для наконечников (например, «Axygen», США) и микропробирок (например, «ИнтерЛабСервис», Россия).
7. Холодильник от 2 до 8 °С с морозильной камерой не выше минус 16 °С.
8. Отдельный халат и одноразовые перчатки.
9. Емкость с дезинфицирующим раствором.
10. Комплект средств для обработки рабочего места.

ПРИМЕЧАНИЕ: допускается применение оборудования другого типа, по своим характеристикам не уступающего рекомендуемому.

ПРОВЕДЕНИЕ ПЦР-АНАЛИЗА.

ЭТАП 1. ВЫДЕЛЕНИЕ ДНК ИЗ ИССЛЕДУЕМОГО МАТЕРИАЛА (Комплект реагентов «ДНК-сорб-В» вариант 50). (проводится в ЗОНЕ 1 – помещении для обработки исследуемого материала).

Порядок работы.

1. Подготовить **отрицательный контроль выделения ДНК (ОК).** В пробирку объемом 1,5 мл внести **300 мкл лизирующего раствора и 100 мкл ОКО** – отрицательного контрольного образца**.**
2. Отдельными наконечниками с аэрозольным барьером внести в каждую пробирку с пробами (см. раздел «Обеззараживание биологического материала»), включая **ОК**, по **10 мкл ВКО STI-704**.
3. Пробы тщательно перемешать на вортексе, прогреть 5 мин при температуре 65 °С, осадить на вортексе 5 с.
   Если в пробирках находятся взвешенные частицы (не растворившийся полностью материал), то необходимо процентрифугировать пробирку на микроцентрифуге 5 мин при 8-10 тыс об/мин (10-13 тыс об/мин при радиусе ротора 70 мм) и использовать для выделения ДНК надосадочную жидкость, перенеся ее в новую пробирку.
4. Тщательно ресуспендировать **сорбент универсальный** на вортексе. В каждую пробирку отдельным наконечником добавить по **25 мкл** ресуспендированного **сорбента универсального**. Перемешать на вортексе, поставить в штатив на 5 мин, еще раз перемешать и оставить в штативе на 5 мин.
5. Осадить сорбент универсальный в пробирках центрифугированием при 8-10 тыс об/мин (10-13 тыс об/мин при радиусе ротора 70 мм) в течение 30 с. Удалить надосадочную жидкость, используя вакуумный отсасыватель и отдельный наконечник для каждой пробы.
6. Добавить в пробы по **300 мкл раствора для отмывки 1**, перемешать на вортексе до полного ресуспендирования сорбента, процентрифугировать 30 с при 8-10 тыс об/мин (10-13 тыс об/мин при радиусе ротора 70 мм) на

микроцентрифуге. Удалить надосадочную жидкость, используя вакуумный отсасыватель и отдельный наконечник для каждой пробы. При работе с образцами крови допустимо применение дозатора с индивидуальным наконечником с аэрозольным барьером для механического разбивания осадка.

7. Добавить в пробы по **500 мкл раствора для отмывки 2**, перемешать на вортексе до полного ресуспендирования сорбента универсального, процентрифугировать 30 с при 8-10 тыс об/мин (10-13 тыс об/мин при радиусе ротора 70 мм) на микроцентрифуге. Удалить надосадочную жидкость, используя вакуумный отсасыватель и отдельный наконечник для каждой пробы.
8. Повторить отмывку еще раз, следуя п. **7**, удалить надосадочную жидкость полностью.
9. Поместить пробирки в термостат при температуре 65 °С на 5-10 мин для подсушивания сорбента универсального. При этом крышки пробирок должны быть открыты.
10. В пробирки добавить по **50 мкл ТЕ-буфера для элюции ДНК**. Перемешать на вортексе. Поместить в термостат при температуре 65 °С на 5 мин, периодически встряхивая на вортексе.
11. Процентрифугировать пробирки при 8-10 тыс об/мин (10-13 тыс об/мин при радиусе ротора 70 мм) в течение 1 мин на микроцентрифуге. Надосадочная жидкость содержит очищенную ДНК. Пробы готовы к постановке ПЦР.

Допускается хранение очищенной ДНК в течение 7 сут при температуре от 2 до 8 °С и в течение года при температуре не выше минус 16 °С.

<u>ЭТАП 2.</u> ПРОВЕДЕНИЯ ПЦР-АМПЛИФИКАЦИИ И ДЕТЕКЦИИ ПРОДУКТОВ АМПЛИФИКАЦИИ (Комплект реагентов «ПЦР-комплект» вариант FRT).

(проводится в ЗОНЕ 2 – помещении для проведения ПЦР-амплификации).

Общий объем реакции – 25 мкл, объем ДНК-пробы – 10 мкл.

В комплекте реагентов применяется «горячий старт», который обеспечивается разделением нуклеотидов и Taq-

полимеразы прослойкой воска. Плавление воска и перемешивание реакционных компонентов происходит только при температуре 95 °С, что значительно снижает количество неспецифически затравленных реакций.

Порядок работы:

А. Подготовка пробирок для проведения ПЦР.

1. Отобрать необходимое количество пробирок с **ПЦР-смесью-1-FRT *Bacillus anthracis*** для амплификации ДНК исследуемых и контрольных проб (1 – отрицательная и 3 – положительные контрольные пробы).
2. На поверхность воска внести по **7 мкл ПЦР-смеси-2-FL**, при этом она не должна проваливаться под воск и смешиваться с **ПЦР-смесью-1-FRT *Bacillus anthracis.***

Б. Проведение амплификации.

1. В подготовленные для ПЦР пробирки внести отдельными наконечниками с аэрозольным барьером по **10 мкл ДНК-проб**, выделенных из исследуемых или контрольных проб этапа выделения ДНК.
2. Поставить **контрольные реакции амплификации**:
   - **а) отрицательный контроль (К-) –** внести в подготовленную пробирку **10 мкл ДНК-буфера**.
   - **б) положительный контроль (К1+)** – внести в подготовленную пробирку **10 мкл ПКО ДНК *Bacillus anthracis* pXO1**.
   - **в) положительный контроль (К2+)** – внести в подготовленную пробирку **10 мкл ПКО ДНК *Bacillus anthracis* pXO2**.
   - **г) положительный контроль (ВК+) –** внести в подготовленную пробирку **10 мкл ПКО STI.**

В. Программирование амплификатора:

Для работы с прибором «Rotor-Gene» 3000 следует использовать программу Rotor-Gene версии 6, с прибором «Rotor-Gene» 6000- программу Rotor-Gene 6000 версии 1.7 (build 67) или выше.

Далее по тексту термины, соответствующие разным версиям приборов и программного обеспечения указаны в следующем порядке: для прибора «Rotor-Gene» 3000 / для англоязычной версии программы «Rotor-Gene» 6000 / для

русскоязычной версии программы «Rotor-Gene» 6000.

1. Нажать кнопку «New»/«Новый» в основном меню программы.
2. В открывшемся окне выбрать меню «Advanced»/«Детальный мастер» и шаблон запуска эксперимента «Dual Labeled Probe»/«Hydrolysis probes»/«Флуоресцентные зонды (TaqMan)». Нажать кнопку «New»/«Новый».
3. Выбрать тип ротора «36-Well Rotor»/«36-луночный ротор». Поставить отметку в окне рядом с надписью «No Domed 0.2 ml Tubes»/«Locking ring attached»/«Кольцо закреплено».
4. Нажать кнопку «Next»/«Далее».
5. Выбрать объем реакционной смеси: Reaction volume/Объем реакции -25 мкл. Для прибора «Rotor-Gene» 6000 должно быть активно (отмечено галочкой) окно «15 µl oil layer volume»/«15 µL объем масла/воска». (Если галочка не стоит в окне по умолчанию, поставить ее с помощью мышки).
6. Нажать кнопку «Next»/«Далее».
7. В верхней части окна нажать кнопку «Edit profile»/«Редактор профиля».
8. Задать следующие параметры эксперимента:
   1. «Hold»/«Удерж. темп-ры» 95 °C – 5 мин
   2. «Cycling»/«Циклирование» 95 °C – 10 с
      60 °C – 25 с
      72 °C – 10 с
      Cycle repeats/Цикл повторить – 10 times/раз.
   3. «Cycling 2»/«Циклирование 2» 95 °C – 10 с
      56 °C – 25 с – Детекция
      72 °C – 10 с
      Cycle repeats/Цикл повторить – 35 times/раз.
   4. Флуоресценцию измеряют при температуре **56 °C** (во втором блоке циклирования) на каналах **FAM/Green, JOE/Yellow и ROX/Orange.**
   5. Нажать дважды кнопку «OK»/«Да».
9. В нижней части окна нажать кнопку «Calibrate»/«Gain Optimisation…»/«Опт. уровня сигн.». В открывшемся окне нажать кнопку «Calibrate Acquiring»/«Optimise Acquiring»/«Опт. Детек-мых». Для канала FAM/Green установить параметры «**Min Reading»/«Миним Сигнал»** –

20Fl и «**Max Reading»/«Максим Сигнал»** – 30Fl. Для канала JOE/Yellow установить параметры «**Min Reading»/«Миним Сигнал»** – 10Fl и «**Max Reading»/«Максим Сигнал»** – 15Fl. Для канала ROX/Orange установить параметры «**Min Reading»/«Миним Сигнал»** – 5Fl и «**Max Reading»/«Максим Сигнал»** – 10Fl. В графе «Tube position»/«Позиция Пробирки» указан номер пробирки, по которой будет автоматически выбран параметр «gain»/«усиление сигнала», по умолчанию это 1-я пробирка в роторе. Поэтому в 1-ой позиции в роторе должна ставиться пробирка с реакционной смесью. Пометить галочкой бокс в строке «Perform Calibration Before 1st Acquisition»/«Perform Optimisation Before 1st Acquisition»/«Выполнить оптимизацию при 1-м шаге детекции». Окно закрыть, нажав кнопку «**Close»/«Закрыть».** Нажать кнопку **«Next»/«Далее»**.

10. Поместить предварительно подготовленные пробирки в амплификатор. Запустить амплификацию кнопкой **«Start run»/«Старт»**.
11. Дать название эксперимента и сохранить его на диске (в этом файле будут автоматически сохранены результаты данного эксперимента).

В процессе работы амплификатора или по окончании его работы необходимо запрограммировать положение пробирок в карусели. Для этого надо использовать кнопку **«Edit samples»/«Правка образцов»** (в нижней правой части основного окна). Все пробы и контроли обозначить в меню «**Samples»/«Образцы как Unknown»/«Образец**».

АНАЛИЗ РЕЗУЛЬТАТОВ.

Анализ результатов амплификации ВКО по каналу ROX/ Orange.

1. Нажать в меню кнопку «Analysis»/«Анализ», выбрать режим анализа «Quantitation»/«Количественный», нажать кнопку «Cycling A. ROX»/«Cycling A. Orange», «Show»/«Показать».
2. Отменить автоматический выбор «Threshold»/«Порог».
3. Выбрать линейную шкалу графического изображения результатов, нажав кнопку «**Linear scale»/«Линейная Шкала»** в нижней части окна справа (если эта шкала активна по умолчанию, вместо кнопки «Linear scale»/

«Линейная Шкала» видна кнопка «Log scale»/ «Лог. Шкала»).
4. В меню основного окна («Quantitation analysis»/«Количественный анализ») должна быть нажата кнопка «Dynamic tube»/«Динамич.фон».
5. В меню «CT Calculation»/«Вычисление CT» (в правой части окна) выставить Threshold/Порог = 0.1.
6. В таблице результатов (окно «Quant. Results»/«Количественные Результаты») появятся значения Ct, которые должны быть не более 31 для исследуемых образцов и контролей.

Анализ результатов амплификации ДНК *Bacillus anthracis* pXO1 по каналу FAM/Green.

1. Нажать в меню кнопку «Analysis»/«Анализ», выбрать режим анализа «Quantitation»/«Количественный», нажать кнопку «Cycling A. FAM»/«Cycling A. Green», «Show»/«Показать».
2. Отменить автоматический выбор «Threshold»/«Порог».
3. Выбрать линейную шкалу графического изображения результатов, нажав кнопку «**Linear scale»/«Линейная Шкала»** в нижней части окна справа (если эта шкала активна по умолчанию, вместо кнопки «Linear scale»/«Линейная Шкала» видна кнопка «Log scale»/ «Лог. Шкала»).
4. В меню основного окна («Quantitation analysis»/«Количественный анализ») должна быть нажата кнопка «Dynamic tube»/«Динамич.фон».
5. В меню «CT Calculation»/«Вычисление CT» выставить «Threshold»/«Порог» = 0.025.
6. В таблице результатов (окно «Quant. Results»/«Количественные Результаты» появятся значения Ct.

Анализ результатов амплификации ДНК *Bacillus anthracis* pXO2 по каналу JOE/Yellow.

1. Нажать в меню кнопку «Analysis»/«Анализ», выбрать режим анализа «Quantitation»/«Количественный», нажать кнопку «Cycling A. JOE»/«Cycling A. Yellow», «Show»/«Показать».
2. Отменить автоматический выбор «Threshold»/«Порог».
3. Выбрать линейную шкалу графического изображения результатов, нажав кнопку «**Linear scale»/«Линейная**

Шкала» в нижней части окна справа (если эта шкала активна по умолчанию, вместо кнопки «Linear scale»/«Линейная Шкала» видна кнопка «Log scale»/ «Лог. Шкала»).

4. В меню основного окна («Quantitation analysis»/«Количественный анализ») должна быть нажата кнопка «Dynamic tube»/«Динамич.фон».
5. В меню «CT Calculation»/«Вычисление CT» выставить «Threshold»/«Порог» = 0.1.
6. В таблице результатов (окно «Quant. Results»/«Количественные Результаты») появятся значения Ct.

УЧЕТ РЕЗУЛЬТАТОВ.

Результаты интерпретируются на основании наличия (или отсутствия) пересечения кривой флуоресценции с установленной на соответствующем уровне пороговой линией (что соответствует наличию (или отсутствию) значения порогового цикла «Ct» в соответствующей графе в таблице результатов).

Результат считается достоверным только в случае прохождения положительных и отрицательных контролей амплификации и отрицательного контроля выделения ДНК (см. табл. 1).

Таблица 1.

Результаты постановки контролей различных этапов ПЦР-анализа

Контроль	Контролируемый этап ПЦР-анализа	Значение Ct по каналу		
		FAM/Green	JOE/Yellow	ROX/Orange
«ОК»	Выделение ДНК	Нет значений	Нет значений	< 31
«К-»	ПЦР	Нет значений	Нет значений	Нет значений
«К1+»	ПЦР	< 33	Нет значений	Нет значений
«К2+»	ПЦР	Нет значений	< 33	Нет значений
«ВК+»	ПЦР	Нет значений	Нет значений	< 31

1. **Образец считают положительным на наличие ДНК *Bacillus anthracis* pXO1+ и pXO2+**, если значение Ct по каналу FAM/Green и JOE/Yellow менее 33, не зависимо от значения Ct по каналу ROX/Orange.
2. **Образец считают положительным на наличие ДНК *Bacillus anthracis* pXO1+**, если значение Ct по каналу

FAM/Green менее 33, не зависимо от значения Ct по каналу ROX/Orange.

3. **Образец считают положительным на наличие ДНК *Bacillus anthracis* pXO2+**, если значение Ct по каналу JOE/Yellow менее 33, не зависимо от значения Ct по каналу ROX/Orange.
4. **Образец считают отрицательным**, если по каналам FAM/Green и JOE/Yellow для него значение Ct отсутствует, а по каналу ROX/Orange для него определено значение Ct, не превышающее 31.

Таблица 2.

Оценка результатов исследуемых проб

	Значение Ct по каналу			Результат анализа
	FAM/Green	JOE/Yellow	ROX/Orange	
1	Нет	Нет	≤ 31	*Bacillus anthracis* не обнаружена
2	< 33	Нет	≤ 31 (или Нет)	*Bacillus anthracis* (pXO1+/pXO2-)
3	< 33	< 33	≤ 31 (или Нет)	*Bacillus anthracis* (pXO1+/pXO2+)
4	Нет	<33	≤ 31 (или Нет)	*Bacillus anthracis* (pXO1-/pXO2+)
5	Нет	Нет	Нет (или > 31)	Проба подлежит повторному анализу с этапа выделения ДНК

Результаты не подлежат учету:

1. Отсутствие положительного сигнала в пробах с положительными контролями ПЦР может свидетельствовать о неправильно выбранной программе амплификации и о других ошибках, допущенных на этапе постановки ПЦР. В таком случае необходимо провести ПЦР еще раз.
2. Если значение Ct по каналу FAM/Green больше 33, а значение Ct по каналу ROX/Orange не превышает 31, требуется повторить ПЦР и считать его положительным в случае повторения результата или получения значения Ct на канале FAM/Green менее 33.
3. Если значение Ct на канале JOE/Yellow больше 33, а значение Ct по каналу ROX/Orange не превышает 31, требуется повторить ПЦР и считать его положительным в случае повторения результата или получения значения Ct на канале JOE/Yellow менее 33.
4. Если в образце отсутствует значение Ct по каналам

FAM/Green и JOE/Yellow, а значение Ct по каналу ROX/Orange более 31 или отсутствует, требуется повторное проведение ПЦР и детекции. В случае если повторно получен аналогичный результат, требуется повторить анализ образца, начиная с этапа выделения нуклеиновых кислот.

5. Появление любого значения Ct в таблице результатов для отрицательного контрольного образца на канале JOE/Yellow и/или FAM/Green и для отрицательного контроля ПЦР (ДНК-буфер) на любом из каналов свидетельствует о наличии контаминации реактивов или образцов. В этом случае результаты анализа по всем пробам считаются недействительными. Требуется повторить анализ всех проб, а также предпринять меры по выявлению и ликвидации источника контаминации.

ОБЕЗЗАРАЖИВАНИЕ.

Обеззараживание биоматериала и реагентов следует проводить на каждой стадии отдельно, помещая одноразовую пластиковую посуду (пробирки, наконечники), колбы-ловушки вакуумных отсасывателей на 20-24 ч в специальные контейнеры, содержащие дезинфицирующий 0,2 % раствор ДП-2Т.

СРОК ГОДНОСТИ. УСЛОВИЯ ТРАНСПОРТИРОВАНИЯ И ХРАНЕНИЯ.

Срок годности 6 мес. Набор реагентов с истекшим сроком годности применению не подлежит.

Транспортирование. Набор реагентов транспортировать при температуре от 2 до 8 °С не более 5 сут. При получении разукомплектовать в соответствии с указанными температурами хранения.

Хранение. Комплект реагентов «ДНК-сорб-В» хранить при температуре от 2 до 25 °С. Комплект реагентов «ПЦР-комплект» хранить при температуре от 2 до 8 °С в защищенном от света месте.

Рекламации на качество набора реагентов **«АмплиСенс®** ***Bacillus anthracis*-FRT»** направлять в адрес ФГУН ГИСК им. Л.А. Тарасевича Роспотребнадзора (119002, г. Москва, пер. Сивцев Вражек, д. 41, тел. (495) 241-39-22, факс (495) 241-92-38), в адрес предприятий-изготовителей: ФГУН «ЦНИИЭ» Роспотребнадзора (111123, г. Москва, ул. Новогиреевская, д. 3а, тел. (495) 305-39-39, факс (495) 305-54-23), ФГУЗ РосНИПЧИ «Микроб» Роспотребнадзора (410005. г. Саратов, ул.Университетская, д.46, тел (8452) 26-21-31, факс (8452) 51-52-12) и в адрес официального дилера **–** компанию ООО «ИнтерЛабСервис» (тел. (495) 105-0554, факс (495) 916-18-18, e-mail: products@pcr.ru).

Директор ФГУН «ЦНИИ эпидемиологии» Роспотребнадзора — В.И. Покровский

Директор ФГУЗ Российского научно-исследовательского противочумного института «Микроб» Роспотребнадзора — В.В. Кутырев

Руководитель приемочных технических и медицинских испытаний
Зав. лабораторией препаратов против чумы и других особо опасных инфекций
ФГУН ГИСК им. Л.А.Тарасевича Роспотребнадзора — Л.В.Саяпина

УТВЕРЖДЕНА

Приказом Росздравнадзора

от 04.05.2012 № 2084-Пр/12

УТВЕРЖДАЮ

Директор Федерального бюджетного учреждения науки «Центральный научно-исследовательский институт эпидемиологии» Федеральной службы по надзору в сфере защиты прав потребителей и благополучия человека

_____ В.И.Покровский

«15» февраля 2012 г.

ИНСТРУКЦИЯ

по применению набора реагентов

для выявления РНК вируса Западного Нила

в биологическом материале методом полимеразной цепной реакции (ПЦР) с гибридизационно-флуоресцентной детекцией

«АмплиСенс® *WNV*-FL»

АмплиСенс®

Федеральное бюджетное учреждение науки
«Центральный научно-исследовательский институт эпидемиологии»,
Российская Федерация, 111123,
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IVD

ОГЛАВЛЕНИЕ

СПИСОК СОКРАЩЕНИЙ

В настоящей инструкции применяются следующие сокращения и обозначения:

ВКО	- внутренний контрольный образец
К–	- отрицательный контроль ПЦР
К+	- положительный контроль ПЦР
кДНК	- комплементарная ДНК, получаемая в реакции обратной транскрипции на матрице РНК
НК	- нуклеиновые кислоты (РНК/ДНК)
ОКО	- отрицательный контрольный образец
ОК	- отрицательный контрольный образец экстракции РНК
ПК	-положительный контроль экстракции РНК
ПКО	- положительный контрольный образец
ПЦР	- полимеразная цепная реакция
СМЖ	- спинномозговая жидкость
ФБУН ЦНИИ Эпидемиологии Роспотребнадзора	- федеральное бюджетное учреждение науки «Центральный научно-исследовательский институт эпидемиологии» Федеральной службы по надзору в сфере защиты прав потребителей и благополучия человека
FRT	- флуоресцентная детекция в режиме «реального времени»
WNV	- *West Nile virus*, вирус Западного Нила

НАЗНАЧЕНИЕ

Набор реагентов **«АмплиСенс®** ***WNV*-FL»** предназначен для выявления РНК вируса Западного Нила (*WNV)* в клиническом (плазма и сыворотка крови, лейкоцитарная фракция крови, спинномозговая жидкость, моча) и аутопсийном материале от людей (ткани мозга, печени, селезенки, лимфоузлов), материале от животных (ткани мозга), в комарах и клещах методом ПЦР с гибридизационно-флуоресцентной детекцией.

ВНИМАНИЕ! Результаты ПЦР-исследования учитываются в комплексной диагностике заболевания[1].

ПРИНЦИП МЕТОДА

Выявление *WNV* методом полимеразной цепной реакции (ПЦР) с гибридизационно-флуоресцентной детекцией включает в себя следующие этапы: экстракцию РНК из образцов клинического материала, проведение обратной транскрипции РНК и амплификацию участка кДНК *WNV* с гибридизационно-флуоресцентной детекцией, которая производится непосредственно в ходе ПЦР.

Экстракция РНК из клинического материала проводится в

[1] В соответствии с Директивой Европейского Союза 98/79/ЕС.

присутствии внутреннего контрольного образца (ВКО STI-87-rec), который позволяет контролировать выполнение процедуры исследования для каждого образца. Затем с полученными пробами проводятся: обратная транскрипции РНК с помощью фермента ТМ-Ревертазы и амплификация участков кДНК *WNV* при помощи специфичных к этому участку кДНК праймеров и фермента Taq-полимеразы. В составе реакционной смеси присутствует флуоресцентно-меченый олигонуклеотидный зонд, который гибридизуется с комплементарным участком амплифицируемой кДНК-мишени, в результате чего происходит нарастание интенсивности флуоресценции. Это позволяет регистрировать накопление специфических продуктов амплификации путем измерения интенсивности флуоресцентных сигналов. Детекция флуоресцентных сигналов при использовании формата FRT происходит непосредственно в ходе ПЦР с помощью амплификатора с системой детекции флуоресцентного сигнала в режиме «реального времени».

ФОРМАТЫ И ФОРМЫ ВЫПУСКА НАБОРА РЕАГЕНТОВ

Набор реагентов выпускается в 1 формате.

Формат FRT

Набор реагентов выпускается в 6 формах комплектации:

Форма 1 включает комплект реагентов «ПЦР-комплект» вариант FRT.

Форма 2 включает комплекты реагентов «РИБО-преп» вариант 50, «ПЦР-комплект» вариант FRT.

Форма 3 включает комплекты реагентов для комбинированного метода экстракции РНК: «РИБО-преп» вариант 50, «РИБО-золь-С» вариант 50, «ПЦР-комплект» вариант FRT.

Форма 4 включает комплекты реагентов для комбинированного метода экстракции РНК: «РИБО-сорб» вариант 50, «РИБО-золь-С» вариант 50, «ПЦР-комплект» вариант FRT.

Форма 5 включает 1 комплект реагентов «МАГНО-сорб» вариант 100-1000, 2 комплекта реагентов «ПЦР-комплект» вариант FRT.

Форма 6 включает наборы реагентов оптом, расфасованные по отдельным реагентам, с маркировкой реагентов на их оптовой фасовке.

Форма комплектации 1 предназначена для проведения обратной транскрипции РНК и амплификации кДНК с

гибридизационно-флуоресцентной детекцией в режиме «реального времени». Для проведения полного ПЦР-исследования необходимо дополнительно использовать комплекты реагентов для экстракции РНК, рекомендованные ФБУН ЦНИИ Эпидемиологии Роспотребнадзора в зависимости от вида исследуемого материала.

Формы комплектации 2, 3, 4, 5 предназначены для проведения полного ПЦР-исследования, включая экстракцию РНК из клинического материала, проведение обратной транскрипции РНК и амплификацию кДНК с гибридизационно-флуоресцентной детекцией в режиме «реального времени».

Форма комплектации 6 предназначена для производственных целей для последующей маркировки на языке заказчика и комплектации по наборам.

ВНИМАНИЕ! Форма комплектации 6 используется только в соответствии с регламентом, утвержденным ФБУН ЦНИИ Эпидемиологии Роспотребнадзора.

АНАЛИТИЧЕСКИЕ ХАРАКТЕРИСТИКИ

Аналитическая чувствительность

Вид биологического материала (объем исследуемой пробы)	Комплект для экстракции РНК/ДНК	Комплект для амплификации и детекции	Аналитическая чувствительность, копий/мл	Пробоподготовка материала
сыворотка крови (200 мкл), СМЖ (200 мкл), лейкоци-тарная фракция крови (200 мкл), 10 % суспензия тканей мозга (30 мкл), комары (100 мкл)	**«РИБО-преп»**	«ПЦР-комплект» вариант FRT	$5x10^3$	Данная чувствительность достигается при соблюдении нижеизложенных правил пробоподготовки биоматериала и рекомендуемом исследуемом объеме пробы
лейкоцитарная фракция крови (200 мкл), 10 % суспензия тканей мозга (30 мкл), комары (100 мкл)	**«РИБО-преп» и «РИБО-золь-С»**	«ПЦР-комплект» вариант FRT	$5x10^3$	
лейкоцитарная фракция крови (200 мкл), 10 % суспензия тканей мозга (30 мкл), комары (100 мкл)	**«РИБО-сорб» и «РИБО-золь-С»**	«ПЦР-комплект» вариант FRT	$5x10^3$	
Сыворотка и плазма крови, СМЖ – 1 мл	**«МАГНО-сорб»**	«ПЦР-комплект» вариант FRT	$5x10^2$	

Аналитическая специфичность

Аналитическая специфичность изучена на:

- флавивирусах (вирус клещевого энцефалита, Лангат, Повассан, Японского энцефалита, Омской геморрагической лихорадки);
- герпесвирусах (I и II типов, *CMV*, *EBV*, *VZV*, IV типа), энтеровирусах (*ECHO*, *Coxsackie*);
- риккетсиях группы пятнистых лихорадок (*Rickettsia conorii* ssp*. caspia*, *R.heilongiangensis*; *Coxiella burnetii*; *Bartonella henselae*, *B.quintana*);
- спирохетах (*Borrelia miyamotoi*; *Treponema pallidum*; *Leptospira interrogans*, *L.kirshneri*, *L.borgpetersenii*).

При работе с РНК/ДНК вышеперечисленных организмов, ДНК человека, ДНК птиц, ДНК клещей и комаров, ДНК грызунов не выявлено ложноположительных результатов.

МЕРЫ ПРЕДОСТОРОЖНОСТИ

Работа должна проводиться в лаборатории, выполняющей молекулярно-биологические (ПЦР) исследования клинического материала на наличие возбудителей инфекционных болезней, с соблюдением санитарно-эпидемических правил СП 1.3.2322-08 «Безопасность работы с микроорганизмами III–IV групп патогенности (опасности) и возбудителями паразитарных болезней», СанПиН 2.1.7.2790-10 «Санитарно-эпидемиологические требования к обращению с медицинскими отходами» и методических указаний МУ 1.3.2569-09 «Организация работы лабораторий, использующих методы амплификации нуклеиновых кислот при работе с материалом, содержащим микроорганизмы I–IV групп патогенности».

При работе всегда следует выполнять следующие требования:

- Следует рассматривать исследуемые образцы как инфекционно-опасные, организовывать работу и хранение в соответствии с СП 1.3.2322-08 «Безопасность работы с микроорганизмами III–IV групп патогенности (опасности) и возбудителями паразитарных болезней».

ВНИМАНИЕ! При работе с клещом с высокой степенью напитанности рекомендуется перед гомогенизацией проколоть его одноразовой иглой для выхода крови и предупреждения разбрызгивания материала при растирании в ступке.

- Убирать и дезинфицировать разлитые образцы или реактивы, используя дезинфицирующие средства в соответствии с СП 1.3.2322-08 «Безопасность работы с

микроорганизмами III–IV групп патогенности (опасности) и возбудителями паразитарных болезней».

- Лабораторный процесс должен быть однонаправленным. Анализ проводится в отдельных помещениях (зонах). Работу следует начинать в Зоне Выделения, продолжать в Зоне Амплификации и Детекции. Не возвращать образцы, оборудование и реактивы в зону, в которой была проведена предыдущая стадия процесса.
- Удалять неиспользованные реактивы в соответствии с СанПиН 2.1.7.2790-10 «Санитарно-эпидемиологические требования к обращению с медицинскими отходами».

ВНИМАНИЕ! При удалении отходов после амплификации (пробирок, содержащих продукты ПЦР) недопустимо открывание пробирок и разбрызгивание содержимого, поскольку это может привести к контаминации продуктами ПЦР лабораторной зоны, оборудования и реагентов.

- Применять набор строго по назначению, согласно данной инструкции.
- Допускать к работе с набором только специально обученный персонал.
- Не использовать набор по истечении срока годности.
- Использовать одноразовые перчатки, лабораторные халаты, защищать глаза во время работы с образцами и реактивами. Тщательно вымыть руки по окончании работы.
- Избегать контакта с кожей, глазами и слизистой оболочкой. При контакте немедленно промыть пораженное место водой и обратиться за медицинской помощью.
- Листы безопасности материалов (MSDS – material safety data sheet) доступны по запросу.

ДОПОЛНИТЕЛЬНЫЕ МАТЕРИАЛЫ И ОБОРУДОВАНИЕ

1. 0,15 М NaCl или фосфатный буферный раствор (PBS) (натрия хлорид, 137 мМ; калия хлорид, 2,7 мМ; натрия монофосфат, 10 мМ; калия дифосфат, 2 мМ; pH=7,5±0,2).
2. Комплекты реагентов для выделения РНК/ДНК (в зависимости от типа исследуемого биоматериала) при работе с формой комплектации 1.
3. Дополнительные материалы и оборудование для экстракции РНК/ДНК – согласно инструкции к комплекту реагентов для выделения РНК/ДНК.

4. Гомогенизатор TissueLyser LT (Qiagen, Германия) рекомендуется использовать для гомогенизации аутопсийного материала, клещей и комаров.
5. Металлические шарики из нержавеющей стали диаметром 5 мм и 7 мм.
6. Бокс абактериальной воздушной среды (ПЦР-бокс).
7. Центрифуга/вортекс.
8. Автоматические дозаторы переменного объема (от 5 до 20 мкл и от 20 до 200 мкл).
9. Одноразовые наконечники с фильтром до 100 мкл в штативах, до 200 мкл и до 1000 мкл.
10. Штативы для пробирок объемом 0,2 мл.
11. Одноразовые полипропиленовые завинчивающиеся или плотно закрывающиеся пробирки объемом 1,5 мл.
12. Штативы для наконечников и пробирок объемом 1,5 мл.
13. Холодильник от 2 до 8 °С с морозильной камерой не выше минус 16 °С для выделенных проб ДНК.
14. Отдельный халат, шапочки, обувь и одноразовые перчатки по МУ 1.3.2569-09.
15. Емкость для сброса наконечников.
16. Емкость с дезинфицирующим раствором.
17. Программируемый амплификатор с системой детекции флуоресцентного сигнала в режиме «реального времени» (например, Rotor-Gene 3000/6000 (Corbett Research, Австралия), Rotor-Gene Q (Qiagen, Германия), iCycler iQ5 (Bio-Rad, США), Mx3000P (Stratagene, США) и рекомендованные ФБУН ЦНИИ Эпидемиологии Роспотребнадзора в методических рекомендациях по применению данного набора реагентов).
18. Одноразовые полипропиленовые пробирки для ПЦР объемом 0,2 мл:
    а) тонкостенные пробирки для ПЦР объемом 0,2 мл с выпуклой крышкой (например, Axygen, США) – при использовании прибора планшетного типа;
    б) тонкостенные пробирки для ПЦР объемом 0,2 мл с плоской крышкой (например, Axygen, США) – при использовании прибора роторного типа.

При использовании комплекта реагентов «МАГНО-сорб» дополнительно:

1. Термостат для пробирок объемом 5 мл, диаметром 12 мм от 25 до 100 °С.
2. Термостат для пробирок типа «Эппендорф» от 25 до 100 °С.
3. Магнитный штатив для пробирок типа «Эппендорф» на 1,5 мл.
4. Магнитный штатив для пробирок на 5 мл, диаметр 12 мм.
5. Одноразовые полипропиленовые или полистирольные пробирки объемом до 5 мл диаметром 12 мм, круглодонные.
6. Одноразовые полипропиленовые крышки для пробирок объемом до 5 мл диаметром 12 мм.
7. Автоматический дозатор переменного объема с возможностью дозирования от 1000 до 5000 мкл.
8. Одноразовые наконечники до 200 мкл, до 1000 мкл и до 5000 мкл.

ВЗЯТИЕ, ТРАНСПОРТИРОВАНИЕ И ХРАНЕНИЕ ИССЛЕДУЕМОГО МАТЕРИАЛА

Перед началом работы следует ознакомиться с методическими рекомендациями «Взятие, транспортировка, хранение клинического материала для ПЦР-диагностики», разработанными ФГУН ЦНИИЭ Роспотребнадзора, Москва, 2008 г.

ПОДГОТОВКА ИССЛЕДУЕМОГО МАТЕРИАЛА К ЭКСТРАКЦИИ РНК

1. Плазма крови, сыворотка крови, СМЖ. Взятие цельной периферической крови проводится утром натощак в пробирку с 6 % раствором ЭДТА из расчета 1:20. Закрытую пробирку с цельной периферической кровью несколько раз переворачивают. Для отбора плазмы пробирку с кровью центрифугируют в течение 20 мин при 1600 g. Сыворотку крови получают стандартными методами. СМЖ не проходит стадию пробоподготовки. Для исследования отбирают 200 мкл клинического материала при экстракции РНК с использованием комплекта «РИБО-преп» или 1 мл клинического материала при использовании комплекта «МАГНО-сорб».
2. Лейкоцитарная фракция крови. Для исследования

лейкоцитарной фракции крови (данный тип клинического материала рекомендуется для исследования на 2-й нед заболевания) 1,5 мл крови с раствором ЭДТА переносят в пробирку типа «Эппендорф» и центрифугируют на микроцентрифуге при 400 g в течение 10 мин. Затем отбирают приблизительно 500-600 мкл плазмы и центрифугируют при 7000 g в течение 10 мин. После чего оставляют для экстракции РНК осадок клеток и 200 мкл надосадочной плазмы над клетками при экстракции с использованием комплекта «РИБО-преп» и при использовании комбинированных методов экстракции (комплекты «РИБО-золь-С» и «РИБО-сорб» или комплекты «РИБО-золь-С» и «РИБО-преп»).

3. Внутренние органы животных и секционный материал. Данный материал гомогенизируют с использованием стерильных фарфоровых ступок и пестиков, затем готовят 10 % суспензию на стерильном физиологическом растворе или фосфатном буфере. При наличии автоматического гомогенизатора TissueLyser LT применяют следующие параметры для гомогенизации тканей внутренних органов: объем PBS-буфера или 0,15 М раствора NaCl для гомогенизации определяется объемом гомогенизируемой ткани – соотношение ткань-буфер определяется как 1:9, то есть готовится 10 % суспензия. Общий объем пробы для пробирок объемом 1,5 мл не должен превышать 1 мл, условия гомогенизации: для тканей мозга: диаметр шариков – 5 мм; частота – 50 Гц/сек; время гомогенизации – 2-3 минуты; для тканей печени, селезенки, лимфоузлов: диаметр шариков – 7 мм; частота – 50 Гц/сек; время гомогенизации – 10 минут; для лимфоузлов: диаметр шариков – 5 мм; частота – 50 Гц/сек; время гомогенизации – 5 минут.

   Для экстракции РНК берут 30 мкл суспензии в случае экстракции комплектом «РИБО-преп» и комбинированным методом (комплекты «РИБО-золь-С» и «РИБО-сорб») и 100 мкл суспензии в случае экстракции комбинированным методом (комплекты «РИБО-золь-С» и «РИБО-преп»).
4. Моча. Для исследования моча собирается в чистую посуду. Если нет возможности исследовать материал в течение

1 суток после взятия, моча переносится в центрифужную пробирку на 30 мл или пробирку типа «Эппендорф», затем в нее вносят глицерин, 10 % от объема пробы, перемешивают для равномерного распределения глицерина и замораживают при минус 20 °С для хранения в течение 1 нед или при минус 70 °С в течение более длительного времени.

При наличии центрифуги с охлаждением до 4 °С для пробирок объемом 30 мл и ускорением 8000 g используется следующий алгоритм пробоподготовки. Пробу центрифугируют при 8000-9000 g в течение 10 мин, затем надосадочную жидкость переносят в емкость с дезинфицирующим раствором, а осадок и 1 мл надосадочной жидкости над ним – в пробирку типа «Эппендорф». После чего снова концентрируют пробу при 8000 g в течение 10 мин. 900 мкл надосадочной жидкости переносят в емкость с дезинфицирующим раствором, а осадок и 100 мкл надосадочной жидкости используют для экстракции РНК. В случае наличия большого количества солей, для экстракции РНК в отдельную пробирку типа «Эппендорф» переносят 100 мкл надосадочной жидкости.

При отсутствии центрифуги для пробирок объемом 30 мл и ускорением 8000 g, проводят концентрирование бактерий только из 1 мл мочи как описано выше. Экстракцию РНК также проводят из осадка и 100 мкл надосадочной жидкости.

5. Комары. Для приготовления суспензий комаров используют стерильную фарфоровую чашку и стерильный пестик. При наличии автоматического гомогенизатора TissueLyser LT применяют следующие параметры для гомогенизации комаров (диаметр шариков – 5 мм; частота – 50 Гц/с; время гомогенизации – 5 мин; объем буфера – 700 мкл (пул из 25 комаров), 1000-1500 мкл (пул из 50 комаров).

   Предварительно формируют пулы комаров (не более 50 особей). Комаров гомогенизируют в стерильном физиологическом растворе или фосфатном буфере из расчета 1 комар – 30 мкл раствора. Центрифугируют пробы при 10 000 g в течение 1 мин. Затем отбирают 100 мкл надосадочной жидкости для экстракции РНК.
6. Клещи. Предварительно формируют пулы клещей: голодных объединяют по 5-7 особей, полунапитавшихся – по 2-3;

полностью напитавшихся – по 1. Для приготовления суспензий клещей используют стерильную фарфоровую чашку и стерильный пестик. При наличии автоматического гомогенизатора TissueLyser LT применяют следующие параметры для гомогенизации клещей рода *Hyalomma* (диаметр шариков – 7 мм; частота – 50 Гц/с; время гомогенизации – 10-12 мин; объем буфера – 700 мкл (ненапитавшийся клещ), 1000-1500 мкл (напитавшийся клещ и пулы клещей). В случае гомогенизации напитавшихся клещей в ступке их предварительно прокалывают стерильной одноразовой иглой в нескольких местах для выхода крови. Клещей растирают в 700 мкл (если проба состоит из одного ненапитавшегося клеща) или в 1-1,5 мл (если гомогенизируют пул клещей или напитавшегося клеща) 0,15 М раствора хлорида натрия, смешивая раствор с клещами небольшими объемами, затем полученную суспензию центрифугируют при 10 000 g в течение 1 мин и отбирают 100 мкл надосадочной жидкости для экстракции РНК.

Допускается хранение вышеперечисленного клинического материала до проведения исследования в течение суток при температуре от 2 до 8 °С или 1 нед – при температуре не выше минус 16 °С. Для аутопсийного материала и насекомых предусмотрены следующие режимы хранения: ткани внутренних органов и комаров хранят 1 нед при температуре не выше минус 16 °С, далее – при температуре минус 70 °С. Клещей хранят или живыми (до 1 мес) или 1 нед при температуре не выше минус 16 °С, далее – при температуре минус 70 °С.

ФОРМАТ FRT
СОСТАВ

Комплект реагентов «РИБО-сорб» вариант 50 (ТУ 9398-004-01897593-2008) **–** комплект реагентов для выделения РНК/ДНК из клинического материала **– включает:**

Реактив	*Описание*	*Объем, мл*	*Кол-во*
Лизирующий раствор	Прозрачная бесцветная жидкость[2]	22,5	1 флакон
Раствор для отмывки 1	Прозрачная бесцветная жидкость[4]	20	1 флакон
Раствор для отмывки 3	Прозрачная бесцветная жидкость	50	1 флакон
Раствор для отмывки 4	Прозрачная бесцветная жидкость	20	1 флакон
Сорбент	Суспензия белого цвета	1,25	1 пробирка
РНК-буфер	Прозрачная бесцветная жидкость	0,5	5 пробирок

Комплект реагентов рассчитан на выделение РНК/ДНК из 50 проб, включая контроли. Входит в состав формы комплектации 4.

Комплект реагентов «РИБО-золь-С» вариант 50 (ТУ 9398-074-01897593-2008) **–** комплект реагентов для первого этапа выделения РНК из биологического материала **– включает:**

Реактив	*Описание*	*Объем,*	*Кол-во*
Раствор D	Прозрачная бесцветная жидкость	20	1 флакон
Раствор E	Прозрачная бесцветная жидкость	1,5	1 пробирка
Раствор A	Прозрачная жидкость оранжевого цвета	15	1 флакон
Раствор B	Прозрачная бесцветная жидкость	5	1 флакон

Комплект реагентов рассчитан на выделение РНК из 50 проб, включая контроли. Входит в состав форм комплектации 3, 4.

Комплект реагентов «РИБО-преп» вариант 50 (ТУ 9398-071-01897593-2008) **–** комплект реагентов для выделения РНК/ДНК из клинического материала **– включает:**

[2] При хранении лизирующего раствора, раствора для отмывки 1 и раствора для лизиса при температуре от 2 до 8 °С возможно образование осадка в виде кристаллов.

Реактив	*Описание*	*Объем, мл*	*Кол-во*
Раствор для лизиса	Прозрачная жидкость голубого цвета[4]	15	1 флакон
Раствор для преципитации	Прозрачная бесцветная жидкость	20	1 флакон
Раствор для отмывки 3	Прозрачная бесцветная жидкость	25	1 флакон
Раствор для отмывки 4	Прозрачная бесцветная жидкость	10	1 флакон
РНК-буфер	Прозрачная бесцветная жидкость	1,2	4 пробирки

Комплект реагентов рассчитан на выделение РНК/ДНК из 50 проб, включая контроли. Входит в состав форм комплектации 2, 3.

Комплект реагентов «МАГНО-сорб» вариант 100-1000 (ТУ 9398-106-01897593-12) **–** комплект реагентов для выделения РНК/ДНК из клинического материала **– включает:**

Реактив	*Описание*	*Объем, мл*	*Кол-во*
Лизирующий раствор МАГНО-сорб	Прозрачная бесцветная жидкость[3]	70	4 флакона
Компонент А	Прозрачная бесцветная жидкость	0,6	4 пробирки
Раствор для отмывки 5	Прозрачная бесцветная жидкость[5]	60	4 флакона
Раствор для отмывки 6	Прозрачная бесцветная жидкость	20	4 флакона
Раствор для отмывки 7	Прозрачная бесцветная жидкость	6,0	4 флакона
Магнетизированная силика	Суспензия черного цвета	0,9	4 пробирки
Буфер для элюции	Прозрачная бесцветная жидкость	1,2	12 пробирок

Комплект реагентов рассчитан на выделение РНК/ДНК из 100 проб, включая контроли. Объем исследуемого материала 1000 мкл. Входит в состав формы комплектации 5.

Комплект реагентов «ПЦР-комплект» вариант FRT – комплект реагентов для проведения обратной транскрипции РНК и амплификации кДНК участка генома вируса Западного Нила с гибридизационно-флуоресцентной детекцией в режиме «реального времени» **– включает**:

[3] При хранении лизирующего раствора и раствора для отмывки 5 при температуре ниже 20 °С возможно образование осадка в виде кристаллов.

Реактив	*Описание*	*Объем, мл*	*Кол-во*
RT-G-mix-2	Прозрачная бесцветная жидкость	0,015	1 пробирка
ОТ-ПЦР-смесь-1-FRT *WNV*	Прозрачная бесцветная жидкость	0,6	1 пробирка
ОТ-ПЦР-смесь-2-FEP/FRT	Прозрачная бесцветная жидкость	0,3	1 пробирка
Полимераза (TaqF)	Прозрачная бесцветная жидкость	0,03	1 пробирка
ТМ-Ревертаза (MMlv)	Прозрачная бесцветная жидкость	0,015	1 пробирка
ПКО кДНК *WNV* / STI	Прозрачная бесцветная жидкость	0,1	1 пробирка
РНК-буфер	Прозрачная бесцветная жидкость	0,6	2 пробирки

Комплект реагентов рассчитан на проведение 60 реакций амплификации, включая контроли. 1 комплект реагентов входит в состав форм комплектации 1, 2, 3, 4. 2 комплекта реагентов входят в состав формы комплектации 5.

К комплекту реагентов прилагаются следующие реагенты:

Реактив	*Описание*	*Объем, мл*	*Кол-во*
ОКО	Прозрачная бесцветная жидкость	1,6	8 пробирок
ПКО *WNV*-rec	Прозрачная бесцветная жидкость	0,03	5 пробирок
ВКО STI-87-rec	Прозрачная бесцветная жидкость	0,12	5 пробирок

ПРОВЕДЕНИЕ ПЦР-ИССЛЕДОВАНИЯ

ПЦР-исследование состоит из следующих этапов:

- Экстракция РНК из исследуемых образцов.
- Проведение обратной транскрипции и амплификации с гибридизационно-флуоресцентной детекцией в режиме «реального времени».
- Анализ и интерпретация результатов.

Детальная информация по процедуре проведения ПЦР-исследования в зависимости от типа используемого оборудования изложена в методических рекомендациях по применению набора реагентов для выявления РНК вируса Западного Нила в биологическом материале методом полимеразной цепной реакции (ПЦР) с гибридизационно-флуоресцентной детекцией «АмплиСенс® *WNV*-FL»,

разработанных ФБУН ЦНИИ Эпидемиологии Роспотребнадзора.

ЭКСТРАКЦИЯ РНК ИЗ ИССЛЕДУЕМЫХ ОБРАЗЦОВ

Для экстракции РНК *WNV* из различных биологических объектов рекомендуется использовать следующие комплекты реагентов производства ФБУН ЦНИИ Эпидемиологии Роспотребнадзора:

- **«РИБО-преп»** – экстракция РНК из плазмы и сыворотки крови, СМЖ, лейкоцитарной фракции крови, гомогенатов тканей внутренних органов и комаров, осадков мочи, содержащих только эпителиальные клетки и не содержащих соли;
- **«РИБО-золь-С»** – экстракция РНК на первом этапе выделения из лейкоцитарной фракции крови, суспензий тканей внутренних органов, комаров и клещей, осадков мочи (в том числе содержащих соли). Второй этап выделения проводится с использованием комплекта **«РИБО-преп»** или **«РИБО-сорб»**;
- **«МАГНО-сорб»** – экстракция РНК из 1 мл плазмы и сыворотки крови, СМЖ.

При использовании формы комплектации 2 экстракция РНК проводится с помощью комплекта «РИБО-преп» в соответствии с Приложением 1. При использовании формы комплектации 3 экстракция РНК проводится с помощью комплектов «РИБО-золь-С» и «РИБО-преп» в соответствии с Приложением 2. При использовании формы комплектации 4 экстракция РНК проводится с помощью комплектов «РИБО-золь-С» и «РИБО-сорб» в соответствии с Приложением 3. При использовании формы комплектации 5 экстракция РНК проводится с помощью комплекта «МАГНО-сорб» в соответствии с инструкцией к набору.

ПРОВЕДЕНИЕ ОБРАТНОЙ ТРАНСКРИПЦИИ РНК И АМПЛИФИКАЦИИ кДНК С ДЕТЕКЦИЕЙ В РЕЖИМЕ «РЕАЛЬНОГО ВРЕМЕНИ»

А. Подготовка пробирок для амплификации

Выбор пробирок для амплификации зависит от используемого амплификатора с системой детекции в

режиме «реального времени».

Для внесения в пробирки реагентов, проб и контрольных образцов используются одноразовые наконечники с фильтрами.

Общий объем реакционной смеси – 25 мкл, включая объем пробы РНК – 10 мкл.

Способы внесения реактивов в пробирки:

1. Приготовить реакционную смесь на необходимое количество реакций - смешать в отдельной пробирке **ОТ-ПЦР-смесь-1-FRT *WNV***, **ОТ-ПЦР-смесь-2-FEP/FRT, полимеразу (TaqF), ТМ-Ревертазу (MMlv)** и **RT-G-mix-2**, из расчета на каждую реакцию:
   – **10 мкл ОТ-ПЦР-смеси-1-FRT *WNV*;**
   – **5 мкл ОТ-ПЦР-смеси-2-FEP/FRT;**
   – **0,5 мкл полимеразы (TaqF);**
   – **0,25 мкл ТМ-Ревертазы (MMlv);**
   – **0,25 мкл RT-G-mix-2.**

При расчете следует учитывать, что постановка сопровождается амплификацией как минимум четырех контрольных образцов: положительного контроля экстракции (ПК), отрицательного контроля экстракции (ОК), положительного и отрицательного контролей ОТ-ПЦР (К+ и К–).

2. Раскапать приготовленные смеси в пробирки по **15 мкл.**

ВНИМАНИЕ! Приготовленную смесь не хранить.

3. Используя наконечник с фильтром, добавить **10 мкл РНК-пробы** в пробирки с каждой реакционной смесью. Осторожно перемешать пипетированием.
4. Для каждой панели исследуемых образцов необходимо поставить контроль амплификации кДНК:
   а) отрицательный контроль ПЦР (К–) – внести в пробирку **10 мкл РНК-буфера.**
   б) положительный контроль ПЦР (К+) – внести в пробирку **10 мкл ПКО кДНК *WNV* / STI.**

ВНИМАНИЕ! Пробы амплифицировать сразу после соединения реакционной смеси и РНК-пробы и контролей.

Б. Проведение обратной транскрипции РНК и амплификации кДНК с детекцией в режиме «реального времени»

1. Запрограммировать прибор (амплификатор с системой детекции в режиме «реального времени») для выполнения соответствующей программы обратной транскрипции, амплификации и детекции флуоресцентного сигнала (табл.1).

Таблица 1

	Приборы роторного типа[4]			**Приборы планшетного типа**[5]		
Цикл	Температура, °С	Время	Кол-во циклов	Температура, °С	Время	Кол-во циклов
1	**50**	**30 мин**	**1**	**50**	**30 мин**	**1**
2	**95**	**15 мин**	**1**	**95**	**15 мин**	**1**
3	**95**	**5 с**	**5**	**95**	**5 с**	**5**
	56	**25 с**		**56**	**30 с**	
	72	**15 с**		**72**	**15 с**	
4	**95**	**5 с**	**40**	**95**	**5 с**	**40**
	56	**25 с** детекция флуоресц. сигнала		**56**	**30 с** детекция флуоресц. сигнала	
	72	**15 с**		**72**	**15 с**	

Детекция флуоресцентного сигнала назначается по каналам для флуорофоров FAM и JOE.

2. Установить пробирки в ячейки реакционного модуля прибора. **Лунка №1 обязательно должна быть заполнена какой-либо исследуемой пробиркой.**
3. Запустить выполнение программы амплификации с детекцией флуоресцентного сигнала.
4. По окончании выполнения программы приступить к анализу и интерпретации результатов.

АНАЛИЗ И ИНТЕРПРЕТАЦИЯ РЕЗУЛЬТАТОВ

Анализ результатов проводят с помощью программного обеспечения используемого прибора для проведения ПЦР с детекцией в режиме «реального времени». Анализируют кривые накопления флуоресцентного сигнала по двум каналам:

[4] Например, Rotor-Gene 3000/6000 (Corbett Research, Австралия), Rotor-Gene Q (Qiagen, Германия) и рекомендованные ФБУН ЦНИИ Эпидемиологии Роспотребнадзора в методических рекомендациях по применению данного набора реагентов.

[5] Например, iQ5 (Bio-Rad, США), Mx3000P (Stratagene, США) и рекомендованные ФБУН ЦНИИ Эпидемиологии Роспотребнадзора в методических рекомендациях по применению данного набора реагентов.

- по каналу для флуорофора FAM регистрируется сигнал, свидетельствующий о накоплении продукта амплификации фрагмента кДНК ВКО STI-87-rec;
- по каналу для флуорофора JOE регистрируется сигнал, свидетельствующий о накоплении продукта амплификации фрагмента кДНК *WNV*.

Результаты интерпретируются на основании наличия (или отсутствия) пересечения кривой флуоресценции с установленной на соответствующем уровне пороговой линией, что определяет наличие (или отсутствие) для данной пробы кДНК значения порогового цикла *Ct* в соответствующей графе в таблице результатов.

Результаты интерпретируются в соответствии с табл. 2.

Таблица 2

Соответствие мишеней и каналов детекции

ПЦР-смесь-1	**Детекция по каналу**	
	FAM/Green	**JOE/Yellow**
ОТ-ПЦР-смесь-1-FRT *WNV*	ВКО	*WNV*

Принцип интерпретации результатов следующий:

- кДНК *WNV* **обнаружена***,* если для данной пробы в таблице результатов по каналу для флуорофора JOE определено значение порогового цикла *Ct*, не превышающее указанное граничное во вкладыше. При этом кривая флуоресценции каждой исследуемой пробы должна пересекать пороговую линию на участке характерного экспоненциального подъема флуоресценции.
- кДНК *WNV* **не обнаружена,** если для данной пробы в таблице результатов по каналу для флуорофора FAM определено значение порогового цикла *Ct,* не превышающее указанное граничное значение, а по каналу JOE значение порогового цикла не определено или больше указанного во вкладыше.
- Результат анализа **невалидный,** если для данной пробы не определено (отсутствует) значение порогового цикла *Ct* по каналу для флуорофора JOE, и по каналу для флуорофора FAM значение *Ct* также не определено (отсутствует) или превышает указанное граничное значение. В этом случае требуется повторно провести ПЦР-исследование

соответствующего клинического образца, начиная с этапа экстракции.

ВНИМАНИЕ! Граничные значения *Ct* указаны во вкладыше, **прилагаемом к набору реагентов**. См. также методические рекомендации по применению набора реагентов для выявления РНК вируса Западного Нила в биологическом материале методом полимеразной цепной реакции (ПЦР) с гибридизационно-флуоресцентной детекцией «АмплиСенс® *WNV*-FL», разработанные ФБУН ЦНИИ Эпидемиологии.

Результат ПЦР-исследования считается достоверным, если получены правильные результаты для положительного и отрицательного контролей амплификации и положительного и отрицательного контролей экстракции РНК, в соответствии с таблицей оценки результатов контрольных реакций (табл. 3).

Таблица 3

Результаты для контролей различных этапов ПЦР-исследования

Контроль	Контролируемый этап ПЦР-исследования	Значение порогового цикла, *Ct*	
		по каналу для флуорофора JOE	по каналу для флуорофора FAM
ОК	Экстракция РНК	Значение отсутствует	Определено значение меньше граничного
ПК	Экстракция РНК	Определено значение меньше граничного	Определено значение меньше граничного
К–	ПЦР	Значение отсутствует	Значение отсутствует
К+	ПЦР	Определено значение меньше граничного	Определено значение меньше граничного

ВНИМАНИЕ!

1. Если для положительного контроля ПЦР (К+) значение порогового цикла по каналу для флуорофора JOE отсутствует или превышает граничное значение, необходимо повторить амплификацию для всех образцов, в которых не обнаружена специфическая кДНК.
2. Если для положительного контроля экстракции РНК (ПК) значение порогового цикла по каналу для флуорофора JOE отсутствует или превышает граничное значение, необходимо повторить экстракцию для всех образцов, в которых не обнаружена специфическая кДНК.

3. Если для отрицательного контроля экстракции РНК (ОК) по каналу для флуорофора JOE определено значение порогового цикла *Ct*, необходимо повторить ПЦР-исследование для всех образцов, в которых обнаружена кДНК, детектируемая на канале для флуорофора JOE.
4. Если для отрицательного контроля ПЦР (К–) по каналам для флуорофоров FAM и JOE определено значение порогового цикла *Ct*, необходимо повторить амплификацию для всех образцов, в которых обнаружена кДНК, детектируемая на канале для флуорофора JOE, с постановкой К– не менее чем в трех повторах.

СРОК ГОДНОСТИ. УСЛОВИЯ ТРАНСПОРТИРОВАНИЯ И ХРАНЕНИЯ

Срок годности. 9 мес. Набор реагентов с истекшим сроком годности применению не подлежит. Срок годности вскрытых реагентов соответствует сроку годности, указанному на этикетках для невскрытых реагентов, если в инструкции не указано иное.

Транспортирование. Набор реагентов транспортировать при температуре от 2 до 8 °С не более 5 сут. «ПЦР-комплект» вариант FRT при получении разукомплектовать в соответствии с указанными температурами хранения.

Хранение. Комплекты реагентов «РИБО-преп», «РИБО-золь-С», «РИБО-сорб», «ПЦР-комплект» (кроме RT-G-mix-2, ОТ-ПЦР-смеси-1-FRT *WNV*, ОТ-ПЦР-смеси-2-FEP/FRT, полимеразы (TaqF) и ТМ-Ревертазы (MMlv)) хранить при температуре от 2 до 8 °С. RT-G-mix-2, ОТ-ПЦР-смесь-1-FRT *WNV*, ОТ-ПЦР-смесь-2-FEP/FRT, полимеразу (TaqF) и ТМ-Ревертазу (MMlv) хранить при температуре не выше минус 16 °С. Комплект реагентов «МАГНО-сорб» хранить температуре от 2 до 25 °С. ОТ-ПЦР-смесь-1-FRT *WNV* хранить в защищенном от света месте.

Условия отпуска. Для лечебно-профилактических и санитарно-профилактических учреждений.

Рекламации на качество набора реагентов «**АмплиСенс**® ***WNV*-FL**» направлять на предприятие-изготовитель ФБУН ЦНИИ Эпидемиологии Роспотребнадзора (111123 г. Москва, ул. Новогиреевская, д. 3а) в отдел по работе с рекламациями и организации обучения (тел. (495) 974-96-46, факс (495) 916-18-18 e-mail: products@pcr.ru)[6].

Заведующий НПЛ ОМДиЭ Е.Н. Родионова

ФБУН ЦНИИ Эпидемиологии Роспотребнадзора

Главный врач ФГБУ «Поликлиника № 1» Управления делами Президента Российской Федерации Е.Л.Никонов

[6] Отзывы и предложения о продукции «АмплиСенс» вы можете оставить, заполнив анкету потребителя на сайте: www.amplisens.ru.

ПРИЛОЖЕНИЕ 1. Экстракция РНК из плазмы и сыворотки крови, СМЖ, лейкоцитарной фракции крови, мочи (без осадка солей), гомогенатов тканей внутренних органов и комаров с применением комплекта реагентов «РИБО-преп» (проводится в ЗОНЕ 1 – помещении для обработки исследуемого материала)

Порядок работы

1. **Раствор для лизиса** (если он хранился при температуре от 2 до 8 °С) прогреть при температуре 65 °С до полного растворения кристаллов.
2. В случае экстракции РНК из **гомогенатов тканей, плазмы, сыворотки, СМЖ** и **гомогенатов комаров** отобрать необходимое количество одноразовых пробирок на 1,5 мл с плотно закрывающимися крышками (включая отрицательный и положительный контроли экстракции). Внести в каждую пробирку по **10 мкл ВКО STI-87-rec**. Добавить в пробирки по **300 мкл раствора для лизиса**. Промаркировать пробирки.
3. В пробирки с **раствором для лизиса** и **ВКО STI-87-rec** внести по **30 мкл** исследуемых суспензий органов, по **100 мкл** суспензий комаров, по **200 мкл** плазмы, сыворотки, СМЖ.
4. При экстракции из **лейкоцитарной фракции крови** или **осадка мочи** в данные исследуемые пробирки необходимо внести **300 мкл раствора для лизиса и 10 мкл ВКО STI-87-rec.** Затем отобрать две пробирки для отрицательного и положительного контролей экстракции.
5. В пробирку отрицательного контроля (ОК) экстракции и положительного контроля (ПК) экстракции внести **10 мкл ВКО STI-87-rec** и **300 мкл раствора для лизиса**. В пробирку положительного контроля (ПК) экстракции внести также **10 мкл ПКО *WNV*-rec**.
6. Содержимое пробирок тщательно перемешать на вортексе и прогреть **5 мин при 65 °С** в термостате. Добавить в пробирки по **400 мкл раствора для преципитации**, перемешать на вортексе.
7. Процентрифугировать пробирки на микроцентрифуге в течение **5 мин** при **10 000 g.**

8. Аккуратно отобрать надосадочную жидкость, не задевая осадок, используя вакуумный отсасыватель и отдельный наконечник для каждой пробы.
9. Добавить в пробирки по **500 мкл раствора для отмывки 3**, плотно закрыть крышки, осторожно промыть осадок, переворачивая пробирки 3-5 раз. Можно провести процедуру одновременно для всех пробирок, для этого необходимо накрыть пробирки в штативе сверху крышкой или другим штативом, прижать их и переворачивать штатив.
10. Процентрифугировать при **10 000 g в течение 2 мин** на микроцентрифуге.
11. Осторожно, не захватывая осадок, отобрать надосадочную жидкость, используя вакуумный отсасыватель и отдельный наконечник для каждой пробы.
12. Добавить в пробирки по **200 мкл раствора для отмывки 4**, плотно закрыть крышки и осторожно промыть осадок, переворачивая пробирки 3-5 раз.
13. Процентрифугировать при **10 000 g** в течение **2 мин** на микроцентрифуге.
14. Осторожно, не захватывая осадок, отобрать надосадочную жидкость, используя вакуумный отсасыватель и отдельный наконечник для каждой пробы.
15. Поместить пробирки в термостат при температуре **65 °C на 5 мин** для подсушивания осадка (при этом крышки пробирок должны быть открыты).
16. Добавить в пробирки по **50 мкл РНК-буфера**. Перемешать на вортексе. Поместить в термостат при температуре **65 °C на 5 мин**, периодически встряхивая на вортексе.
17. Процентрифугировать пробирки при **10 000 g в течение 1 мин** на микроцентрифуге. Надосадочная жидкость содержит очищенные РНК. Пробы готовы к постановке реакции обратной транскрипции и ПЦР.

ПРИЛОЖЕНИЕ 2. Экстракция РНК из лейкоцитарной фракции крови, осадков мочи, суспензий тканей внутренних органов, комаров и клещей с применением комплектов реагентов «РИБО-золь-С» и «РИБО-преп» (проводится в ЗОНЕ 1 – помещении для обработки исследуемого материала)

Порядок работы

I этап

1. Отобрать необходимое количество одноразовых пробирок объемом 1,5 мл (включая отрицательный и положительный контроли экстракции). Внести в каждую пробирку по **10 мкл ВКО STI-87-rec**, затем добавить по **300 мкл раствора D**. Промаркировать пробирки.
2. В случае изоляции РНК из суспензий клещей, комаров, внутренних органов в пробирки с раствором D и ВКО STI-87-rec добавляют по **100 мкл подготовленных проб**, используя наконечники с фильтром.
3. При экстракции РНК из лейкоцитарной фракции крови или осадков мочи в пробирки с осадками вносят по **300 мкл раствора D** и по **10 мкл ВКО STI-87-rec**.
4. В пробирку отрицательного контроля (ОК) экстракции внести **100 мкл ОКО и 10 мкл ВКО STI-87-rec**. В пробирку положительного контроля (ПК) экстракции внести **10 мкл ПКО *WNV*-rec, 10 мкл ВКО STI-87-rec** и **90 мкл ОКО**. Плотно закрытые пробы тщательно перемешать на вортексе
5. Прогреть пробирки в термостате при 56 °С в течение 5 мин, периодически встряхивая их на вортексе.
6. Добавить к образцам, лизированным в растворе D, **30 мкл раствора E**, перемешать на вортексе и центрифугировать 5 с при 1500 g.
7. В эти же пробирки добавить **300 мкл раствора A**, перемешать на вортексе и центрифугировать 5 с при 1500 g.
8. В эти же пробирки внести **100 мкл раствора B**, перемешать на вортексе в течение 1-2 мин (раствор должен стать молочно-белым), затем поместить пробы на ледяную баню (при температуре от 0 до 4 °С) на 5 мин. После этого центрифугировать пробирки в течение 10 мин при 10 000 g.

9. В новые пробирки объемом 1,5 мл внести по **300 мкл раствора для лизиса (из комплекта реагентов «РИБО-преп»)** и промаркировать соответственно номерам проб.
10. После центрифугирования раствор должен разделиться на 2 фазы: нижнюю (фенольную), содержащую белки и ДНК, и верхнюю (водную), содержащую РНК. Необходимо аккуратно, не захватывая нижний слой, отобрать **200 мкл** верхней фазы и перенести ее в пробирку с раствором для лизиса. Тщательно перемешать смесь.

II этап

11. Содержимое пробирок тщательно перемешать на вортексе и прогреть **5 мин при 65 °С** в термостате. Процентрифугировать в течение **5 с** при **1500 g** для удаления капель с внутренней поверхности крышки пробирки. Добавить в пробирки по **400 мкл раствора для преципитации**, перемешать на вортексе.
12. Процентрифугировать пробирки на микроцентрифуге в течение **5 мин** при **10 000 g.**
13. Аккуратно отобрать надосадочную жидкость, не задевая осадок, используя вакуумный отсасыватель и отдельный наконечник для каждой пробы.
14. Добавить в пробирки по **500 мкл раствора для отмывки 3**, плотно закрыть крышки, осторожно промыть осадок, переворачивая пробирки 3-5 раз. Можно провести процедуру одновременно для всех пробирок, для этого необходимо накрыть пробирки в штативе сверху крышкой или другим штативом, прижать их и переворачивать штатив.
15. Процентрифугировать при **10 000 g в течение 2 мин** на микроцентрифуге.
16. Осторожно, не захватывая осадок, отобрать надосадочную жидкость, используя вакуумный отсасыватель и отдельный наконечник для каждой пробы.
17. Добавить в пробирки по **200 мкл раствора для отмывки 4**, плотно закрыть крышки и осторожно промыть осадок, переворачивая пробирки 3-5 раз.
18. Процентрифугировать при **10 000 g** в течение **2 мин** на микроцентрифуге.
19. Осторожно, не захватывая осадок, отобрать надосадочную жидкость, используя вакуумный отсасыватель и отдельный

наконечник для каждой пробы.

20. Поместить пробирки в термостат при температуре **65 °C на 5 мин** для подсушивания осадка (при этом крышки пробирок должны быть открыты).
21. Добавить в пробирки по **50 мкл РНК-буфера**. Перемешать на вортексе. Поместить в термостат при температуре **65 °C на 5 мин**, периодически встряхивая на вортексе.
22. Процентрифугировать пробирки при **10 000 g в течение 1 мин** на микроцентрифуге. Надосадочная жидкость содержит очищенные РНК. Пробы готовы к постановке реакции обратной транскрипции и ПЦР.

ПРИЛОЖЕНИЕ 3. Экстракция РНК из лейкоцитарной фракции крови, осадков мочи, суспензий тканей внутренних органов, комаров и клещей с применением комплектов реагентов «РИБО-золь-С» и «РИБО-сорб» (проводится в ЗОНЕ 1 – помещении для обработки исследуемого материала)

Порядок работы.
I этап

1. Отобрать необходимое количество одноразовых пробирок объемом 1,5 мл (включая отрицательный и положительный контроли экстракции). Внести в каждую пробирку по **10 мкл ВКО STI-87-rec**, затем добавить по **300 мкл раствора D**. Промаркировать пробирки.
2. В случае изоляции РНК из суспензий клещей, комаров в пробирки с раствором D и ВКО STI-87-rec добавляют по **100 мкл подготовленных проб,** в случае изоляции из суспензий тканей внутренних органов – **30 мкл подготовленных проб**, используя наконечники с фильтром.
3. При экстракции РНК из лейкоцитарной фракции крови или осадков мочи в пробирки с осадками вносят по **300 мкл раствора D** и по **10 мкл ВКО STI-87-rec**.
4. В пробирку отрицательного контроля (ОК) экстракции внести **100 мкл ОКО и 10 мкл ВКО STI-87-rec**. В пробирку положительного контроля (ПК) экстракции внести **10 мкл ПКО *WNV*-rec, 10 мкл ВКО STI-87-rec** и **90 мкл ОКО**. Плотно закрытые пробы тщательно перемешать на вортексе и процентрифугировать в течение 5 с при 1500 g на микроцентрифуге для удаления капель с внутренней поверхности крышки пробирки.
5. Прогреть пробирки в термостате при 56 °С в течение 5 мин, периодически встряхивая их на вортексе.
6. Добавить к образцам, лизированным в растворе D, **30 мкл раствора E**, перемешать на вортексе и центрифугировать 5 с при 1500 g.
7. В эти же пробирки добавить **300 мкл раствора A**, перемешать на вортексе и центрифугировать 5 с при 1500 g.
8. В эти же пробирки внести **100 мкл раствора B**, перемешать на вортексе в течение 1-2 мин (раствор должен стать

молочно-белым), затем поместить пробы на ледяную баню (при температуре от 0 до 4 °С) на 5 мин. После этого центрифугировать пробирки в течение 10 мин при 10 000 g.

9. В новые пробирки объемом 1,5 мл внести по **400 мкл лизирующего раствора (из комплекта реагентов «РИБО-сорб»)** и промаркировать соответственно номерам проб.
10. После центрифугирования раствор должен разделиться на 2 фазы: нижнюю (фенольную), содержащую белки и ДНК, и верхнюю (водную), содержащую РНК. Необходимо аккуратно, не захватывая нижний слой, отобрать верхнюю фазу (**400 мкл при экстракции РНК из осадков мочи и гомогенатов внутренних органов, гомогенатов комаров и клещей, 300 мкл – из осадков крови, 450 мкл при экстракции РНК** из **ОКО** и **ПКО**) и перенести ее в пробирку с лизирующим раствором. Тщательно перемешать смесь.

II этап

11. Тщательно ресуспендировать **сорбент** на вортексе. В каждую пробирку отдельным наконечником добавить по **25 мкл сорбента**. Перемешать на вортексе, поставить в штатив на 1 мин, еще раз перемешать и оставить на 5 мин.
12. Центрифугировать пробирки для осаждения сорбента при 1500 g в течение 30 с на микроцентрифуге. Удалить надосадочную жидкость, используя вакуумный отсасыватель и отдельный наконечник для каждой пробы.
13. Добавить в пробирки по **400 мкл раствора для отмывки 1**. Перемешать на вортексе до полного ресуспендирования сорбента, процентрифугировать 30 с при 1500 g на микроцентрифуге. Удалить надосадочную жидкость, используя вакуумный отсасыватель и отдельный наконечник для каждой пробы.
14. Добавить в пробирки по **500 мкл раствора для отмывки 3**. Тщательно ресуспендировать сорбент на вортексе. Процентрифугировать 45 с при 5000 g на микроцентрифуге. Удалить надосадочную жидкость, используя вакуумный отсасыватель и отдельный наконечник для каждой пробы.
15. Повторить отмывку **раствором для отмывки 3**, следуя пункту 13.
16. Добавить в пробирки по **400 мкл раствора для отмывки 4**. Тщательно ресуспендировать сорбент на вортексе,

процентрифугировать 1 мин при 6000 g на микроцентрифуге. Полностью удалить надосадочную жидкость из каждой пробирки отдельным наконечником, используя вакуумный отсасыватель.

17. Поместить пробирки в термостат при температуре 56 °С на 10 мин для подсушивания сорбента. При этом крышки пробирок должны быть открыты.
18. В пробирки добавить по **50 мкл РНК-буфера**, используя свободный от РНКаз наконечник с фильтром.
    ВНИМАНИЕ! Вскрытую пробирку с РНК-буфером хранить при температуре не выше минус 16 °С.
19. Перемешать содержимое пробирок на вортексе. Поместить в термостат при температуре 56 °С на 5 мин (встряхивая пробы на вортексе каждую мин). Процентрифугировать пробирки на максимальных оборотах микроцентрифуги (10 000 g) в течение 1 мин. Надосадочная жидкость содержит очищенные РНК. Пробы готовы к постановке реакции обратной транскрипции и ПЦР.

СИМВОЛЫ, ИСПОЛЬЗУЕМЫЕ В ПЕЧАТНОЙ ПРОДУКЦИИ

REF	Номер в каталоге		Осторожно! Обратитесь к сопроводительной документации
LOT	Код партии		Максимальное число тестов
IVD	Изделие для in vitro диагностики		Использовать до
VER	Дата изменения		Обратитесь к руководству по эксплуатации
	Ограничение температуры		Не допускать попадания солнечного света
	Верхнее ограничение температуры		Дата изготовления
	Производитель		

ИНСТРУКЦИЯ

по применению набора реагентов
для выявления РНК/ДНК возбудителей инфекций,
передающихся иксодовыми клещами
TBEV, *Borellia burgdorferi sl*, *Anaplasma phagocytophilum*,
Ehrlichia chaffeensis / Ehrlichia muris,
в биологическом материале
методом полимеразной цепной реакции (ПЦР)
с гибридизационно-флуоресцентной детекцией

«АмплиСенс® *TBEV, B.burgdorferi sl, A.phagocytophilum, E.chaffeensis / E.muris*-FL»

АмплиСенс®

ФБУН ЦНИИ Эпидемиологии
Роспотребнадзора,
Российская Федерация, 111123,
город Москва, улица Новогиреевская, дом 3А

RUO
Только для исследовательских и
иных немедицинских целей

ОГЛАВЛЕНИЕ

СПИСОК СОКРАЩЕНИЙ

В настоящей инструкции применяются следующие сокращения и обозначения:

ВКО STI-87-rec	- внутренний контрольный образец
В–	- отрицательный контроль экстракции РНК/ДНК
К–	- отрицательный контроль ПЦР
К+	- положительный контроль ПЦР
кДНК	- комплементарная ДНК, получаемая в реакции обратной транскрипции на матрице РНК
НК	- нуклеиновые кислоты (РНК/ДНК)
ПКО	- положительный контрольный образец
ПЦР	- полимеразная цепная реакция
ФБУН ЦНИИ Эпидемиологии Роспотребнадзора	- федеральное бюджетное учреждение науки «Центральный научно-исследовательский институт эпидемиологии» Федеральной службы по надзору в сфере защиты прав потребителей и благополучия человека
FRT	- флуоресцентная детекция в режиме «реального времени»

НАЗНАЧЕНИЕ

Набор реагентов **«АмплиСенс® *TBEV, B.burgdorferi sl, A.phagocytophilum, E.chaffeensis / E.muris*-FL»** предназначен для выявления РНК *TBEV* – вируса клещевого энцефалита (Tick-borne encephalitis virus), *Borrelia burgdorferi sl* – возбудителя иксодовых клещевых боррелиозов (ИКБ), *Ehrlichia chaffeensis u Ehrlichia muris* – возбудителей моноцитарного эрлихиоза человека (МЭЧ), ДНК *Anaplasma phagocytophilum* – возбудителя гранулоцитарного анаплазмоза человека (ГАЧ) в клещах, крови, ликворе, аутоптатах методом ПЦР с гибридизационно-флуоресцентной детекцией продуктов амплификации.

ПРИНЦИП МЕТОДА

Выявление *TBEV, B.burgdorferi sl, A.phagocytophilum, E.chaffeensis / E.muris* методом полимеразной цепной реакции (ПЦР) с гибридизационно-флуоресцентной детекцией включает в себя следующие этапы: экстракцию РНК/ДНК из образцов биологического материала, проведение реакции обратной транскрипции и получение кДНК на матрице РНК, амплификацию участка кДНК/ДНК данных микроорганизмов и гибридизационно-флуоресцентную детекцию, которая производится непосредственно в ходе ПЦР. Экстракция РНК/ДНК из биологического материала проводится с использованием наборов реагентов, рекомендованных ФБУН

ЦНИИ Эпидемиологии Роспотребнадзора, в присутствии внутреннего контрольного образца (**ВКО-STI-87-rec**), который позволяет контролировать выполнение процедуры исследования для каждого образца. Затем с полученными пробами проводятся реакции обратной транскрипции РНК и амплификации участков кДНК/ДНК *TBEV, B.burgdorferi sl, E.chaffeensis / E.muris* при помощи специфичных к этим участкам кДНК/ДНК праймеров и фермента TaqF-полимеразы. В составе реакционной смеси присутствуют флуоресцентно-меченые олигонуклеотидные зонды, которые гибридизуются с комплементарными участками амплифицируемых кДНК/ДНК-мишеней, в результате чего происходит нарастание интенсивности флуоресценции. Это позволяет регистрировать накопление специфических продуктов амплификации путем измерения интенсивности флуоресцентных сигналов. Детекция флуоресцентных сигналов происходит непосредственно в ходе ПЦР с помощью амплификатора с системой детекции флуоресцентного сигнала в режиме «реального времени».

ФОРМАТЫ И ФОРМЫ ВЫПУСКА НАБОРА РЕАГЕНТОВ

Набор реагентов выпускается в 1 формате.

Формат FRT

Набор реагентов выпускается в 3 формах комплектации:

Форма 1 включает комплекты реагентов «РИБО-преп» вариант 100, «РЕВЕРТА-L» вариант 100, «ПЦР-комплект» вариант FRT-100 F.

Форма 2 включает комплект реагентов «ПЦР-комплект» вариант FRT-100 F.

Форма 3 включает наборы реагентов оптом, расфасованные по отдельным реагентам, с маркировкой реагентов на их оптовой фасовке.

Форма комплектации 1 предназначена для полного ПЦР-исследования, включающего экстракцию РНК/ДНК из биологического материала, проведение реакции обратной транскрипции и получение кДНК на матрице РНК и амплификацию кДНК/ДНК *TBEV, B.burgdorferi sl, A.phagocytophilum, E.chaffeensis / E.muris* с гибридизационно-флуоресцентной детекцией.

Форма комплектации 2 предназначена для амплификации кДНК/ДНК *TBEV, B.burgdorferi sl, A.phagocytophilum, E.chaffeensis / E.muris* с гибридизационно-флуоресцентной детекцией в режиме «реального времени». Для проведения полного ПЦР-исследования необходимо использовать комплекты реагентов для проведения реакции обратной транскрипции и комплекты реагентов для экстракции РНК/ДНК, рекомендованные ФБУН ЦНИИ Эпидемиологии Роспотребнадзора.

Форма комплектации 3 предназначена для производственных целей для последующей маркировки на языке заказчика и комплектации по наборам.

ВНИМАНИЕ! Форма комплектации 3 используется только в соответствии с регламентом, утвержденным ФБУН ЦНИИ Эпидемиологии Роспотребнадзора.

АНАЛИТИЧЕСКИЕ ХАРАКТЕРИСТИКИ

Аналитическая чувствительность

Вид биологического материала	Комплект для экстракции РНК/ДНК	Комплект для обратной транскрипции	Комплект для амплификации и детекции	Аналитическая чувствительность	Пробоподготовка материала
Клещи родов Ixodes, Dermacentor	«РИБО-преп»	«РЕВЕРТ А-L»	«ПЦР-комплект» вариант FRT	$5x10^3$ ГЭ/мл для всех заявленных микроорганизмов[1]	Данная чувствительность достигается при соблюдении нижеизложенных правил пробоподготовки биоматериала и рекомендуемом исследуемом объеме пробы

Аналитическая специфичность

Аналитическая специфичность изучена на:

– флавивирусах (вирус Западного Нила, Лангат, Повассан, Японского энцефалита, Омской геморрагической лихорадки);

– спирохетах (*Borrelia miyamotoi*, *Treponema pallidum*, *Leptospira interrogans*, *Leptospira kirshneri*, *Leptospira*

[1] Количество геномных эквивалентов микроорганизма (ГЭ) в 1 мл образца клинического материала, помещенного в указанную транспортную среду.

borgpetersenii);

– риккетсиях группы пятнистых лихорадок (*Rickettsia conorii subsp caspia*, *R.heilongiangensis*, *Coxiella burnetii*, *Bartonella henselae*, *Bartonella quintana*).

При работе с ДНК вышеперечисленных организмов, ДНК человека, ДНК клещей *Ixodes persulcatus*, *Ixodes ricinus*, *Dermacentor reticulatus*, *Dermacentor marginatus*, ДНК грызунов *Clethrionomys glareolus* и *Apodemus agrarius* не выявлено ложноположительных результатов.

МЕРЫ ПРЕДОСТОРОЖНОСТИ

Работа должна проводиться в лаборатории, выполняющей молекулярно-биологические (ПЦР) исследования клинического материала на наличие возбудителей инфекционных болезней, с соблюдением санитарно-эпидемических правил СП 1.3.2322-08 «Безопасность работы с микроорганизмами III–IV групп патогенности (опасности) и возбудителями паразитарных болезней», СанПиН 2.1.7.2790-10 «Санитарно-эпидемиологические требования к обращению с медицинскими отходами» и методических указаний МУ 1.3.2569-09 «Организация работы лабораторий, использующих методы амплификации нуклеиновых кислот при работе с материалом, содержащим микроорганизмы I–IV групп патогенности».

При работе всегда следует выполнять следующие требования:

- Следует рассматривать исследуемые образцы как инфекционно-опасные, организовывать работу и хранение в соответствии с СП 1.3.2322-08 «Безопасность работы с микроорганизмами III–IV групп патогенности (опасности) и возбудителями паразитарных болезней».

ВНИМАНИЕ! При работе с клещом с высокой степенью напитанности рекомендуется перед гомогенизацией проколоть его одноразовой иглой для выхода крови и предупреждения разбрызгивания материала при растирании в ступке.

- Убирать и дезинфицировать разлитые образцы или реактивы, используя дезинфицирующие средства в соответствии с СП 1.3.2322-08 «Безопасность работы с микроорганизмами III–IV групп патогенности (опасности) и возбудителями паразитарных болезней».
- Лабораторный процесс должен быть однонаправленным.

Анализ проводится в отдельных помещениях (зонах). Работу следует начинать в Зоне Выделения, продолжать в Зоне Амплификации и Детекции. Не возвращать образцы, оборудование и реактивы в зону, в которой была проведена предыдущая стадия процесса.

- Удалять неиспользованные реактивы в соответствии с СанПиН 2.1.7.2790-10 «Санитарно-эпидемиологические требования к обращению с медицинскими отходами».

ВНИМАНИЕ! При удалении отходов после амплификации (пробирок, содержащих продукты ПЦР) недопустимо открывание пробирок и разбрызгивание содержимого, поскольку это может привести к контаминации продуктами ПЦР лабораторной зоны, оборудования и реагентов.

- Применять набор строго по назначению, согласно данной инструкции.
- Допускать к работе с набором только специально обученный персонал.
- Не использовать набор по истечении срока годности.
- Использовать одноразовые перчатки, лабораторные халаты, защищать глаза во время работы с образцами и реактивами. Тщательно вымыть руки по окончании работы.
- Избегать контакта с кожей, глазами и слизистой оболочкой. При контакте немедленно промыть пораженное место водой и обратиться за медицинской помощью.
- Листы безопасности материалов (MSDS – material safety data sheet) доступны по запросу.

ДОПОЛНИТЕЛЬНЫЕ МАТЕРИАЛЫ И ОБОРУДОВАНИЕ

1. 0,15 М NaCl или фосфатный буферный раствор (PBS) (натрия хлорид, 137 мМ; калия хлорид, 2,7 мМ; натрия монофосфат, 10 мМ; калия дифосфат, 2мМ; pH=7,5±0,2) и 96% этиловый спирт для проведения пробоподготовки клещей и суспензии органов, глицерин.
2. Комплект реагентов для выделения РНК/ДНК – «РИБО-преп» (ТУ 9398-071-01897593-2008) – при работе с формой комплектации 2.
3. Дополнительные материалы и оборудование для экстракции РНК/ДНК – согласно инструкции к комплекту реагентов для выделения РНК/ДНК.

4. Комплект реагентов для проведения реакции обратной транскрипции «РЕВЕРТА-L» (ТУ 9398-005-01897593-2008) – при работе с формой комплектации 2.
5. Бокс абактериальной воздушной среды (ПЦР-бокс).
6. Центрифуга/вортекс.
7. Автоматические дозаторы переменного объема (от 5 до 20 мкл и от 20 до 200 мкл).
8. Одноразовые наконечники с фильтром до 100 мкл в штативах.
9. Штативы для пробирок объемом 0,1 мл, 0,2 мл или 0,5 мл (в соответствии с используемыми комплектами реагентов).
10. Холодильник от 2 до 8 °С с морозильной камерой от минус 24 до минус 16 °С.
11. Отдельный халат, шапочки, обувь и одноразовые перчатки в соответствии с МУ 1.3.2569-09.
12. Емкость для сброса наконечников.
13. Программируемый амплификатор с системой детекции флуоресцентного сигнала в режиме «реального времени» (например, Rotor-Gene 3000/6000 (Corbett Research, Австралия), iCycler iQ5 (Bio-Rad, США), Mx3000P (Stratagene, США), «ДТ-96» («ДНК-Технология», Россия) и рекомендованные ФБУН ЦНИИ Эпидемиологии Роспотребнадзора в методических рекомендациях по применению данного набора реагентов).
14. Одноразовые полипропиленовые пробирки для ПЦР объемом 0,2 мл или 0,1 мл:
    а) тонкостенные пробирки для ПЦР объемом 0,2 мл с выпуклой крышкой (например, Axygen, США) – при использовании прибора планшетного типа;
    б) тонкостенные пробирки для ПЦР объемом 0,2 мл с плоской крышкой (например, Axygen, США), или пробирки для ПЦР к Rotor-Gene, объемом 0,1 мл в стрипах по 4 шт. с крышками (например, Corbett Research, Австралия) – при использовании прибора роторного типа.

ВЗЯТИЕ, ТРАНСПОРТИРОВАНИЕ И ХРАНЕНИЕ ИССЛЕДУЕМОГО МАТЕРИАЛА

Осуществляется в соответствии с методическими рекомендациями «Взятие, транспортировка, хранение

клинического материала для ПЦР-диагностики», разработанными ФГУН ЦНИИЭ Роспотребнадзора, Москва, 2008 г.

ПОДГОТОВКА ИССЛЕДУЕМОГО МАТЕРИАЛА К ЭКСТРАКЦИИ РНК/ДНК

Подготовка суспензий клещей.

При исследовании пулов клещей число особей в одном пуле не должно превышать 10. Особей клещей рода *Dermacentor* предпочтительнее исследовать индивидуально. Исследуемых клещей помещают в пробирки типа «Эппендорф», добавляют 500 мкл 96 % этанола и встряхивают на вортексе. Пробирку центрифугируют в течение 3-5 с на микроцентрифуге типа вортекс для удаления капель с внутренней поверхности крышки пробирки, после чего жидкость аккуратно забирают с помощью вакуумного отсасывателя. Затем в пробирку с клещами добавляют 500 мкл 0,15 М раствора хлорида натрия или фосфатного буфера, встряхивают на вортексе, центрифугируют в течение 3-5 с на микроцентрифуге для удаления капель с внутренней поверхности крышки пробирки, после чего жидкость аккуратно забирают с помощью вакуумного отсасывателя. Для приготовления суспензий клещей используют стерильную фарфоровую чашку и стерильный пестик. Клещей растирают в 300 мкл (если проба состоит из одного клеща *Ixodes*), в 500 мкл (при исследовании клеща рода *Dermacentor*) или в 1 мл (если гомогенизируют пул клещей) 0,15 М раствора хлорида натрия или фосфатного буфера, затем полученную суспензию центрифугируют при 5 тыс об/мин в течение 2 мин и отбирают 100 мкл надосадочной жидкости для экстракции РНК/ДНК из клещей *Ixodes* и 50 мкл для экстракции РНК/ДНК из клещей *Dermacentor*. В оставшийся объем суспензии вносят глицерол (10% по объему), пробу перемешивают и замораживают при температуре не выше минус 16 °С для последующего исследования.

Лейкоцитарная фракция крови и СМЖ

Взятие цельной периферической крови проводится утром натощак в пробирку с 6% раствором ЭДТА в соотношении 1:20. Закрытую пробирку несколько раз переворачивают. Для

получения лейкоцитарной фракции крови в пробирку типа «Эппендорф» вносят 1,5 мл цельной крови, взятой с ЭДТА, и центрифугируют при 800 об/мин в течение 10 мин; затем верхний слой плазмы (500-600 мкл) с лейкоцитами переносят во вторую пробирку типа «Эппендорф» и центрифугируют при 13 000 об/мин в течение 10 мин. Оставшуюся надосадочную жидкость переносят в контейнер с дезраствором, а осадок клеток и 200 мкл надосадочной жидкости используют для экстракции РНК/ДНК.

1-1,5 мл ликвора центрифугируют при 13 000 об/мин в течение 10 мин. Надосадочную жидкость переносят в контейнер с дезраствором, а осадок клеток и 200 мкл надосадочной жидкости используют для экстракции РНК/ДНК.

Внутренние органы животных и секционный материал гомогенизируют в стерильной фарфоровой ступке и готовят 10 % суспензию на стерильном физиологическом растворе (0,15 М раствор хлорида натрия), или фосфатном буфере. Для экстракции РНК/ДНК берут 50 мкл суспензии.

ФОРМАТ FRT
СОСТАВ

«РИБО-преп» вариант 100 – комплект реагентов для выделения РНК/ДНК из клинического материала – **включает:**

Реактив	*Описание*	*Объем,мл*	*Кол-во*
Раствор для лизиса	Прозрачная жидкость от бесцветного до серо-голубого цвета[2]	30	1 флакон
Раствор для преципитации	Прозрачная бесцветная жидкость	40	1 флакон
Раствор для отмывки 3	Прозрачная бесцветная жидкость	50	1 флакон
Раствор для отмывки 4	Прозрачная бесцветная жидкость	20	1 флакон
РНК-буфер	Прозрачная бесцветная жидкость	1,2	8 пробирок

Комплект реагентов рассчитан на выделение РНК/ДНК из 100 проб, включая контроли. Входит в форму комплектации 1.

«РЕВЕРТА-L» вариант 100 – комплект реагентов для получения кДНК на матрице РНК – **включает:**

Реактив	*Описание*	*Объем, мл*	*Кол-во*
RT-G-mix-1	Прозрачная бесцветная жидкость	0,01	10 пробирок
RT-mix	Прозрачная бесцветная жидкость	0,125	10 пробирок
Ревертаза (MMlv)	Прозрачная бесцветная жидкость	0,06	1 пробирка
ДНК-буфер	Прозрачная бесцветная жидкость	1,2	2 пробирки

Комплект реагентов рассчитан на проведение 120 реакций обратной транскрипции, включая контроли. Входит в форму комплектации 1.

[2] При хранении раствора для лизиса при температуре от 2 до 8 °С возможно образование осадка в виде кристаллов.

«ПЦР-комплект» вариант FRT-100 F – комплект реагентов для амплификации фрагментов кДНК/ДНК *TBEV, B.burgdorferi sl, A.phagocytophilum, E.chaffeensis / E.muris* с гибридизационно-флуоресцентной детекцией в режиме «реального времени» – **включает:**

Реактив	*Описание*	*Объем, мл*	*Кол-во*
ПЦР-смесь-1-FRT ***TBEV, A.ph., E.ch. / E.m.***	Прозрачная жидкость от бесцветного до светло-лилового цвета	0,6	2 пробирки
ПЦР-смесь-1-FRT ***B.b. sl* / ВКО**	Прозрачная жидкость от бесцветного до светло-лилового цвета	0,6	2 пробирки
ОТ-ПЦР-смесь-2-FEP/FRT	Прозрачная бесцветная жидкость	0,3	4 пробирки
Полимераза (TaqF)	Прозрачная бесцветная жидкость	0,03	4 пробирки
ПКО кДНК *TBEV, B.b. sl, A.ph., E.ch. / E.m.* / STI	Прозрачная бесцветная жидкость	0,2	2 пробирки
ДНК-буфер	Прозрачная бесцветная жидкость	0,5	2 пробирки

Комплект реагентов рассчитан на проведение 120 реакций амплификации, включая контроли.

К комплекту реагентов прилагается контрольный образец этапа экстракции РНК/ДНК:

Реактив	*Описание*	*Объем, мл*	*Кол-во*
ВКО STI-87-rec	Прозрачная бесцветная жидкость	0,12	10 пробирок

ПРОВЕДЕНИЕ ПЦР-ИССЛЕДОВАНИЯ

ПЦР-исследование состоит из следующих этапов:

- ЭкстракцияРНК/ДНК из исследуемых образцов.
- Получение кДНК на матрице РНК.
- Проведение амплификации с гибридизационно-флуоресцентной детекцией в режиме «реального времени».
- Анализ и интерпретация результатов.

Детальная информация по процедуре проведения ПЦР-исследования в зависимости от типа используемого оборудования изложена в методических рекомендациях ФБУН ЦНИИ Эпидемиологии Роспотребнадзора по применению набора реагентов для выявления РНК/ДНК возбудителей инфекций, передающихся иксодовыми клещами *TBEV*, *Borellia*

burgdorferi sl, *Anaplasma phagocytophilum*, *Ehrlichia chaffeensis / Ehrlichia muris*, в биологическом материале методом полимеразной цепной реакции (ПЦР) с гибридизационно-флуоресцентной детекцией «АмплиСенс® *TBEV, B.burgdorferi sl, A.phagocytophilum, E.chaffeensis / E.muris*-FL».

ЭКСТРАКЦИЯ РНК/ДНК ИЗ ИССЛЕДУЕМЫХ ОБРАЗЦОВ

Для экстракции РНК/ДНК используется комплект реагентов, рекомендованный ФБУН ЦНИИ Эпидемиологии Роспотребнадзора, в соответствии с **приложением** к данной инструкции. Экстракция РНК/ДНК из каждого клинического образца проводится в присутствии внутреннего контрольного образца – **ВКО-STI-87-rec**.

При использовании формы комплектации набора 1 для экстракции РНК/ДНК используется входящий в набор комплект реагентов «РИБО-преп».

ПРОВЕДЕНИЕ РЕАКЦИИ ОБРАТНОЙ ТРАНСКРИПЦИИ

Для получения кДНК на матрице РНК используется комплект реагентов, рекомендованный ФБУН ЦНИИ Эпидемиологии Роспотребнадзора, в соответствии с инструкцией к используемому набору.

При использовании формы комплектации 1 для реакции обратной транскрипции используется входящий в набор комплект реагентов «РЕВЕРТА-L».

ПРОВЕДЕНИЕ АМПЛИФИКАЦИИ С ДЕТЕКЦИЕЙ В РЕЖИМЕ «РЕАЛЬНОГО ВРЕМЕНИ»

Выбор пробирок для амплификации зависит от используемого амплификатора с системой детекции в режиме «реального времени».

Для внесения в пробирки реагентов, проб кДНК/ДНК и контрольных образцов используются одноразовые наконечники с фильтрами.

Общий объем реакционной смеси – 25 мкл, включая объем пробы кДНК/ДНК – 10 мкл.

ВНИМАНИЕ! Полученная кДНК/ДНК каждой пробы исследуется в двух пробирках: с **ПЦР-смесью-1-FRT *TBEV, A.ph., E.ch. / E.m.*** и с **ПЦР-смесью-1-FRT *B.b. sl* / ВКО.**

А. Подготовка пробирок для амплификации

1. Приготовить реакционную смесь на необходимое количество реакций: смешать в отдельной пробирке **ПЦР-смесь-1-FRT *TBEV, A.ph., E.ch. / E.m.*, полимеразу (TaqF), ОТ-ПЦР-смесь-2-FEP/FRT**, в другой пробирке смешать **ПЦР-смесь-1-FRT *B.b. sl* / ВКО, полимеразу (TaqF)** и **ОТ-ПЦР-смесь-2-FEP/FRT** из расчета на каждую реакцию:
   - **10 мкл ПЦР-смеси-1-FRT *TBEV, A.ph., E.ch. / E.m.*** или **ПЦР-смеси-1-FRT *B.b. sl* / ВКО**;
   - **5 мкл ОТ-ПЦР-смеси-2-FEP/FRT**;
   - **0,5 мкл полимеразы (TaqF)**.

ВНИМАНИЕ! Приготовленную смесь не хранить.

ВНИМАНИЕ! При расчете следует учитывать, что постановка сопровождается амплификацией как минимум шести контрольных точек: отрицательного контроля экстракции (В–), положительного и отрицательного контролей ОТ-ПЦР (К+ и К–) для двух смесей – **ПЦР-смеси-1-FRT *TBEV, A.ph., E.ch. / E.m.*** и **ПЦР-смеси-1-FRT *B.b. sl* / ВКО**.

2. Раскапать приготовленные смеси в пробирки по **15 мкл**.
3. Используя наконечник с фильтром, добавить **10 мкл пробы кДНК/ДНК** в пробирки с каждой реакционной смесью. Осторожно перемешать пипетированием.
4. Для каждой панели исследуемых образцов необходимо поставить контроли амплификации кДНК/ДНК:
   а) **отрицательный контроль ПЦР (К–)** – внести в пробирку **10 мкл ДНК-буфера**;
   б) **положительный контроль ПЦР (К+)** – внести в пробирку **10 мкл ПКО кДНК *TBEV, B.b. sl, A.ph., E.ch. / E.m.* / STI**.

ВНИМАНИЕ! Пробы амплифицировать сразу после соединения реакционной смеси, с пробами кДНК/ДНК и контролями.

Б. Проведение амплификации с детекцией в режиме «реального времени»

1. Запрограммировать прибор (амплификатор с системой детекции в режиме «реального времени») для выполнения соответствующей программы амплификации и детекции флуоресцентного сигнала:

	Приборы роторного типа[3]			Приборы планшетного типа[4]		
Цикл	***Температура, °С***	***Время***	***Кол-во циклов***	***Температура, °С***	***Время***	***Кол-во циклов***
1	**95**	15 мин	1	**95**	15 мин	1
2	**95**	10 с	5	**95**	10 с	5
	60	30 с		**60**	35 с	
	72	15 с		**72**	15 с	
3	**95**	10 с	40	**95**	10 с	40
	56	30 с детекция флуоресц. сигнала		**56**	35 с детекция флуоресц. сигнала	
	72	15 с		**72**	15 с	

Детекция флуоресцентного сигнала назначается по каналам для флуорофоров FAM, JOE, ROX для пробирок с **ПЦР-смесью-1-FRT *TBEV, A.ph., E.ch. / E.m.*** и по каналам FAM, JOE для пробирок с **ПЦР-смесью-1-FRT *B.b. sl* / ВКО**.

2. Установить пробирки в ячейки реакционного модуля прибора. Первыми в ячейки прибора ставятся пробирки с **ПЦР-смесью-1-FRT *TBEV, A.ph., E.ch. / E.m.*** в том случае, если амплификация будет проводиться одновременно с двумя видами ПЦР-смесей.
3. Запустить выполнение программы амплификации с детекцией флуоресцентного сигнала.
4. По окончании выполнения программы приступить к анализу и учету результатов.

АНАЛИЗ И ИНТЕРПРЕТАЦИЯ РЕЗУЛЬТАТОВ

Анализ результатов проводят с помощью программного обеспечения используемого прибора для проведения ПЦР с детекцией в режиме «реального времени». Кривые накопления флуоресцентного сигнала анализируют по трем и двум каналам соответственно для каждого вида ПЦР-смеси:

- Для **ПЦР-смеси-1-FRT *TBEV, A.ph., E.ch. / E.m.*** по каналу для флуорофора FAM регистрируется сигнал, свидетельствующий о накоплении продукта амплификации

[3] Например, Rotor-Gene 3000, Rotor-Gene 6000 и рекомендованные ФБУН ЦНИИ Эпидемиологии Роспотребнадзора в методических рекомендациях по применению данного набора реагентов.

[4] Например, iCycler iQ5, Mx3000P, Mx3000, «ДТ-96» и рекомендованные ФБУН ЦНИИ Эпидемиологии Роспотребнадзора в методических рекомендациях по применению данного набора реагентов.

фрагмента кДНК *TBEV,* по каналу JOE – ДНК *A.phagocytophilum*, по каналу ROX – кДНК *E.chaffeensis/E.muris*.

- Для **ПЦР-смеси-1-FRT *B.b. sl* / ВКО** по каналу для флуорофора FAM регистрируется сигнал, свидетельствующий о накоплении продукта амплификации кДНК ВКО, по каналу JOE – кДНК *B.burgdorferi sl***.**

Результаты интерпретируются на основании наличия (или отсутствия) пересечения кривой флуоресценции с установленной на соответствующем уровне пороговой линией, что определяет наличие (или отсутствие) для данной пробы кДНК/ДНК значения порогового цикла *Ct* в соответствующей графе в таблице результатов.

Принцип интерпретации результатов следующий:

- кДНК *TBEV* **обнаружена***,* если при использовании **ПЦР-смеси-1-FRT *TBEV, A.ph., E.ch. / E.m.*** для данной пробы в таблице результатов по каналу для флуорофора FAM определено значение порогового цикла *Ct*.
- ДНК *A.phagocytophilum* **обнаружена**, если при использовании **ПЦР-смеси-1-FRT *TBEV, A.ph., E.ch. / E.m.*** для данной пробы в таблице результатов по каналу для флуорофора JOE определено значение порогового цикла *Ct*.
- кДНК *E.chaffeensis/E.muris* **обнаружена**, если при использовании **ПЦР-смеси-1-FRT *TBEV, A.ph., E.ch. / E.m.*** для данной пробы в таблице результатов по каналу для флуорофора ROX определено значение порогового цикла *Ct*.
- кДНК *B.burgdorferi sl* **обнаружена**, если при использовании **ПЦР-смеси-1-FRT *B.b. sl* / ВКО** для данной пробы в таблице результатов по каналу для флуорофора JOE определено значение порогового цикла *Ct*.

При этом кривая флуоресценции каждой исследуемой пробы должна пересекать пороговую линию на участке характерного экспоненциального подъема флуоресценции.

- кДНК *B.burgdorferi sl* **не обнаружена,** если при использовании **ПЦР-смеси-1-FRT *B.b. sl* / ВКО** в таблице результатов по каналу для флуорофора JOE не определено значение порогового цикла *Ct,* а по каналу FAM определено значение, не превышающее граничное.

- кДНК/ДНК *TBEV, A.phagocytophilum* и *E.chaffeensis/E.muris* **не обнаружена** если при использовании **ПЦР-смеси-1-FRT *TBEV, A.ph., E.ch. / E.m.*** для данной пробы в таблице результатов по каналу, по которому осуществляется детекция специфического сигнала, не определено значение порогового цикла.
- Результат анализа **невалидный,** если для данной пробы значение порогового цикла *Ct* по каналу для регистрации специфического сигнала не определено (отсутствует) и если при использовании **ПЦР-смеси-1-FRT *B.b. sl* / ВКО** значение *Ct* по каналу для флуорофора FAM также не определено (отсутствует) или превышает указанное граничное значение. В этом случае требуется повторно провести ПЦР-исследование соответствующего клинического образца.

ВНИМАНИЕ! Граничные значения *Ct* указаны во вкладыше к ПЦР-комплекту. См. также методические рекомендации ФБУН ЦНИИ Эпидемиологии Роспотребнадзора по применению набора реагентов для выявления РНК/ДНК возбудителей инфекций, передающихся иксодовыми клещами *TBEV*, *Borellia burgdorferi sl*, *Anaplasma phagocytophilum*, *Ehrlichia chaffeensis / Ehrlichia muris*, в биологическом материале методом полимеразной цепной реакции (ПЦР) с гибридизационно-флуоресцентной детекцией «АмплиСенс® *TBEV, B.burgdorferi sl, A.phagocytophilum, E.chaffeensis / E.muris*-FL».

Результат ПЦР-исследования считается достоверным, если получены правильные результаты для положительного и отрицательного контролей амплификации и отрицательного контроля экстракции РНК/ДНК, в соответствии с таблицей оценки результатов контрольных реакций (табл. 1).

Таблица 1

Результаты для контролей различных этапов ПЦР-исследования

ПЦР-смесь-1	Контроль	Контролируемый этап ПЦР-исследования	Значение порогового цикла, *Ct* (по всем каналам)
ПЦР-смесь-1-FRT *TBEV, A.ph., E.ch./E.m.*	В–	**Экстракция РНК/ДНК**	По всем каналам **значение отсутствует**
	К–	**ПЦР**	По всем каналам **значение отсутствует**
	К+	**ПЦР**	По всем каналам **определено значение меньше граничного**
ПЦР-смесь-1-FRT *B.b. sl*/ВКО	В–	**Экстракция РНК/ДНК**	По каналу JOE **значение отсутствует**, по каналу FAM **определено значение меньше граничного**
	К–	**ПЦР**	По всем каналам **значение отсутствует**
	К+	**ПЦР**	По всем каналам **определено значение меньше граничного**

ВНИМАНИЕ!

1. Если для положительного контроля ПЦР (К+) значение порогового цикла по какому-либо каналу (или каналам) для флуорофоров FAM, JOE, ROX при использовании **ПЦР-смеси-1-FRT *TBEV, A.ph., E.ch. / E.m.*** и по каналам для флуорофоров FAM и JOE при использовании **ПЦР-смеси-1-FRT *B.b. sl* / ВКО** отсутствует или превышает граничное значение, необходимо повторить амплификацию для всех образцов, в которых не обнаружена специфическая кДНК или ДНК, детектируемая по данному каналу (или каналам).
2. Если для отрицательного контроля экстракции РНК/ДНК (В–) (по каналам для флуорофоров FAM, JOE, ROX при использовании **ПЦР-смеси-1-FRT *TBEV, A.ph., E.ch. / E.m.*** и по каналу для флуорофора JOE при использовании **ПЦР-смеси-1-FRT *B.b. sl* / ВКО**) и/или отрицательного контроля ПЦР (К–) (по всем каналам) определено значение порогового цикла *Ct,* необходимо повторить ПЦР-исследование для всех образцов, в которых обнаружена ДНК или кДНК, детекируемая по данному каналу (или каналам).

СРОК ГОДНОСТИ. УСЛОВИЯ ТРАНСПОРТИРОВАНИЯ И ХРАНЕНИЯ

Срок годности. 9 мес. Набор реагентов с истекшим сроком годности применению не подлежит. Срок годности вскрытых реагентов соответствует сроку годности, указанному на этикетках для невскрытых реагентов, если в инструкции не указано иное.

Транспортирование. Набор реагентов транспортировать при температуре от 2 до 8 °С не более 5 сут. «ПЦР-комплект» вариант FRT-100 F при получении разукомплектовать в соответствии с указанными температурами хранения.

Хранение. Комплект реагентов «РЕВЕРТА-L» хранить при температуре от минус 24 °С до минус 16 °С. Комплекты реагентов «РИБО-преп» и «ПЦР-комплект» хранить при температуре от 2 до 8 °С. ПЦР-смесь-1-FRT *TBEV, A.ph., E.ch. / E.m.*, ПЦР-смесь-1-FRT *B.burgdorferi sl* / ВКО, ОТ-ПЦР-смесь-2-FEP/FRT и полимеразу (TaqF) (из «ПЦР-комплекта») хранить при температуре от минус 24 °С до минус 16 °С. ПЦР-смесь-1-FRT *TBEV, A.ph., E.ch. / E.m.* и ПЦР-смесь-1-FRT *B.b. sl* / ВКО хранить в защищенном от света месте.

Рекламации на качество набора реагентов направлять по адресу 111123, г.Москва, ул. Новогиреевская, дом 3А, e-mail: cs@pcr.ru[5].

[5] Отзывы и предложения о продукции «АмплиСенс» вы можете оставить, заполнив анкету потребителя на сайте: www.amplisens.ru.

ПРИЛОЖЕНИЕ

Экстракция РНК/ДНК из исследуемого материала

1. **Раствор для лизиса** (если он хранился при температуре от 2 до 8 °С) прогреть при температуре 65 °С до полного растворения кристаллов.
2. В пробирки с исследуемым материалом **(концентрированный осадок клеток СМЖ, лейкоцитарный осадок крови, гомогенат внутренних органов, осветленная суспензия клещей)** и в пробирку В– (отрицательный контроль экстракции) внести по **300 мкл раствора для лизиса**. Промаркировать пробирки. Содержимое пробирок тщательно перемешать и центрифугировать при **5 тыс об/мин** в течение **5 с** для стряхивания капель с крышек пробирок.
3. Внести в пробирки отдельными наконечниками по **10 мкл** внутреннего контрольного образца (**ВКО STI-87-rec)**. Содержимое пробирок тщательно перемешать на вортексе и прогреть **5 мин** при 65 °С в термостате.
4. Добавить в пробирки по **400 мкл раствора для преципитации**, перемешать на вортексе.
5. Процентрифугировать пробирки на микроцентрифуге в течение **5 мин** при **13 тыс об/мин.**
6. Аккуратно отобрать надосадочную жидкость, не задевая осадок, используя вакуумный отсасыватель и отдельный наконечник на 200 мкл для каждой пробы.
7. Добавить в пробирки по **500 мкл раствора для отмывки 3**, плотно закрыть крышки, осторожно промыть осадок, переворачивая пробирки 3-5 раз. Можно провести процедуру одновременно для всех пробирок; для этого необходимо накрыть пробирки в штативе сверху крышкой или другим штативом, прижать их и переворачивать штатив.
8. Процентрифугировать при **13 тыс об/мин** в течение **2 мин** на микроцентрифуге.
9. Осторожно, не захватывая осадок, отобрать супернатант, используя вакуумный отсасыватель и отдельный наконечник на **200 мкл** для каждой пробы.

10. Добавить в пробирки по **200 мкл раствора для отмывки 4**, плотно закрыть крышки и осторожно промыть осадок, переворачивая пробирки 3-5 раз.
11. Процентрифугировать при **13 тыс об/мин** в течение **2 мин** на микроцентрифуге.
12. Осторожно, не захватывая осадок, отобрать надосадочную жидкость, используя вакуумный отсасыватель и отдельный наконечник на 200 мкл для каждой пробы.
13. Поместить пробирки в термостат при температуре 65 °С на **5 мин** для подсушивания осадка (при этом крышки пробирок должны быть открыты).
14. При экстракция РНК/ДНК из **сконцентрированного осадка клеток СМЖ, лейкоцитарного осадка крови** внести в пробирки по **100 мкл РНК-буфера** , при экстракции из гомогената тканей и клещей – по **50 мкл РНК-буфера**. Перемешать на вортексе. Поместить в термостат при температуре 65 °С на **5-10 мин**, периодически встряхивая на вортексе.

Процентрифугировать пробирки при **13 тыс об/мин** в течение **1 мин** на микроцентрифуге. Надосадочная жидкость содержит очищенные РНК и ДНК. Пробы готовы к постановке реакции обратной транскрипции и ПЦР.

СИМВОЛЫ, ИСПОЛЬЗУЕМЫЕ В ПЕЧАТНОЙ ПРОДУКЦИИ

REF	Номер в каталоге		Осторожно! Обратитесь к сопроводительной документации
LOT	Код партии		Максимальное число тестов
RUO	Только для исследовательских и иных немедицинских целей		Использовать до
VER	Дата изменения		Обратитесь к руководству по эксплуатации
	Ограничение температуры		Не допускать попадания солнечного света
	Производитель		Дата изготовления

Лист вносимых изменений

Редакция	Место внесения изменений	Суть вносимых изменений
28.08.14 PM	Титульный лист. Срок годности. Условия транспортирования и хранения	Удалены печати и подписи
	Символы, используемые в печатной продукции	Символ IVD заменен на RUO
28.01.15 ChA	Титульный лист	Для символа RUO добавлена надпись «Только для научно-исследовательских целей»
	Срок годности. Условия транспортирования и хранения	Изменен адресат для направления рекламаций
	Символы, используемые в печатной продукции	Для символа RUO изменена фраза с «Только для исследовательских целей» на «Только для научно-исследовательских целей»
04.02.15 BS	Назначение	Удалено: «ВНИМАНИЕ! Результаты ПЦР-исследования учитываются в комплексной диагностике заболевания»
	Титульный лист	Для символа RUO изменена надпись «Только для научно-исследовательских целей» на «Только для исследовательских и иных немедицинских целей»
	Символы, используемые в печатной продукции	
09.02.15 PM	Срок годности. Условия транспортирования и хранения	Удален подраздел «Условия отпуска»
28.09.18 EM	Состав	Уточнены цвета реагентов
22.02.19 PM	Состав	Уточнен цвет реагента
	По тексту	Изменено форматирование текста
14.01.20 EM	Нижний колонтитул	Добавлен новый каталожный номер REF H-3932-1-0

AmpliSens® *Leptospira*-FRT PCR kit

IVD

For Professional Use Only

Instruction Manual

KEY TO SYMBOLS USED

REF	Catalogue number	⚠	Caution
LOT	Batch code	Σ	Sufficient for
IVD	*In vitro* diagnostic medical device		Use-by Date
VER	Version		Consult instructions for use
	Temperature limit		Keep away from sunlight
	Manufacturer	**NCA**	Negative control of amplification
	Date of manufacture	**C–**	Negative control of extraction
EC REP	Authorized representative in the European Community	**C+**	Positive control of amplification
PCE	Positive control of extraction	**IC**	Internal control

1. INTENDED USE

AmpliSens® *Leptospira*-FRT PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of 16S RNA of pathogenic *Leptospira* genospecies in the clinical material (blood and cerebrospinal fluid), autopsy material (brain, kidney, liver, lung tissue, and mesenterial lymph nodes) and biological material (material obtained from died animals (lung, brain, and kidney tissue) and animals suffering from acute leptospirosis (blood) or *Leptospira* persisting in kidneys (urine)) using real-time hybridization-fluorescence detection of amplified products.

NOTE: The results of PCR analysis are taken into account in complex diagnostics of disease.

2. PRINCIPLE OF PCR DETECTION

Detection of 16S RNA of pathogenic *Leptospira genospecies* by the polymerase chain reaction (PCR) is based on the amplification of the pathogen genome specific region using specific primers. In the real-time PCR, the amplified product is detected with the use of fluorescent dyes. These dyes are linked to oligonucleotide probes, which bind specifically to the amplified product during thermocycling. The real-time monitoring of the fluorescence intensities during the real-time PCR allows the detection of accumulating product without re-opening the reaction tubes after the PCR run.

AmpliSens® *Leptospira*-FRT PCR kit is a qualitative test that contains the Internal Control (Internal Control STI-87-rec (IC)). It must be used in the isolation procedure in order to control the extraction process of each individual sample and to identify possible reaction inhibition.

AmpliSens® *Leptospira*-FRT PCR kit uses "hot-start", which greatly reduces the frequency of nonspecifically primed reactions. "Hot-start" is guaranteed by separation of nucleotides and Taq-polymerase using chemically modified polymerase (TaqF). Chemically modified polymerase (TaqF) is activated by heating at 95 ºC for 15 min.

The results of amplification are registered in the following fluorescence channels:

Table 1

Channel for fluorophore	FAM	JOE
DNA-target	IC STI-87-rec cDNA	*Leptospira* cDNA
Target gene	Artificially synthesized sequence	16S RNA

3. CONTENT

AmpliSens® *Leptospira*-FRT PCR kit is produced in 1 form:
variant FRT REF R-B49(RG,iQ)-CE.
Variant FRT includes:

Reagent	Description	Volume, ml	Quantity
RT-G-mix-2	colorless clear liquid	0.01	2 tubes
RT-PCR-mix-1-FRT *Leptospira*	colorless clear liquid	0.6	1 tube
RT-PCR-mix-2-FEP/FRT	colorless clear liquid	0.3	1 tube
Polymerase (TaqF)	colorless clear liquid	0.03	1 tube
TM-Revertase (MMlv)	colorless clear liquid	0.015	1 tube
Positive Control cDNA *Leptospira* (C+$_{Leptospira}$)	colorless clear liquid	0.1	1 tube
RNA-eluent	colorless clear liquid	0.07	2 tubes
Negative Control (C–)*	colorless clear liquid	1.6	2 tubes
Positive Control *Leptospira*-rec	colorless clear liquid	0.03	5 tubes
Internal Control STI-87-rec (IC)**	colorless clear liquid	0.12	5 tubes

* must be used in the extraction procedure as Negative Control of Extraction

** add 10 µl of Internal Control during the DNA extraction procedure directly to the sample/lysis mixture (see **RIBO-sorb** REF K2-1-Et-50-CE, **RIBO-zol-C** REF K2-13-50-CE, **RIBO-prep** REF K2-9-Et-50-CE protocols).

Variant FRT PCR kit is intended for 60 reactions, including controls.

4. ADDITIONAL REQUIREMENTS

- RNA extraction kit.
- Disposable powder-free gloves and laboratory coat.
- Pipettes (adjustable).
- Sterile RNase-free pipette tips with aerosol filters (up to 200 µl).
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with a rotor for 2 ml tubes.
- PCR box.
- Real-time instruments (for example, Rotor-Gene 3000/6000 (Corbett Research, Australia); iCycler iQ5 (Bio-Rad, USA); or equivalent).
- Disposable polypropylene PCR tubes (0.2-ml)
- Refrigerator for 2 to 8 °C.
- Deep-freezer at the temperature from minus 24 to minus 16 °C.
- Reservoir for used tips.

5. GENERAL PRECAUTIONS

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol barriers and use a new tip for every procedure.
- Store all extracted positive material (specimens, controls and amplicons) away from all other reagents and add it to the reaction mix in a distantly separated facility.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Use disposable protective gloves and laboratory cloths, and protect eyes while samples and reagents handling. Thoroughly wash hands afterwards.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
- Do not use the PCR kit if the internal packaging was damaged or its appearance was changed.
- Do not use the PCR kit if the transportation and storage conditions according to the Instruction Manual were not observed.
- Do not use a kit after its expiration date.
- Dispose of all specimens and unused reagents in accordance with local regulations.
- Samples should be considered potentially infectious and handled in biological cabinet in compliance with appropriate biosafety practices.
- Clean and disinfect all samples or reagents spills using a disinfectant, such as 0.5 % sodium hypochlorite or another suitable disinfectant.
- Avoid samples and reagents contact with the skin, eyes, and mucous membranes. If these solutions come into contact, rinse the injured area immediately with water and seek medical advice immediately.
- Safety Data Sheets (SDS) are available on request.
- The PCR kit is intended for single use for PCR analysis of specified number of samples (see the section "Content").
- The PCR kit is ready for use in accordance with the Instruction Manual. Use the PCR kit strictly for intended purpose.
- Use of this product should be limited to personnel trained in DNA amplification techniques.
- Workflow in the laboratory must be one-directional, beginning in the Extraction Area and moving to the Amplification and Detection Area. Do not return samples, equipment and reagents in the area where the previous step was performed.

⚠ Some components of this kit contain sodium azide as a preservative. Do not use metal tubing for reagent transfer.

6. SAMPLING AND HANDLING

NOTE: Obtaining samples of biological materials for PCR-analysis, transportation and storage is described in manufacturer's handbook [1]. It is recommended that this handbook is read before starting work.

AmpliSens® *Leptospira*-FRT PCR kit is intended for analysis of RNA extracted with RNA extraction kits from the following material.

Human material
- clinical material (blood, cerebrospinal fluid);
- autopsy material (brain, kidney, liver, and lung tissue and mesenterial lymph nodes).

Animal material (biological material)
- urine;
- blood;
- brain, kidney, and lung tissue.

The material can be stored at 2-8 °C for 1 day. The autopsy material can be stored at the temperature not more than minus 16 °C for 1 week, at the temperature not more than minus 68 °C for a long time.

Sampling and pretreatment

6.1. *Blood and cerebrospinal fluid.* Whole blood is taken in the morning after overnight fasting into the tube with 6 % EDTA solution in the ratio 1 : 20. The closed tube with whole peripheral blood should be rotated several times. The tube with blood should be centrifugated at 1,000 g for 10 min to obtain blood plasma (if the blood was stored at 2–8 °C more than 1 hour, it should be mixed carefully by inverting the tube). Transfer 1 ml of plasma into two tubes. Two tubes with 1.0 ml of plasma should be centrifuged at 13,000 rpm for 10 min to concentrate bacterial cells. Then, 900 µl of the supernatant plasma should be removed with a filter tip into the container with disinfectant. The pellet and 100 µl of the supernatant are tested for the presence of *Leptospira* 16S RNA. The second pellet prepared in the same way should be stored at the temperature not more than minus 16 °C for repeated extraction (if any technological procedure is performed incorrectly). The pellet obtained from blood plasma can be stored at the temperature not more than minus 16 °C for 1 week or at the temperature not more than minus 68 °C for a long time.

When cerebrospinal fluid is analyzed, the pellet is obtained by the same procedure by centrifugation at 13,000 rpm for 10 min. The pellet and 100 µl of the supernatant are analyzed.

6.2. *Urine* for analyses is taken into a sterile container. If there is no chance to test material within 24 h after sampling, urine is transferred to a centrifuge tube or an Eppendorf tube. The contents of the tube is mixed with glycerol (~10 % v/v) and frozen. It can be stored at the temperature not more than minus 16 °C for 1 week or at the temperature not more than minus 68 °C for a long time.

If a cooling centrifuge (4 ºC) with a speed of 9,000–10,000 g intended for 30-ml tubes is available, the following sample preparation procedure is used. The sample is centrifuged at 9,000–10,000 g for 10 min, the supernatant is transferred to a container with disinfectant. Leave ~1 ml of the supernatant over the pellet in the tube and resuspend it. Transfer the suspension to a new tube and concentrate it by centrifugation at 13 000,rpm for 10 min. Then, 900 µl of the supernatant is transferred to the container with disinfectant, and the pellet and 100 µl of the supernatant is used for RNA isolation. In case of large quantities of salts and mucus, 100 µl of the supernatant and the upper layer of cells should be carefully taken from the salt pellet and transferred into a new tube for RNA isolation.

If you have no centrifuge for 30-ml tubes and a speed of 9,000–10,000 g, bacteria are concentrated from 1 ml of urine as described above using 1.5-ml tubes and a microcentrifuge for Eppendorf tubes. The remaining urine should be decontaminated in a disinfectant.

6.3. *Animal internal organs and autopsy material* is to be homogenized in sterile porcelain mortars with pestles. Then, 10 % suspension in sterile saline or phosphate buffer is prepared; 30 µl of the suspension is taken for RNA extraction.

7. WORKING CONDITIONS

AmpliSens® *Leptospira*-FRT PCR kit should be used at 18–25 °C.

8. PROTOCOL

8.1. RNA Extraction

It is recommended to use the following nucleic acid extraction kits:
- **RIBO-prep**, REF K2-9-Et-50-CE,
- **RIBO-zol-C**, REF K2-13-50-CE,
- **RIBO-sorb**, REF K2-1-Et-50-CE.

NOTE: Extract the RNA according to the manufacturer's protocol.

NOTE: **If using the RIBO-prep kit** extract the RNA/DNA according to the manufacturer's protocol taking into account next additions and improvements:
- If RNA is extracted from the tissues homogenates. Add **10 µl** of **Internal Control STI-87-rec (IC)** to each tube and then add **300 µl** of **Solution for Lysis**. Label test tubes. Then, add **50 µl** of test suspensions to the tubes with **Solution for Lysis** and **Internal Control STI-87-rec (IC).**
- If RNA is extracted from the cell pellets after *Leptospira* concentration, **300 µl** of **Solution for Lysis** and **10 µl** of **Internal Control STI-87-rec (IC)** should be added directly into the tubes with the pellets.
- Add only **10 µl** of **Internal Control STI-87-rec (IC)** and **300 µl** of **Solution for Lysis** into the tube for the Negative Control of extraction (C–).
- Add **300 µl** of **Solution for Lysis,** and then add **90 µl** of **Negative Control (C–), 10 µl** of **Internal Control STI-87-rec (IC)** and **10 µl** of **Positive Control *Leptospira*-rec** into the tube for the Positive Control of extraction (PCE).
- **40 µl** of **RNA-buffer** is to be used.

NOTE: **If using the RIBO-zol-C and RIBO-sorb kit** extract the RNA/DNA according to the manufacturer's protocol taking into account next additions and improvements:
- Add **300 µl** of **Solution D**, **10 µl** of **Internal Control STI-87-rec (IC)** and **100 µl** of **Negative control (C–)** into the tube for the Negative Control of extraction (C–).
- Add **300 µl** of **Solution D,** and then add **90 µl** of **Negative Control (C–), 10 µl** of **Internal Control STI-87-rec (IC)** and **10 µl** of **Positive Control *Leptospira*-rec** into the tube for the Positive Control of extraction (PCE).
- After the first stage of RNA extraction with **RIBO-zol-C** kit the top phase of obtained samples (**400 µl** when extracting RNA from cerebrospinal fluid, urine pellets and tissues homogenates, **200 µl** – from blood pellets, 450 µl – from C– and PCE samples) should be transferred into new 1.5-ml tubes with **400 µl** of **Lysis Solution (**from RIBO-sorb kit).
- **40 µl** of **RNA-buffer** is to be used.

8.2. Preparing reverse transcription and PCR

The total reaction volume is **25 µl**, the volume of DNA sample is **10 µl**.

NOTE: Only RNase-free, DNase-free disposable plastic consumables must be used when working with RNA.

8.2.1 Preparing tubes for reverse transcription and PCR

1 Prepare the required number of tubes for amplification of cDNA obtained from clinical and control samples. The type of tubes depends on the PCR instrument used for analysis. For carrying out N reactions with 2 controls, N+2 tubes are required.
2 Prepare the reaction mixture, calculating per one reaction:
   – **10 µl** of **RT-PCR-mix-1-FRT *Leptospira***
   – **5 µl** of **RT-PCR-mix-2-FEP/FRT**
   – **0.5 µl** of **polymerase (TaqF)**
   – **0.25 µl** of **TM-Revertase (MMlv)**
   – **0.25 µl** of **RT-G-mix-2**
3 Transfer **15 µl** of the prepared mixture to each tube. Discard the unused mixture.
4 Using filter tips add **10 µl** of **RNA** samples obtained at the RNA extraction stage into prepared tubes.
5 Carry out the control amplification reactions:
   NCA – Add **10 µl** of **RNA-eluent** to the tube labeled NCA (Negative Control of Amplification).
   C+ – Add **10 µl** of **Positive Control cDNA *Leptospira* (C+$_{Leptospira}$)** to the tube labeled C+ (Positive Control of Amplification).
   C– – Add **10 µl** of **the sample extracted from the Negative Control of Extraction sample** to the tube labeled C– (Negative control of Extraction).
   PCE – Add **10 µl** of **the sample extracted from the Positive control of Extraction sample** to the tube labeled PCE (Positive control of Extraction).

NOTE: Amplification is to be carried immediately after mixing the reaction mixture, RNA-sample and controls. The time period between addition of RNA-samples into the reaction mixture and amplification starting is to be not more than 10-15 min.

8.2.2. Amplification

1 Create a temperature profile on your instrument as follows:

Table 2

Amplification program for *Leptospira* cDNA

	Rotor-type instruments[1]			Plate-type instruments[2]		
Step	**Temperature, °C**	**Time**	**Cycles**	**Temperature, °C**	**Time**	**Cycles**
1	**50**	**30 min**	**1**	**50**	**30 min**	**1**
2	**95**	**15 min**	**1**	**95**	**15 min**	**1**
3	**95**	**20 s**	**10**	**95**	**20 s**	**10**
	65	**50 s**		**65**	**50 s**	
	72	**20 s**				
4	**95**	**20 s**	**38**	**95**	**20 s**	**40**
	61	**50 s Fluorescence acquiring**		**61**	**50 s Fluorescence acquiring**	
	72	**20 s**		**65**	**20 s**	

Fluorescent signal is detected in the channels for the FAM and JOE fluorophores.
2 Adjust the fluorescence channel sensitivity according to the Guidelines [2].
3 Insert tubes into the reaction module of the device.
4 Run the amplification program with fluorescence detection.
5 Analyze results after the amplification program is completed

9. DATA ANALYSIS

Analysis of results is performed by the software of the real-time PCR instrument used by measuring fluorescence signal accumulation in two channels:
– The signal of the IC cDNA amplification product is detected in the channel for the FAM fluorophore.
– The signal of the *Leptospira* cDNA amplification product is detected in the channel for the JOE fluorophore.

Results are interpreted by the crossing (or not-crossing) the fluorescence curve with the threshold line set at the specific level that corresponds to the presence (or absence) of a *Ct* value of the cDNA sample in the corresponding column of the results grid.

Principle of interpretation is the following:
– *Leptospira* cDNA is **detected** if the *Ct* value determined in the results grid in the channel for the JOE fluorophore is less than the boundary *Ct* value specified in the Guidelines.
– *Leptospira* cDNA is **not detected** in a sample if the *Ct* value is not determined (absent) in the channel for the JOE fluorophore, whereas the *Ct* value determined in the channel for the FAM fluorophore is less than the boundary *Ct* value specified in the Guidelines.
– The result is **invalid** if the *Ct* value is not determined (absent) in the channel for the JOE fluorophores whereas the *Ct* value in the channel for the FAM fluorophore is greater than the specified boundary *Ct* value. In such cases, the PCR analysis of this sample should be repeated starting from the RNA extraction stage.
– The result is **equivocal** if the *Ct* value determined in the channel for the JOE fluorophore is greater than the boundary *Ct* value specified in the Guidelines, whereas the *Ct* value determined in the channel for the FAM fluorophore is less than the boundary *Ct* value specified in the Guidelines. In such cases, the PCR analysis of this sample should be repeated two times starting from the RNA extraction stage.

NOTE: Boundary *Ct* values are specified in the Guidelines [2]

The result of the analysis is considered reliable only if the results obtained for the Positive and Negative Controls of amplification and extraction are correct (see Table 3).

Table 3

Results for controls

Control	Stage for control	*Ct* value in the channel for the fluorophore	
		FAM	JOE
PCE	DNA extraction	Present	< boundary value
C–	DNA extraction	Present	Absent
NCA	PCR	Absent	Absent
C+	PCR	Absent	< boundary value

[1] For example, Rotor-Gene 3000/Rotor-Gene 6000 (Corbett Research, Australia).
[2] For example, iCycler iQ5 (Bio-Rad, USA).

10. TROUBLESHOOTING

Results of analysis are not being registered in the following cases:

1. If the *Ct* value is determined for the Negative Control of extraction (C–) in the channel for the JOE fluorophore and/or for the Negative Control of amplification (NCA) in the channels for the FAM and JOE fluorophores in the results grid, it indicates contamination of reagents or samples. In such cases, the results of analysis are considered to be irrelevant. Analysis should be repeated and measures to detect and eliminate the source of contamination should be taken.
2. If no signal is detected for the Negative Control of extraction (C–) in the channel for the FAM fluorophore and/or for the Positive Control of extraction (PCE) in the channels for the FAM and JOE fluorophores, the results of analysis are considered invalid. Analysis of all samples should be repeated starting from the extraction stage.
3. If no signal is detected for Positive Control of amplification (C+) in the channel for the JOE fluorophore, the results of analysis are considered invalid. Analysis of all samples should be repeated starting from the RT-PCR stage..

If you have any further questions or if encounter problems, please contact our Authorized representative in the European Community.

11. TRANSPORTATION

AmpliSens® *Leptospira*-FRT PCR kit should be transported at 2–8 ºC for no longer than 5 days.

12. STABILITY AND STORAGE

All components of the **AmpliSens® *Leptospira*-FRT** PCR kit are to be stored at 2–8 ºC when not in use (except for RT-G-mix-2, RT-PCR-mix-1-FRT *Leptospira*, RT-PCR-mix-2-FEP/FRT, Polymerase (TaqF), and TM-Revertase (MMlv)). All components of the **AmpliSens® *Leptospira*-FRT** PCR kit are stable until the expiry date stated on the label. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

NOTE: RT-G-mix-2, RT-PCR-mix-1-FRT *Leptospira*, RT-PCR-mix-2-FEP/FRT, Polymerase (TaqF), and TM-Revertase (MMlv) are to be stored at the temperature from minus 24 to minus 16 °C.

NOTE: RT-PCR-mix-1-FRT *Leptospira* is to be stored away from light

13. SPECIFICATIONS

13.1. Sensitivity

Analytical Sensitivity of **AmpliSens® *Leptospira*-FRT** PCR kit is not less than $5x10^3$ copies per 1 ml of sample (copies/ml).

NOTE: The claimed analytical features of **AmpliSens® *Leptospira*-FRT** PCR kit are guaranteed only when additional reagents kits **RIBO-sorb** and **RIBO-zol-C** or **RIBO-prep** (manufactured by Federal Budget Institute of Science "Central Research Institute for Epidemiology") are used.

13.2. Specificity

The analytical specificity of **AmpliSens® *Leptospira*-FRT** PCR kit is ensured by the selection of specific primers and probes as well as stringent reaction conditions. The primers and probes have been checked for possible homologies to all sequences published in gene banks by sequence comparison analysis.

The clinical specificity of **AmpliSens® *Leptospira*-FRT** PCR kit was confirmed in laboratory clinical trials.

14. REFERENCES

1. Handbook "Sampling, Transportation, and Storage of Clinical Material for PCR diagnostics", developed by Federal Budget Institute of Science "Central Research Institute for Epidemiology" of Federal Service for Surveillance on Consumers' Rights Protection and Human Well-Being.
2. Guidelines to the **AmpliSens® *Leptospira*-FRT** PCR kit for qualitative detection of 16S RNA of pathogenic *Leptospira* genospecies in the clinical material, autopsy material and biological material by the polymerase chain reaction (PCR) with real-time hybridization-fluorescence detection developed by Federal Budget Institute of Science "Central Research Institute for Epidemiology".

15. QUALITY CONTROL

In compliance with Federal Budget Institute of Science "Central Research Institute for Epidemiology" ISO 13485-Certified Quality Management System, each lot of the **AmpliSens® *Leptospira*-FRT** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
13.12.10	Cover page	The phrase "For Professional Use Only" was added
	Intended use	The phrase "The results of PCR analysis are taken into account in complex diagnostics of disease" was added
	Content	New sections "Working Conditions" and "Transportation" were added
		The "Explanation of Symbols" section was renamed to "Key to Symbols Used"
	Stability and Storage	The information about the shelf life of reagents before and after the first use was added
		Information that RT-PCR-mix-1-FRT *Leptospira* is kept away from light was added
	Key to Symbols Used	The explanation of symbols was corrected
	Footer	Reference number was changed from R-B49(RG)-CE to R-B49(RG,iQ)-CE
	Sampling and Handling	Duration of the urine sample storage was changed
		Information about storage and disposal of urine samples was added
01.07.11 RT	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science "Central Research Institute for Epidemiology"
16.10.15 ChA	3. Content	Quantity of Negative Control (C-) tubes was changed from 1 to 2
	8.1. RNA Isolation	For RIBO-prep the procedure of positive control of extraction preparation was changed: 90 µl of Negative Control (C-) is to be added additionally
17.01.17 ME	Text	Corrections according to the template
	1. Intended use	Types of biological material was specified
	8.1. RNA extraction	The sections were rewritten
	8.2.2. Amplification	
	9. Data analysis	
	14. References	The reference to Guidelines was added
23.04.20 KK	Through the text	The text formatting was changed
	2. Principle of PCR detection	The table with targets was added.
	Footer	The phrase "Not for use in the Russian Federation" was added

AmpliSens®

EC REP

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УТВЕРЖДЕНА
Приказом Росздравнадзора
от 03.02.2012г № 340-Пр/12

УТВЕРЖДАЮ
Директор Федерального бюджетного учреждения науки «Центральный научно-исследовательский институт эпидемиологии» Федеральной службы по надзору в сфере защиты прав потребителей и благополучия человека
_____ В.И.Покровский
«01» августа 2011 г.

ИНСТРУКЦИЯ

по применению набора реагентов
для выявления РНК вируса Крымской-Конго геморрагической лихорадки (ККГЛ, *Crimean-Congo hemorrhagic fever virus, CCHFV*) в биологическом материале методом полимеразной цепной реакции (ПЦР) с гибридизационно-флуоресцентной детекцией

«АмплиСенс® *CCHFV*-FL»

АмплиСенс®

Федеральное бюджетное учреждение науки
«Центральный научно-исследовательский институт эпидемиологии»,
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IVD

ОГЛАВЛЕНИЕ

СПИСОК СОКРАЩЕНИЙ

В настоящей инструкции применяются следующие сокращения и обозначения:

ВКО	- внутренний контрольный образец
К–	- отрицательный контроль ПЦР
К+	- положительный контроль ПЦР
кДНК	- комплементарная ДНК, получаемая в реакции обратной транскрипции на матрице РНК
НК	- нуклеиновые кислоты (РНК/ДНК)
ОК	- отрицательный контроль экстракции РНК
ОКО	- отрицательный контрольный образец
ПК	-положительный контроль экстракции РНК
ПКО	- положительный контрольный образец
ПЦР	- полимеразная цепная реакция
ФБУН ЦНИИ Эпидемиологии Роспотребнадзора	- федеральное бюджетное учреждение науки «Центральный научно-исследовательский институт эпидемиологии» Федеральной службы по надзору в сфере защиты прав потребителей и благополучия человека
CCHFV	- *Crimean-Congo hemorrhagic fever virus*, вирус Крымской-Конго геморрагической лихорадки
FRT	- флуоресцентная детекция в режиме «реального времени»

НАЗНАЧЕНИЕ

Набор реагентов **«АмплиСенс® *CCHFV*-FL»** предназначен для выявления РНК вируса Крымской-Конго геморрагической лихорадки (*CCHFV*) в клиническом материале (плазма и сыворотка крови) и клещах методом полимеразной цепной реакции (ПЦР) с гибридизационно-флуоресцентной детекцией.

ВНИМАНИЕ! Результаты ПЦР-исследования учитываются в комплексной диагностике заболевания[1].

ПРИНЦИП МЕТОДА

Выявление *CCHFV* методом полимеразной цепной реакции (ПЦР) с гибридизационно-флуоресцентной детекцией включает в себя следующие этапы: экстракция РНК из образцов биологического материала, обратная транскрипция РНК и ПЦР-амплификация участка кДНК *CCHFV* и гибридизационно-флуоресцентной детекцией, которая производится непосредственно в ходе ПЦР (формат FRT).

Экстракция РНК из биологического материала проводится в присутствии внутреннего контрольного образца (ВКО STI-87-rec), который позволяет контролировать выполнение

[1] В соответствии с Директивой Европейского Союза 98/79/ЕС.

процедуры исследования для каждого образца. Затем с полученными пробами проводится обратная транскрипции РНК с помощью фермента ТМ-ревертазы и амплификация участка кДНК *CCHFV* при помощи специфичных к этому участку кДНК праймеров и фермента Taq-полимеразы. В составе реакционной смеси присутствует флуоресцентно-меченый олигонуклеотидный зонд, который гибридизуется с комплементарным участком амплифицируемой кДНК-мишени, в результате чего происходит нарастание интенсивности флуоресценции. Это позволяет регистрировать накопление специфических продуктов амплификации путем измерения интенсивности флуоресцентных сигналов. Детекция флуоресцентных сигналов при использовании формата FRT происходит непосредственно в ходе ПЦР с помощью амплификатора с системой детекции флуоресцентного сигнала в режиме «реального времени».

ФОРМАТЫ И ФОРМЫ ВЫПУСКА НАБОРА РЕАГЕНТОВ

Набор реагентов выпускается в 1 формате.

Формат FRT

Набор реагентов выпускается в 4 формах комплектации:

Форма 1 включает комплект реагентов «ПЦР-комплект» вариант FRT;

Форма 2 включает комплекты реагентов «РИБО-преп» вариант 50 и «ПЦР-комплект» вариант FRT;

Форма 3 включает комплекты реагентов «РИБО-золь-В» вариант 50 и «ПЦР-комплект» вариант FRT;

Форма 4 включает наборы реагентов оптом, расфасованные по отдельным реагентам, с маркировкой реагентов на их оптовой фасовке.

Форма комплектации 1 предназначена для проведения обратной транскрипции РНК и амплификации кДНК с гибридизационно-флуоресцентной детекцией в режиме «реального времени». Для проведения полного ПЦР-исследования необходимо дополнительно использовать комплекты реагентов для экстракции РНК, рекомендованные ФБУН ЦНИИ Эпидемиологии Роспотребнадзора в зависимости от вида исследуемого материала.

Формы комплектации 2 и 3 предназначены для проведения полного ПЦР-исследования, включающего экстракцию РНК из биологического материала, обратную транскрипции РНК и амплификацию кДНК с гибридизационно-флуоресцентной детекцией в режиме «реального времени».

Форма комплектации 4 предназначена для производственных целей для последующей маркировки на языке заказчика и комплектации по наборам.

ВНИМАНИЕ! Использование формы комплектации 4 производится только в соответствии с регламентом, утвержденным ФБУН ЦНИИ Эпидемиологии Роспотребнадзора.

АНАЛИТИЧЕСКИЕ ХАРАКТЕРИСТИКИ

Аналитическая чувствительность

Вид биологического материала (объем исследуемой пробы)	Комплект для выделения РНК/ДНК	Комплект для амплификации и детекции	Аналитическая чувствительность, копий/мл	Пробоподготовка материала
Сыворотка крови (100 мкл) Клещи *H.marginatum* пулы (50 мкл)	«РИБО-преп»	«ПЦР-комплект» вариант FRT	$5x10^3$	Данная чувствительность достигается при соблю-дении нижеизло-женных правил пробоподготовки биоматериала и рекомендуемом объеме исследуемой пробы
Клещи *H.marginatum* пулы (100 мкл)	«РИБО-золь-В»	«ПЦР-комплект» вариант FRT	$5x10^3$	

Аналитическая специфичность

Аналитическая специфичность изучена на:

- флавивирусах (вирус Западного Нила, Омской геморрагической лихорадки);
- герпесвирусах (I и II типов, *CMV*, *EBV*, *VZV*, IV типа), энтеровирусах (*ECHO*, *Coxsackie*);
- риккетсиях группы пятнистых лихорадок (*Rickettsia conorii* ssp. *caspia*, *Coxiella burnetii*);
- ортобуньявирусах (вирус Тягини, Батаи);
- хантавирусах (Пумала, Добрава);

- тоготовирусах (Баткен).

При работе с РНК/ДНК вышеперечисленных организмов, ДНК человека и ДНК клещей ложноположительных результатов выявлено не было.

МЕРЫ ПРЕДОСТОРОЖНОСТИ

Работа должна проводиться в лаборатории, выполняющей молекулярно-биологические (ПЦР) исследования клинического материала на наличие возбудителей инфекционных болезней, с соблюдением санитарно-эпидемических правил СП 1.3.2322-08 «Безопасность работы с микроорганизмами III–IV групп патогенности (опасности) и возбудителями паразитарных болезней», СанПиН 2.1.7.2790-10 «Санитарно-эпидемиологические требования к обращению с медицинскими отходами» и методических указаний МУ 1.3.2569-09 «Организация работы лабораторий, использующих методы амплификации нуклеиновых кислот при работе с материалом, содержащим микроорганизмы I–IV групп патогенности».

При работе всегда следует выполнять следующие требования:

- Следует рассматривать исследуемые образцы как инфекционно-опасные, организовывать работу и хранение в соответствии с СП 1.3.2322-08 «Безопасность работы с микроорганизмами III–IV групп патогенности (опасности) и возбудителями паразитарных болезней».
- Убирать и дезинфицировать разлитые образцы или реактивы, используя дезинфицирующие средства в соответствии с СП 1.3.2322-08 «Безопасность работы с микроорганизмами III–IV групп патогенности (опасности) и возбудителями паразитарных болезней».
- Лабораторный процесс должен быть однонаправленным. Анализ проводится в отдельных помещениях (зонах). Работу следует начинать в Зоне Выделения, продолжать в Зоне Амплификации и Детекции. Не возвращать образцы, оборудование и реактивы в зону, в которой была проведена предыдущая стадия процесса.
- Удалять неиспользованные реактивы в соответствии с СанПиН 2.1.7.2790-10 «Санитарно-эпидемиологические требования к обращению с медицинскими отходами».

ВНИМАНИЕ! При удалении отходов после амплификации (пробирок, содержащих продукты ПЦР) недопустимо открывание пробирок и разбрызгивание содержимого, поскольку это может привести к контаминации продуктами ПЦР лабораторной зоны, оборудования и реагентов.

- Применять набор строго по назначению, согласно данной инструкции.
- Допускать к работе с набором только специально обученный персонал.
- Не использовать набор по истечении срока годности.
- Использовать одноразовые перчатки, лабораторные халаты, защищать глаза во время работы с образцами и реактивами. Тщательно вымыть руки по окончании работы.
- Избегать контакта с кожей, глазами и слизистой оболочкой. При контакте немедленно промыть пораженное место водой и обратиться за медицинской помощью.
- Листы безопасности материалов (MSDS – material safety data sheet) доступны по запросу.

ВНИМАНИЕ! В соответствии с Директивой Европейского Союза 67/548/EEC следующие реагенты подлежат маркировке, как содержащие опасные вещества, а также требуют указания факторов риска (R) и мер предосторожности (S):

Наименование реагента	Наименование комплекта, в который входит реагент	Наименование опасного (в соответствии с директивой 67/548/EEC) вещества	Код опасности, перечень факторов риска (R) и мер предосторожности (S) в соответствии с директивой 67/548/EEC	по ГН 2.2.5.1313-03[2]			
				ПДК макс разовая/среднесменная	основная опасность	класс опасности	автоматический контроль над содержанием вещества в воздухе рабочей зоны
Раствор для лизиса	«РИБО-преп»	Гуанидин тиоцианат	Harmful[3] R:20/21/22-32-52/53 S:13-61	Нет данных			
Раствор D	«РИБО-золь-В»						
Раствор для преципитации	«РИБО-преп»	Изопропанол	Highly flammable. Irritant[3] R:11-36-67 S:7-16-24/25-26	50/ 10	Пары	Класс опасности 3	не требуется
Раствор С	«РИБО-золь-В»						
Раствор А	«РИБО-золь-В»	Фенол	Toxic, Corrosive[3] R: 23/24/25-34-48/20/21/22-68 S: 24/25-26-28-36/37/39-45	1/ 0,3	Пары	Класс опасности 2	не требуется
Раствор В	«РИБО-золь-В»	Хлороформ	Harmful[3] R: 22-38-40-48/20/22 S: 36/37	10/ 5	Пары	Класс опасности 2	не требуется
Раствор для отмывки 3	«РИБО-преп» «РИБО-золь-В»	Этанол	Highly flammable[3] R:11 S:7-16	2000/ 1000	Пары	Класс опасности 4	не требуется
раствор для отмывки 4	«РИБО-преп»						

Расшифровка обозначений факторов риска (R) и мер предосторожности (S).

R11: легко воспламеняется.

R20/21/22: опасен при проглатывании, контакте с кожей или вдыхании.

R22: опасен при проглатывании.

R23/24/25: ядовит при вдыхании, контакте с кожей и при проглатывании.

[2] Данные ГН 2.2.5.1313-03 «Предельно допустимые концентрации (ПДК) вредных веществ в воздухе рабочей зоны». Класс опасности по ГОСТ 12.1.007-76. ССБТ. «Вредные вещества. Классификация. Общие требования безопасности».

[3] Использованы данные о коде опасности, факторах риска (R) и мерах предосторожности (S) фирмы Sigma-Aldrich (harmful - вредный для здоровья, highly flammable – легковоспламеняющийся, irritant - вызывающий раздражение, toxic- токсичный, corrosive-коррозивный).

R32: при контакте с кислотой образует токсичный газ.
R34: вызывает ожоги.
R36: раздражает слизистую глаз.
R38: раздражает кожу.
R40: ограниченное число доказательств канцерогенного эффекта.
R48/20/22: опасность серьезного вреда для организма при длительном вдыхании и приеме внутрь.
R48/20/21/22: опасность серьезных повреждений организма при длительном вдыхании, контакте с кожей или при приеме внутрь
R52/53: опасен для водных организмов, может вызывать долговременное нежелательное воздействие на водную среду.
R67: пары вещества могут вызывать сонливость и головокружение.
R68: риск необратимых последствий.
S7: держать емкость плотно закрытой.
S13: держать вдали от пищевых продуктов и напитков, продуктов для животных.
S16: держать вдали от источников огня, не курить.
S24/25: избегать контакта с кожей и глазами.
S26: в случае попадания в глаза немедленно промыть большим количеством воды и обратиться за медицинской помощью.
S28: после попадания на кожу промыть большим количеством воды.
S36/37: использовать соответствующую защитную одежду и перчатки.
S36/37/39: использовать соответствующую защитную одежду, перчатки и маску/очки.
S45: в случае происшествия или ухудшения самочувствия немедленно обратиться за медицинской помощью.
S61: избегать попадания в окружающую среду.
ВНИМАНИЕ! При работе с легковоспламеняющимися веществами соблюдать правила пожарной безопасности для учреждений здравоохранения ППБО 07-91 от 30.08.91

ДОПОЛНИТЕЛЬНЫЕ МАТЕРИАЛЫ И ОБОРУДОВАНИЕ

1. 0,15 M NaCl или фосфатный буферный раствор (PBS) (натрия хлорид, 137 мМ; калия хлорид, 2,7 мМ; натрия

монофосфат, 10 мМ; калия дифосфат, 2 мМ; pH=7,5±0,2).

2. Комплекты реагентов для выделения РНК/ДНК (в зависимости от типа исследуемого биоматериала) – «РИБО-преп» (ТУ 9398-071-01897593-2008) или «РИБО-золь-В» (ТУ 9398-073-01897593-2008), или другие рекомендованные ФБУН ЦНИИ Эпидемиологии Роспотребнадзора – при работе с формой комплектации 1.
3. Дополнительные материалы и оборудование для экстракции РНК/ДНК – согласно инструкции к комплекту реагентов для выделения РНК/ДНК.
4. Гомогенизатор TissueLyser LT (Qiagen, Германия) рекомендуется использовать для гомогенизации клещей.
5. Металлические шарики из нержавеющей стали диаметром 7 мм.
6. Бокс абактериальной воздушной среды (ПЦР-бокс).
7. Центрифуга/вортекс.
8. Автоматические дозаторы переменного объема (от 5 до 20 мкл и от 20 до 200 мкл).
9. Одноразовые наконечники с фильтром до 100 мкл и до 200 мкл в штативах.
10. Штативы для пробирок объемом 0,2 и 0,1 мл.
11. Одноразовые полипропиленовые завинчивающиеся или плотно закрывающиеся пробирки объемом 1,5 мл.
12. Холодильник от 2 до 8 °С с морозильной камерой не выше минус 16 °С для выделенных проб ДНК/РНК.
13. Отдельный халат, шапочки, обувь и одноразовые перчатки по МУ 1.3.2569-09.
14. Емкость для сброса наконечников.
15. Программируемый амплификатор с системой детекции флуоресцентного сигнала в режиме «реального времени» (например, Rotor-Gene 3000/6000 (Corbett Research, Австралия), Rotor-Gene Q (Qiagen, Германия), iQ5 (Bio-Rad, США), Mx3000P (Stratagene, США), ДТ-96 (ДНК-технологии, Россия) или аналогичные).
16. Одноразовые полипропиленовые пробирки для ПЦР:
    а) тонкостенные пробирки для ПЦР объемом 0,2 мл с выпуклой крышкой (например, Axygen, США) – при использовании прибора планшетного типа;
    б) тонкостенные пробирки для ПЦР объемом 0,2 мл с

плоской крышкой или 0,1 мл (например, Axygen, США) – при использовании прибора роторного типа.

ВЗЯТИЕ, ТРАНСПОРТИРОВАНИЕ И ХРАНЕНИЕ ИССЛЕДУЕМОГО МАТЕРИАЛА

Перед началом работы следует ознакомиться с методическими рекомендациями «Взятие, транспортировка, хранение клинического материала для ПЦР-диагностики», разработанными ФГУН ЦНИИЭ Роспотребнадзора, Москва, 2008 г.

ПОДГОТОВКА ИССЛЕДУЕМОГО МАТЕРИАЛА К ЭКСТРАКЦИИ РНК

1. Плазма крови, сыворотка крови. Взятие цельной периферической крови проводится утром натощак в пробирку с 6 % раствором ЭДТА из расчета 1:20. Закрытую пробирку с цельной периферической кровью несколько раз переворачивают. Для отбора плазмы пробирку с кровью центрифугируют в течение 20 мин при 1600 g. Сыворотку крови получают стандартными методами. Для исследования отбирают 100 мкл клинического материала.
2. Клещи. Предварительно формируют пулы клещей: голодных объединяют по 5-7 особей, полунапитавшихся – по 2-3; полностью напитавшихся – по 1. Для приготовления суспензий клещей используют стерильную фарфоровую чашку и стерильный пестик. При наличии автоматического гомогенизатора TissueLyser LT применяют следующие параметры для гомогенизации клещей рода *Hyalomma* (диаметр шариков – 7 мм; частота – 50 Гц/с; время гомогенизации – 12-15 мин; объем буфера – 700 мкл (ненапитавшийся клещ), 1000-1500 мкл (напитавшийся клещ и пулы клещей). В случае гомогенизации напитавшихся клещей в ступке их предварительно прокалывают стерильной одноразовой иглой в нескольких местах для выхода крови. Клещей предварительно отмывают в 70 % этаноле в случае если клещ загрязнен маслом. Клещей растирают в 700 мкл (если проба состоит из одного ненапитавшегося клеща) или в 1-1,5 мл (если гомогенизируют пул клещей или напитавшегося клеща) 0,15 М раствора хлорида натрия или PBS-буфера, смешивая

раствор с клещами небольшими объемами, затем полученную суспензию центрифугируют при 10 000 g в течение 1 мин и отбирают 50 мкл надосадочной жидкости для выделения РНК с набором «РИБО-преп». РНК из полностью напитавшихся клещей рекомендуется выделять с применением набора реагентов «РИБО-золь-В». В этом случае для выделения РНК отбирают 100 мкл осветленной клещевой суспензии.

Допускается хранение вышеперечисленного клинического материала до проведения исследования в течение суток при температуре от 2 до 8 °С или 1 нед – при температуре не выше минус 16 °С. Клещей хранят или живыми (до 1 мес) или 1 нед при температуре не выше минус 16 °С, далее - при температуре минус 70 °С.

ФОРМАТ FRT
СОСТАВ

Комплект реагентов «ПЦР-комплект» вариант FRT – комплект реагентов для обратной транскрипции РНК и амплификации кДНК участка генома вируса Крымской-Конго геморрагической лихорадки с гибридизационно-флуоресцентной детекцией в режиме «реального времени» – **включает**:

Реактив	*Описание*	*Объем, мл*	*Кол-во*
ОТ-ПЦР-смесь-1-FRT *CCHFV*	Прозрачная бесцветная жидкость	0,6	1 пробирка
ОТ-ПЦР-смесь-2-FEP/FRT	Прозрачная бесцветная жидкость	0,3	1 пробирка
RT-G-mix-2	Прозрачная бесцветная жидкость	0,015	1 пробирка
Полимераза (TaqF)	Прозрачная бесцветная жидкость	0,03	1 пробирка
ТМ-Ревертаза (MMlv)	Прозрачная бесцветная жидкость	0,015	1 пробирка
ПКО кДНК *CCHFV* / STI	Прозрачная бесцветная жидкость	0,1	1 пробирка
РНК-буфер	Прозрачная бесцветная жидкость	0,6	2 пробирки

Комплект реагентов рассчитан на проведение 60 реакций обратной транскрипции и амплификации, включая контроли.

К комплекту реагентов «ПЦР-комплект» прилагаются следующие реагенты:

Реактив	*Описание*	*Объем, мл*	*Кол-во*
ОКО	Прозрачная бесцветная жидкость	1,6	1 пробирка
ПКО *CCHFV*-FL-rec	Прозрачная бесцветная жидкость	0,03	5 пробирок
ВКО STI-87-rec	Прозрачная бесцветная жидкость	0,12	5 пробирок
тРНК 1 мкг/мкл	Прозрачная бесцветная жидкость	0,06	5 пробирок

Комплект реагентов «РИБО-преп» вариант 50 (ТУ 9398-071-01897593-2008) – комплект реагентов для выделения РНК/ДНК из клинического материала – **включает:**

Реактив	*Описание*	*Объем, мл*	*Кол-во*
Раствор для лизиса	Прозрачная жидкость голубого цвета[4]	15	1 флакон
Раствор для преципитации	Прозрачная бесцветная жидкость	20	1 флакон
Раствор для отмывки 3	Прозрачная бесцветная жидкость	25	1 флакон
Раствор для отмывки 4	Прозрачная бесцветная жидкость	10	1 флакон
РНК-буфер	Прозрачная бесцветная жидкость	1,2	4 пробирки

Комплект реагентов рассчитан на выделение РНК из 50 образцов, включая контроли. Входит в состав формы комплектации 2.

Комплект реагентов «РИБО-золь-В» вариант 50 (ТУ 9398-073-01897593-2008) – комплект реагентов для выделения РНК из клинического материала – **включает:**

Реактив	*Описание*	*Объем, мл*	*Кол-во*
Раствор D	Прозрачная бесцветная жидкость	20	1 флакон
Раствор E	Прозрачная бесцветная жидкость	1,5	1 пробирка
Раствор A	Прозрачная жидкость оранжевого цвета	15	1 флакон
Раствор B	Прозрачная бесцветная жидкость	5,0	1 пробирка
Раствор C	Прозрачная бесцветная жидкость	20	1 флакон
Раствор для отмывки 3	Прозрачная бесцветная жидкость	50	1 флакон
РНК-элюент	Прозрачная бесцветная жидкость	0,5	5 пробирок

Комплект реагентов вариант 50 рассчитан на выделение РНК из 50 образцов, включая контроли. Входит в состав формы комплектации 3.

[4] При хранении раствора для лизиса при температуре от 2 до 8 °С возможно образование осадка в виде кристаллов.

ПРОВЕДЕНИЕ ПЦР-ИССЛЕДОВАНИЯ

ПЦР-исследование состоит из следующих этапов:

- Экстракция РНК из исследуемых образцов.
- Проведение обратной транскрипции и ПЦР-амплификации с гибридизационно-флуоресцентной детекцией в режиме «реального времени».
- Анализ и интерпретация результатов.

Детальная информация по процедуре проведения ПЦР-исследования в зависимости от типа используемого оборудования изложена в методических рекомендациях по применению набора реагентов для выявления РНК вируса Крымской-Конго геморрагической лихорадки (ККГЛ, *Crimean-Congo hemorrhagic fever virus, CCHFV*) в биологическом материале методом полимеразной цепной реакции (ПЦР) с гибридизационно-флуоресцентной детекцией «АмплиСенс® *CCHFV*-FL», разработанных ФБУН ЦНИИ Эпидемиологии Роспотребнадзора.

ЭКСТРАКЦИЯ РНК ИЗ ИССЛЕДУЕМЫХ ОБРАЗЦОВ

Для экстракции РНК *CCHFV* из различных биологических объектов рекомендуется использовать следующие комплекты реагентов производства ФБУН ЦНИИ Эпидемиологии Роспотребнадзора:

- **«РИБО-преп»** – экстракция РНК из плазмы и сыворотки крови, суспензии голодных и полунапитавшихся клещей;
- **«РИБО-золь-В»** – экстракция РНК из суспензии полностью напитавшихся клещей.

При использовании формы комплектации 2 экстракция РНК проводится с помощью комплекта «РИБО-преп» в соответствии с Приложением 1. При использовании формы комплектации 3 экстракция РНК проводится с помощью комплекта «РИБО-золь-В» в соответствии с Приложением 2.

ПРОВЕДЕНИЕ ОБРАТНОЙ ТРАНСКРИПЦИИ РНК И АМПЛИФИКАЦИИ кДНК С ДЕТЕКЦИЕЙ В РЕЖИМЕ «РЕАЛЬНОГО ВРЕМЕНИ»

А. Подготовка пробирок для амплификации

Выбор пробирок для амплификации зависит от используемого амплификатора с системой детекции в

режиме «реального времени».

Для внесения в пробирки реагентов, проб и контрольных образцов используются одноразовые наконечники с фильтрами.

Общий объем реакционной смеси – 25 мкл, включая объем пробы РНК – 10 мкл.

1. Приготовить реакционную смесь на необходимое количество реакций - смешайте в отдельной пробирке **ОТ-ПЦР-смесь-1-FRT *CCHFV***, **ОТ-ПЦР-смесь-2-FEP/FRT, полимеразу (TaqF), ТМ-Ревертазу (MMlv)** и **RT-G-mix-2**, из расчета на каждую реакцию:
   – **10 мкл ОТ-ПЦР-смеси-1-FRT *CCHFV*;**
   – **5 мкл ОТ-ПЦР-смеси-2-FEP/FRT;**
   – **0,5 мкл полимеразы (TaqF);**
   – **0,25 мкл ТМ-Ревертазы (MMlv);**
   – **0,25 мкл RT-G-mix-2.**

При расчете следует учитывать, что постановка сопровождается амплификацией как минимум четырех контрольных образцов: положительного контроля экстракции (ПК), отрицательного контроля экстракции (ОК), положительного и отрицательного контролей ОТ-ПЦР (К+ и К–).

2. Внести в каждую пробирку по **15 мкл** подготовленной смеси.

ВНИМАНИЕ! Приготовленную смесь не хранить.

3. В подготовленные пробирки внести по **10 мкл проб РНК**, полученных в результате экстракции из исследуемых или контрольных образцов. Осторожно перемешать пипетированием.
4. Поставить контрольные реакции:
   а) **отрицательный контроль ОТ-ПЦР (К–)** – внести в пробирку **10 мкл РНК-буфера.**
   б) **положительный контроль ОТ-ПЦР (К+)** – внести в пробирку **10 мкл ПКО кДНК *CCHFV* / STI.**

ВНИМАНИЕ! Пробы амплифицировать сразу после соединения реакционной смеси и проб РНК и контролей.

Б. Проведение обратной транскрипции РНК и амплификации кДНК с детекцией в режиме «реального времени»

1. Запрограммировать прибор (амплификатор с системой детекции в режиме «реального времени») для выполнения соответствующей программы обратной транскрипции, амплификации и детекции флуоресцентного сигнала (см. табл. 1)

Таблица 1

	Приборы роторного типа[5]			**Приборы планшетного типа**[6]		
Цикл	Температура, °С	Время	Кол-во циклов	Температура, °С	Время	Кол-во циклов
1	**50**	30 мин	1	**50**	30 мин	1
2	**95**	15 мин	1	**95**	15 мин	1
3	**95**	10 с	5	**95**	10 с	5
	54	25 с		**54**	30 с	
	72	15 с		**72**	15 с	
4	**95**	10 с	45	**95**	10 с	45
	50	25 с детекция флуоресц. сигнала		**50**	35 с детекция флуоресц. сигнала	
	72	15 с		**72**	15 с	

Детекция флуоресцентного сигнала назначается по каналам для флуорофоров FAM/Green и JOE/Yellow/HEX.

2. Установить пробирки в ячейки реакционного модуля прибора. **Лунка №1 обязательно должна быть заполнена какой-либо исследуемой пробиркой.**
3. Запустить выполнение программы амплификации с детекцией флуоресцентного сигнала.
4. По окончании выполнения программы приступить к анализу и интерпретации результатов.

АНАЛИЗ И ИНТЕРПРЕТАЦИЯ РЕЗУЛЬТАТОВ

Анализ результатов поводят с помощью программного обеспечения используемого прибора для проведения ПЦР с детекцией в режиме «реального времени». Анализируют кривые накопления флуоресцентного сигнала по двум каналам:

[5] Например, Rotor-Gene 3000/6000 (Corbett Research, Австралия), Rotor-Gene Q (Qiagen, Германия) или аналогичные.

[6] Например, iQ5 (Bio-Rad, США), Mx3000P (Stratagene, США), ДТ-96 (ДНК-технологии, РФ) или аналогичные.

- по каналу для флуорофора FAM регистрируется сигнал, свидетельствующий о накоплении продукта амплификации фрагмента кДНК ВКО STI-87-rec;
- по каналу для флуорофора JOE регистрируется сигнал, свидетельствующий о накоплении продукта амплификации фрагмента кДНК *CCHFV*.

Результаты интерпретируются на основании наличия (или отсутствия) пересечения кривой флуоресценции с установленной на соответствующем уровне пороговой линией, что определяет наличие (или отсутствие) для данной пробы кДНК значения порогового цикла Ct в соответствующей графе в таблице результатов.

Принцип интерпретации результатов следующий:

- кДНК *CCHFV* **обнаружена***,* если для данной пробы в таблице результатов по каналу для флуорофора JOE определено значение порогового цикла *Ct,* не превышающее указанное (граничное) значение. При этом кривая флуоресценции каждой исследуемой пробы должна пересекать пороговую линию на участке характерного экспоненциального подъема флуоресценции.
- кДНК *CCHFV* **не обнаружена,** если для данной пробы в таблице результатов по каналу для флуорофора FAM определено значение порогового цикла *Ct,* не превышающее указанное (граничное) значение, а по каналу JOE значение порогового цикла не определено или превышает указанное граничное значение.
- Результат анализа **невалидный,** если для данной пробы не определено (отсутствует) значение порогового цикла *Ct* по каналу для флуорофора JOE, и по каналу для флуорофора FAM значение *Ct* также не определено (отсутствует) или превышает указанное граничное значение. В этом случае требуется повторно провести ПЦР-исследование соответствующего клинического образца, начиная с этапа экстракции.

ВНИМАНИЕ! Граничные значения *Ct* указаны во вкладыше, **прилагаемом к набору реагентов**. См. также методические рекомендации по применению набора реагентов для выявления РНК вируса Крымской-Конго геморрагической

лихорадки (ККГЛ, *Crimean-Congo hemorrhagic fever virus, CCHFV*) в биологическом материале методом полимеразной цепной реакции (ПЦР) с гибридизационно-флуоресцентной детекцией «АмплиСенс® *CCHFV*-FL».

Результат ПЦР-исследования считается достоверным, если получены правильные результаты для положительного и отрицательного контролей амплификации и положительного и отрицательного контролей экстракции РНК, в соответствии с таблицей оценки результатов контрольных реакций (табл. 2).

Таблица 2

Результаты для контролей различных этапов ПЦР-исследования

Контроль	Контролируемый этап ПЦР-исследования	Значение порогового цикла, Ct	
		по каналу для флуорофора JOE	по каналу для флуорофора FAM
ОК	Экстракция РНК	Значение отсутствует	Определено значение меньше граничного
ПК	Экстракция РНК	Определено значение меньше граничного	Определено значение меньше граничного
К–	ПЦР	Значение отсутствует	Значение отсутствует
К+	ПЦР	Определено значение меньше граничного	Определено значение меньше граничного

ВНИМАНИЕ!

1. Если для положительного контроля ПЦР (К+) значение порогового цикла по каналу для флуорофора JOE отсутствует или превышает граничное значение, необходимо повторить амплификацию для всех образцов, в которых не обнаружена специфическая кДНК.
2. Если для положительного контроля экстракции РНК (ПК) значение порогового цикла по каналу для флуорофора JOE отсутствует или превышает граничное значение, необходимо повторить экстракцию для всех образцов, в которых не обнаружена специфическая кДНК.
3. Если для отрицательного контроля экстракции РНК (ОК) по каналу для флуорофора JOE определено значение порогового цикла *Ct*, необходимо повторить ПЦР-исследование для всех образцов, в которых обнаружена кДНК, детектируемая на канале для флуорофора JOE.

4. Если для отрицательного контроля ПЦР (К–) по каналам для флуорофоров FAM и JOE определено значение порогового цикла *Ct*, необходимо повторить амплификацию для всех образцов, в которых обнаружена кДНК, детектируемая на канале для флуорофора JOE, с постановкой К– не менее чем в трех повторах.

СРОК ГОДНОСТИ. УСЛОВИЯ ТРАНСПОРТИРОВАНИЯ И ХРАНЕНИЯ

Срок годности. 9 мес. Набор реагентов с истекшим сроком годности применению не подлежит. Срок годности вскрытых реагентов соответствует сроку годности, указанному на этикетках для невскрытых реагентов, если в инструкции не указано иное.

Транспортирование. Набор реагентов транспортировать при температуре от 2 до 8 °С не более 5 сут. При получении разукомплектовать в соответствии с указанными температурами хранения.

Хранение. Комплекты реагентов «РИБО-преп», «РИБО-золь-В» и «ПЦР-комплект» хранить при температуре от 2 до 8 °С. РНК-элюент (из комплекта «РИБО-золь-В»), RT-G-mix-2, ОТ-ПЦР-смесь-1-FRT *CCHFV*, ОТ-ПЦР-смесь-2-FEP/FRT, полимеразу (TaqF), ТМ-Ревертазу (MMlv) и тРНК 1 мкг/мкл (из комплекта «ПЦР-комплект») хранить при температуре не выше минус 16 °С. ОТ-ПЦР-смесь-1-FRT *CCHFV* хранить в защищенном от света месте.

Условия отпуска. Для лечебно-профилактических и санитарно-профилактических учреждений.

Рекламации на качество набора реагентов «**АмплиСенс**® ***CCHFV*-FL**» направлять на предприятие-изготовитель ФБУН ЦНИИ Эпидемиологии Роспотребнадзора (111123 г. Москва, ул. Новогиреевская, д. 3а) в отдел по работе с рекламациями и организации обучения (тел. (495) 974-96-42, факс (495) 305-54-23 e-mail: products@pcr.ru)[7].

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[7] Отзывы и предложения о продукции «АмплиСенс» вы можете оставить, заполнив анкету потребителя на сайте: www.amplisens.ru.

ПРИЛОЖЕНИЕ 1

Экстракция РНК из плазмы и сыворотки крови, клещей с применением комплекта реагентов «РИБО-преп»

1. **Раствор для лизиса** (если он хранился при температуре от 2 до 8 °С) прогреть при температуре 65 °С до полного растворения кристаллов.
2. Отобрать необходимое количество одноразовых пробирок на 1,5 мл с плотно закрывающимися крышками (включая отрицательный и положительный контроли экстракции). Внести в каждую пробирку, предназначенную для экстракции исследуемых проб, по **10 мкл ВКО STI-87-rec** и по **300 мкл раствора для лизиса**. Промаркировать пробирки.
3. В пробирки с **раствором для лизиса** и **ВКО STI-87-rec** внести по **50 мкл** суспензий клещей либо по **100 мкл** плазмы, сыворотки.
4. В пробирку положительного контроля экстракции (ПК) внести **10 мкл ВКО STI-87-rec** и **300 мкл раствора для лизиса,** затем добавить **10 мкл ПКО *CCHFV*-FL-rec**.
5. В пробирку отрицательного контроля экстракции (ОК) внести **только 10 мкл ВКО STI-87-rec** и **300 мкл раствора для лизиса**.
6. Содержимое пробирок тщательно перемешать на вортексе и прогреть **5 мин при 65 °С** в термостате. Добавить в пробирки по **400 мкл раствора для преципитации**, перемешать на вортексе.
7. Процентрифугировать пробирки на микроцентрифуге в течение **5 мин** при **10 000 g.**
8. Аккуратно отобрать надосадочную жидкость, не задевая осадок, используя вакуумный отсасыватель и отдельный наконечник для каждой пробы.
9. Добавить в пробирки по **500 мкл раствора для отмывки 3**, плотно закрыть крышки, осторожно промыть осадок, переворачивая пробирки 3-5 раз. Можно провести процедуру одновременно для всех пробирок, для этого необходимо накрыть пробирки в штативе сверху крышкой или другим штативом, прижать их и переворачивать штатив.
10. Процентрифугировать при **10 000 g в течение 2 мин** на микроцентрифуге.

11. Осторожно, не захватывая осадок, отобрать надосадочную жидкость, используя вакуумный отсасыватель и отдельный наконечник для каждой пробы.
12. Добавить в пробирки по **200 мкл раствора для отмывки 4**, плотно закрыть крышки и осторожно промыть осадок, переворачивая пробирки 3-5 раз.
13. Процентрифугировать при **10 000 g** в течение **2 мин** на микроцентрифуге.
14. Осторожно, не захватывая осадок, отобрать надосадочную жидкость, используя вакуумный отсасыватель и отдельный наконечник для каждой пробы.
15. Поместить пробирки в термостат при температуре **65 °C на 5 мин** для подсушивания осадка (при этом крышки пробирок должны быть открыты).
16. Добавить в пробирки по **50 мкл РНК-буфера**. Перемешать на вортексе. Поместить в термостат при температуре **65 °C на 5 мин**, периодически встряхивая на вортексе.
17. Процентрифугировать пробирки при **10 000 g в течение 1 мин** на микроцентрифуге. Надосадочная жидкость содержит очищенные РНК. Пробы готовы к постановке реакции обратной транскрипции и ПЦР.

ПРИЛОЖЕНИЕ 2

Экстракция РНК из клещей с применением комплекта реагентов «РИБО-золь-В»

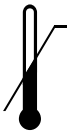
1. Отобрать необходимое количество одноразовых пробирок на 1,5 мл с плотно закрывающимися крышками (включая отрицательный и положительный контроли экстракции). Внести в каждую пробирку, предназначенную для экстракции исследуемых проб, по **10 мкл ВКО STI-87-rec**. Добавить в пробирки по **300 мкл раствора D**. Промаркировать пробирки.
2. В пробирки с **раствором D** и **ВКО STI-87-rec** внести по **100 мкл** суспензий клещей.
3. В пробирку положительного контроля экстракции (ПК) внести **10 мкл ВКО STI-87-rec** и **300 мкл раствора D,** затем **80 мкл ОКО** и **10 мкл ПКО *CCHFV*-FL-rec**.
4. В пробирку отрицательного контроля экстракции (ОК) внести **10 мкл ВКО STI-87-rec** и **300 мкл раствора D,** затем **90 мкл ОКО.**
5. Содержимое пробирок тщательно перемешать на вортексе и прогреть **5 мин при 56 °С** в термостате. Процентрифугировать на вортексе для удаления капель с крышки пробирки.
6. Добавить к раствору **30 мкл раствора Е**. Перемешать на вортексе процентрифугировать для удаления капель с крышки пробирки.
7. Добавить к раствору **300 мкл раствора А**, перемешать на вортексе и процентрифугировать для удаления капель с крышки пробирки.
8. Добавить к раствору **100 мкл раствора В**. Перемешивать на вортексе 1-2 мин (раствор должен стать молочно-белым).
9. Поставить пробирки в холодильник (температура от 2 до 4 °С) на 10 мин.
10. Центрифугировать пробирки 10 мин при 10 тыс g. В процессе центрифугирования раствор разделится на две фазы: нижнюю, содержащую белки и ДНК, и верхнюю – водную, содержащую РНК.
11. Отобрать новые пробирки на 1,5 мл, в которые необходимо внести **300 мкл раствора С.** Промаркировать пробирки. В пробирки с отрицательным и положительным контролем

экстракции (промаркированы **ОК** и **ПК**) внести по **10 мкл тРНК 1 мкг/мкл.**

12. Аккуратно отобрать верхнюю фазу (приблизительно 400 мкл), используя наконечники с фильтром и перенести в пробирку с раствором С. Перемешать на вортексе и выдержать в морозильнике при температуре не выше минус 16 °С в течение 1 ч.
13. Центрифугировать пробирки 10 мин при 10 тыс g. Удалить надосадочную жидкость отдельным наконечником на 1 мл, не задевая осадка.
14. Растворить осадок в **100 мкл раствора D**, добавить **100 мкл раствора С**, перемешать на вортексе. Выдержать в морозильнике при температуре не выше минус 16 °С в течение 1 ч.
15. Центрифугировать пробирки 10 мин при 10 тыс g. Удалить надосадочную жидкость отдельным наконечником на 1 мл, не задевая осадка.
16. Осадок промыть в **800 мкл охлажденного при температуре плюс 2 до 8 °С раствора для отмывки 3**, перемешивая на вортексе. Центрифугировать пробирки 10 мин при 10 тыс g. Удалить надосадочную жидкость отдельным наконечником на 1 мл, не задевая осадка.
17. Добавить **150 мкл** охлажденного **раствора для отмывки 3**. Центрифугировать пробирки 10 мин при 10 тыс g. Удалить надосадочную жидкость отдельным наконечником на 200 мкл, не задевая осадка.
18. Поместить пробирки в термостат при температуре 56 °С на 5 мин для подсушивания осадка (при этом крышки пробирок должны быть открыты).
19. Добавить в пробирки по **50 мкл РНК-элюента**. Растворить осадок РНК в пробирке, перемешивая элюат на вортексе. В случае высокой вязкости раствора увеличить объем элюента до 100 мкл. Прогревать пробирки в термостате в течение 5-7 мин.
20. Центрифугировать пробирки 2 мин при 10 тыс g. Надосадочная жидкость содержит очищенные РНК. Пробы готовы к постановке реакции обратной транскрипции и ПЦР.

Раствор РНК хранить при температуре не выше минус 68 °С.

СИМВОЛЫ, ИСПОЛЬЗУЕМЫЕ В ПЕЧАТНОЙ ПРОДУКЦИИ

REF	Номер в каталоге		Осторожно! Обратитесь к сопроводительной документации
LOT	Код партии		Максимальное число тестов
IVD	Изделие для in vitro диагностики		Использовать до
VER	Дата изменения		Обратитесь к руководству по эксплуатации
	Ограничение температуры		Не допускать попадания солнечного света
	Верхнее ограничение температуры		Дата изготовления
	Производитель		

УТВЕРЖДЕНА
Приказом Росздравнадзора
от 10.02.09г. № 992-Пр/09

«УТВЕРЖДАЮ»
Директор Федерального государственного учреждения науки «Центральный научно-исследовательский институт эпидемиологии» Федеральной службы по надзору в сфере защиты прав потребителей и благополучия человека
_____ В.И. Покровский
«07» ноября 2008 г.

ИНСТРУКЦИЯ

по применению набора реагентов
для выявления ДНК бактерий *Brucella* spp. в биологическом материале и культурах микроорганизмов методом полимеразной цепной реакции (ПЦР) с гибридизационно-флуоресцентной детекцией

«АмплиСенс® *Brucella* spp.-FL»

Набор реагентов выпускается в двух вариантах.

Вариант FEP.

ФОРМА КОМПЛЕКТАЦИИ.

Набор реагентов выпускается в 4 формах комплектации:

Форма 1 включает комплекты реагентов «ДНК-сорб-В» вариант 50, «ПЦР-комплект» вариант FEP (пробирки 0,5 мл).

Форма 2 включает комплекты реагентов «ДНК-сорб-В» вариант 50, «ПЦР-комплект» вариант FEP (пробирки 0,2 мл).

Форма 3 включает комплект реагентов «ПЦР-комплект» вариант FEP (пробирки 0,5 мл).

Форма 4 включает комплект реагентов «ПЦР-комплект» вариант FEP (пробирки 0,2 мл).

ВНИМАНИЕ! Заявленные аналитические характеристики набора реагентов при работе с формами **3** и **4** гарантируются только в случае применения дополнительного комплекта реагентов «ДНК-сорб-В» производства ФГУН ЦНИИЭ Роспотребнадзора.

Вариант FRT.

ФОРМА КОМПЛЕКТАЦИИ.

Набор реагентов выпускается в 2 формах комплектации:

Форма 1 включает комплекты реагентов «ДНК-сорб-В» вариант 50, «ПЦР-комплект» вариант FRT.

Форма 2 включает комплект реагентов «ПЦР-комплект» вариант FRT.

ВНИМАНИЕ! Заявленные аналитические характеристики набора реагентов при работе с формой **2** гарантируются только в случае применения дополнительного комплекта реагентов «ДНК-сорб-В» производства ФГУН ЦНИИЭ Роспотребнадзора.

СОСТАВ.

Комплект реагентов «ДНК-сорб-В» вариант 50 (ТУ 9398-003-01897593-2006) – комплект реагентов для выделения ДНК из клинического материала **включает:**

Реактив	*Описание*	*Объем (мл)*	*Кол-во*
Лизирующий раствор	Прозрачная бесцветная жидкость*	15	1 флакон
Раствор для отмывки 1	Прозрачная бесцветная жидкость*	15	1 флакон
Раствор для отмывки 2	Прозрачная бесцветная жидкость	50	1 флакон
Сорбент универсальный	Суспензия белого цвета	1,25	1 пробирка
ТЕ-буфер для элюции ДНК	Прозрачная бесцветная жидкость	5,0	1 пробирка

Комплект реагентов рассчитан на выделение ДНК из 50 проб, включая контроли.

Комплект реагентов «ПЦР-комплект» вариант FEP – комплект реагентов для ПЦР-амплификации ДНК бактерий *Brucella* spp. с гибридизационно-флуоресцентной детекцией по «конечной точке» **включает:**

Реактив	*Описание*	*Объем (мл)*	*Кол-во*
ПЦР-смесь-1-FEP/FRT *Brucella* spp. раскапана под воск	Прозрачная бесцветная жидкость	0,008	55 пробирок объемом 0,5 или 0,2 мл
ПЦР-смесь-2-FL	Прозрачная бесцветная жидкость	0,77	1 пробирка
ПЦР-смесь-Фон	Прозрачная бесцветная жидкость	0,5	1 пробирка
Минеральное масло для ПЦР	Бесцветная вязкая жидкость	2,0	1 пробирка
ПКО ДНК *Brucella*	Прозрачная бесцветная жидкость	0,1	1 пробирка
ДНК-буфер	Прозрачная бесцветная жидкость	0,5	1 пробирка

Комплект реагентов рассчитан на проведение 55 реакций амплификации, включая контроли.

Дополнительно к комплекту реагентов прилагаются контрольные образцы этапа выделения:

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* При хранении лизирующего раствора и раствора для отмывки 1 при температуре от 2 до 8 °С возможно образование осадка в виде кристаллов.

Реактив	*Описание*	*Объем (мл)*	*Кол-во*
ОКО	Прозрачная жидкость от соломенно-желтого до бесцветного	1,6	1 пробирка
ВКО STI-704	Прозрачная бесцветная жидкость	0,5	1 пробирка

Комплект реагентов «ПЦР-комплект» вариант FRT – комплект реагентов для ПЦР-амплификации ДНК бактерий *Brucella* spp. с гибридизационно-флуоресцентной детекцией в режиме «реального времени» **включает:**

Реактив	*Описание*	*Объем (мл)*	*Кол-во*
ПЦР-смесь-1-FEP/FRT *Brucella* spp. раскапана под воск	Прозрачная бесцветная жидкость	0,008	55 пробирок объемом 0,2 мл
ПЦР-смесь-2-FL	Прозрачная бесцветная жидкость	0,77	1 пробирка
ПКО ДНК *Brucella*	Прозрачная бесцветная жидкость	0,1	1 пробирка
ПКО STI	Прозрачная бесцветная жидкость	0,1	1 пробирка
ДНК-буфер	Прозрачная бесцветная жидкость	0,5	1 пробирка

Комплект реагентов рассчитан на проведение 55 реакций амплификации, включая контроли.

Дополнительно к комплекту реагентов прилагаются контрольные образцы этапа выделения:

Реактив	*Описание*	*Объем (мл)*	*Кол-во*
ОКО	Прозрачная жидкость от соломенно-желтого до бесцветного	1,6	1 пробирка
ВКО STI-704	Прозрачная бесцветная жидкость	0,5	1 пробирка

НАЗНАЧЕНИЕ.

Набор реагентов **«АмплиСенс® *Brucella* spp.-FL»** предназначен для выявления ДНК бактерий *Brucella* spp. *(B.melitensis, B.abortus, B.suis, B.ovis, B.canis, B.neotomae)* в биологическом материале и культурах микроорганизмов методом полимеразной цепной реакции (ПЦР) с гибридизационно-флуоресцентной детекцией.

Рекомендуется ознакомиться с МУ 3.1.7.1189-03 «ПРОФИЛАКТИКА И ЛАБОРАТОРНАЯ ДИАГНОСТИКА

БРУЦЕЛЛЕЗА ЛЮДЕЙ», утвержденными Главным государственным санитарным врачом РФ 30.01.2003.

Вариант FEP. Формы комплектации 1 и 2 предназначены для полного анализа, включая выделение ДНК из клинического материала и проведение ПЦР-амплификации ДНК с гибридизационно-флуоресцентной детекцией по «конечной точке». Формы комплектации 3 и 4 предназначены для проведения ПЦР-амплификации ДНК. Для полного анализа необходимо дополнительно использовать комплект реагентов «ДНК-сорб-В» для выделения ДНК из клинического материала (ТУ 9398-003-01897593-2006) производства ФГУН ЦНИИЭ Роспотребнадзора.

Вариант FRT. Форма комплектации 1 предназначена для полного анализа, включая выделение ДНК из клинического материала и проведения ПЦР-амплификации ДНК с гибридизационно-флуоресцентной детекцией в режиме «реального времени». Форма комплектации 2 предназначена для проведения ПЦР-амплификации ДНК. Для полного анализа необходимо дополнительно использовать комплект реагентов «ДНК-сорб-В» для выделения ДНК из клинического материала производства ФГУН ЦНИИЭ Роспотребнадзора.

ВЗЯТИЕ КЛИНИЧЕСКОГО МАТЕРИАЛА. ТРАНСПОРТИРОВАНИЕ И ХРАНЕНИЕ ПРОБ.

Перед началом работы следует ознакомиться с методическими рекомендациями «Взятие, транспортировка, хранение клинического материала для ПЦР-диагностики», разработанными ФГУН ЦНИИЭ Роспотребнадзора, Москва, 2008 г.

Для проведения анализа используется следующий материал:

Материал от людей:

- цельная периферическая кровь забирается в пробирку с 3 % ЭДТА из расчета 50 мкл ЭДТА на 1 мл крови.
- пунктат из лимфоузлов после взятия помещают в стерильную одноразовую пробирку со 100 мкл транспортной среды (производства ФГУН ЦНИИЭ Роспотребнадзора) или стерильного 0,9 % изотонического раствора натрия хлорида

(физиологического раствора).

- синовиальная жидкость помещают в стерильную одноразовую пробирку.

Материал от животных:

- кровь забирается в пробирку с 6 % ЭДТА из расчета 50 мкл ЭДТА на 1 мл крови.
- молоко отбирают в объеме 10-20 мл в стерильную посуду.
- содержимое брюшной полости и желудка, селезенка, печень абортированного плода.
- плацента и плодовые оболочки от абортировавших животных.
- содержимое бурс, гигром.
- в случае убоя животных для исследования отбирают парные лимфатические узлы с обеих сторон туши целиком (парааортальные, надвыменные, паховые, тазовые) и кусочки паренхиматозных органов (печень, селезенка), от самцов с признаками орхита или эпидидимита отбирают семенники с придатками.

Культуры микроорганизмов.

- культуры в жидких средах использовать без предварительной подготовки.
- подозрительные на *Brucell*a spp. колонии ресуспендировать в 0,5 мл физиологического раствора.

Хранить материал до проведения исследования можно в течение 1 сут при температуре от 2 до 8 °С, 1 мес при температуре не выше минус 16 °С. Допускается однократное замораживание-оттаивание материала.

Подготовка исследуемого материала.

Все работы по сбору, транспортированию и подготовке проб клинического и секционного материала осуществляют в строгом соответствии с требованиями СП 1.3. 1285–03 «Безопасность работы с микроорганизмами I-II групп патогенности (опасности)», СП 1.2.036-95 «Порядок учета, хранения, передачи и транспортирования микроорганизмов I-IV групп патогенности». Все манипуляции, связанные с подготовкой проб, проводятся с использованием стерильных ступок, пестиков, инструментов (ножниц, пинцетов,

скальпелей), дозаторов переменных объемов, одноразовых полипропиленовых пробирок на 1,5 мл и наконечников с аэрозольным барьером. Одноразовая пластиковая посуда (пробирки, наконечники) должна сбрасываться в специальный контейнер, содержащий дезинфицирующий 0,2 % раствор ДП-2Т и утилизироваться в соответствии с вышеуказанными документами. Ступки, пестики и инструменты должны обрабатываться согласно СП 1.3. 1285–03.

Пробы цельной крови, консервированной ЭДТА, синовиальной жидкости, пунктаты из лимфоузлов, содержимое бурс и гигром, культуры микроорганизмов используют для выделения ДНК без предварительной подготовки после стадии обеззараживания (см. раздел «**Обеззараживание материала»**).

Пробы паренхиматозных органов, семенников, плодовых оболочек, плаценты (каждую отдельно) размером 1х1х1см, а лимфатические узлы целиком, гомогенизируют с использованием стерильных фарфоровых ступок и пестиков добавляют равный объем стерильного физиологического раствора и тщательно перемешивают. Образовавшуюся смесь отстаивают при температуре от 20 до 25 ° С в течение 5 мин, затем верхнюю фазу по 0,4-0,5 мл переносят пастеровской пипеткой (или наконечником с аэрозольным барьером) в пробирки на 1,5 мл, проводят обеззараживание (см. раздел «**Обеззараживание материала»**) и 0,1 мл используют для выделения ДНК. Нижнюю фазу вместе с пробиркой утилизируют в соответствии с требованиями СП 1.3. 1285–03 «Безопасность работы с микроорганизмами I-II групп патогенности (опасности)».

Молоко в объеме 10 мл (при необходимости объем проб доводят до требуемого путем добавления физиологического раствора), обеззараживают (см. раздел «**Обеззараживание материала»**) и центрифугируют при 3 тыс об/мин в течение 10-15 мин. Если осадок практически не виден, то в эту же пробирку вносят еще 10 мл материала и повторяют центрифугирование. Надосадочную жидкость осторожно отбирают, оставив над осадком примерно 0,2 мл жидкости. Осадок ресуспендируют в оставшейся надосадочной жидкости

и 0,1 мл суспензии используют для выделения ДНК.

ОБЕЗЗАРАЖИВАНИЕ МАТЕРИАЛА.

Проводят согласно МУ 3.5.5.1034-01 «Обеззараживание исследуемого материала, инфицированного бактериями I-IV групп патогенности, при работе методом ПЦР».

Обработка мертиолятом натрия.

1. В образцы биологического материала и культуры микроорганизмов (при необходимости после предварительной подготовки см. пункт **«Подготовка исследуемого материала»**) добавить 0,1 % натрия мертиолята (разведение 1:1000) до конечной концентрации 0,01 % (разведение 1:10000) и прогревают при температуре (56±1) °С в течение 30 мин. Далее в работе использовать по 100 мкл проб.
2. При работе с подозрительными культурами обработанные мертиолятом бактериальные культуры по 1 мл отдельными дозаторами перенести в пробирки объемом 1,5 мл и центрифугировать при 12000 об/мин в течение 15 мин. Надосадочную жидкость удалить в емкость с дезинфицирующим раствором, осадок ресуспендировать в 100 мкл 0,9 % раствора натрия хлорида и использовать далее в работе.
3. Лизирующий раствор из комплекта реагентов «ДНК-сорб-В» (если он хранился при температуре от 2 до 8 °С) прогреть при температуре от 60 до 65 °С до полного растворения кристаллов.
4. В каждую пробирку со 100 мкл обеззараженного исследуемого материала внести по 300 мкл лизирующего раствора и инкубировать в течение 15 мин при температуре 65 °С.

Дальнейшие исследования проб проводить как с обеззараженным материалом по порядку процедур, описанных в разделе «Выделение ДНК из проб».

МЕРЫ ПРЕДОСТОРОЖНОСТИ.

1. **Необходимо строго соблюдать СП 1.3. 1285–03 «Безопасность работы с микроорганизмами I-II групп**

патогенности (опасности)».

2. **Необходимо строго соблюдать «Правила устройства, техники безопасности, производственной санитарии, противоэпидемического режима и личной гигиены при работе в лабораториях (отделениях, отделах) санитарно-эпидемиологических учреждений системы здравоохранения СССР», Москва, 1981 г.**
3. Анализ проводится в отдельных помещениях (зонах), согласно МУ 1.3.1794-03 «Организация работы при исследованиях методом ПЦР материала, инфицированного микроорганизмами I-II групп патогенности».
4. Работать только в одноразовых перчатках, использовать и менять при каждой операции одноразовые наконечники для автоматических дозаторов с аэрозольным барьером. Одноразовую пластиковую посуду (пробирки, наконечники) необходимо сбрасывать в специальный контейнер, содержащий дезинфицирующий 0,2 % раствор ДП-2Т.
5. Все лабораторное оборудование, в том числе дозаторы, штативы, лабораторная посуда, а также все рабочие растворы должны быть строго стационарными. Запрещается переносить их из одного помещения в другое.
6. Поверхности столов, а также помещения, в которых проводится постановка ПЦР, до начала и после завершения работ необходимо облучать ультрафиолетовым светом в течение 30 мин.

ДОПОЛНИТЕЛЬНЫЕ МАТЕРИАЛЫ И ОБОРУДОВАНИЕ, ТРЕБУЕМЫЕ ДЛЯ ПРОВЕДЕНИЯ ПЦР-АНАЛИЗА. (с указанием фирм-производителей/поставщиков):

ЗОНА 1.

Для выделения ДНК из исследуемого материала требуются:

1. Ламинарный бокс (например, «БАВп-01-«Ламинар-С»-1,2», «Ламинарные системы», Россия, класс биологической безопасности II тип А).
2. Термостат для пробирок типа «Эппендорф» от 25 до 100 °С (например, «ТЕРМО 24-15», «Биоком», Россия).
3. Микроцентрифуга для пробирок типа «Эппендорф» до 16 тыс

об/мин (например, «MiniSpin», «Eppendorf», Германия).
4. Вакуумный отсасыватель медицинский с колбой-ловушкой для удаления надосадочной жидкости (например, «ОМ-1», г. Ульяновск, Россия).
5. Вортекс (например, «ТЭТА-2», «Биоком», Россия).
6. Набор электронных или механических дозаторов переменного объема (например, «Ленпипет», Россия).
7. Одноразовые полипропиленовые завинчивающиеся или плотно закрывающиеся микропробирки объемом 1,5 мл (например, «Axygen», США).
8. Штативы для микропробирок объемом 1,5 мл (например, «ИнтерЛабСервис», Россия) и наконечников (например, «Axygen», США).
9. Одноразовые наконечники для дозаторов переменного объема с аэрозольным барьером до 200 мкл и до 1000 мкл (например, «Axygen», США).
10. Одноразовые наконечники для дозаторов переменного объема до 200 мкл и до 1000 мкл (например, «Axygen», США).
11. Холодильник от 2 до 8 °С с морозильной камерой не выше минус 16 °С.
12. Отдельный халат и одноразовые перчатки.
13. Емкость с дезинфицирующим раствором.

ЗОНА 2.

Для проведения ПЦР-амплификации и гибридизационно-флуоресцентной детекции продуктов ПЦР-амплификации требуются:

(с указанием фирм-производителей / поставщиков):

1. **Вариант FEP:** амплификатор для микропробирок 0,5 мл (например, «Терцик», «ДНК-Технология», Россия или эквивалентный), для микропробирок 0,2 мл (например, «Gradient Palm Cycler», «Corbett Research», Австралия или эквивалентный). Флуоресцентный ПЦР-детектор, например, «АЛА-1/4» («BioSan», Латвия) или эквивалентный.
2. **Вариант FRT:** амплификатор «Rotor-Gene» 3000 или 6000 («Corbett Research», Австралия) или эквивалентный.
3. ПЦР-бокс (например, «БАВ-ПЦР-«Ламинар-С», «Ламинарные системы», Россия).
4. Вортекс (например, «ТЭТА-2», «Биоком», Россия).

5. Набор электронных или механических дозаторов переменного объема (например, «Ленпипет», Россия).
6. Одноразовые наконечники с аэрозольным барьером до 200 мкл (например, «Axygen», США).
7. Штативы для наконечников (например, «Axygen», США) и микропробирок на 0,2 (0,5) мл (например, «ИнтерЛабСервис», Россия).
8. Холодильник от 2 до 8 °С с морозильной камерой не выше минус 16 °С.
9. Отдельный халат и одноразовые перчатки.
10. Емкость с дезинфицирующим раствором.

ПРОВЕДЕНИЕ ПЦР-АНАЛИЗА.

ЭТАП 1. ВЫДЕЛЕНИЕ ДНК ИЗ ПРОБ.

(проводится в ЗОНЕ 1 - помещении для обработки исследуемого материала).

Объем пробы, необходимый для выделения ДНК, – 0,1 мл.

Порядок работы.

1. Подготовить **отрицательный контроль выделения ДНК (ОК).** В пробирку объемом 1,5 мл внести **300 мкл лизирующего раствора и 100 мкл ОКО** – отрицательного контрольного образца**.**
2. Отдельными наконечниками с аэрозольным барьером внести в каждую пробирку с пробами (см. раздел «Обеззараживание материала»), включая **ОК**, по **10 мкл ВКО STI-704**.
3. Пробы тщательно перемешать на вортексе, прогреть 5 мин при температуре 65 °С, осадить на вортексе 5 с. Если в пробирках находятся взвешенные частицы (не растворившийся полностью материал), то необходимо процентрифугировать пробирку на микроцентрифуге 5 мин при 8-10 тыс об/мин (10-13 тыс об/мин при радиусе ротора 70 мм) и использовать для выделения ДНК надосадочную жидкость, перенеся ее в новую пробирку.
4. Тщательно ресуспендировать **сорбент универсальный** на вортексе. В каждую пробирку отдельным наконечником добавить по **25 мкл** ресуспендированного **сорбента универсального**. Перемешать на вортексе, поставить в штатив на 5 мин, еще раз перемешать и оставить в штативе

на 5 мин.

5. Осадить сорбент универсальный в пробирках центрифугированием при 8-10 тыс об/мин (10-13 тыс об/мин при радиусе ротора 70 мм) в течение 30 с. Удалить надосадочную жидкость, используя вакуумный отсасыватель и отдельный наконечник для каждой пробы.
6. Добавить в пробы по **300 мкл раствора для отмывки 1**, перемешать на вортексе до полного ресуспендирования сорбента, процентрифугировать 30 с при 8-10 тыс об/мин (10-13 тыс об/мин при радиусе ротора 70 мм) на микроцентрифуге. Удалить надосадочную жидкость, используя вакуумный вакуумный отсасыватель и отдельный наконечник для каждой пробы.
7. Добавить в пробы по **500 мкл раствора для отмывки 2**, перемешать на вортексе до полного ресуспендирования сорбента универсального, процентрифугировать 30 с при 8-10 тыс об/мин (10-13 тыс об/мин при радиусе ротора 70 мм) на микроцентрифуге. Удалить надосадочную жидкость, используя вакуумный отсасыватель и отдельный наконечник для каждой пробы.
8. Повторить отмывку раствором для отмывки 2, следуя п. **7**, удалить надосадочную жидкость полностью.
9. Поместить пробирки в термостат при температуре 65 °С на 5-10 мин для подсушивания сорбента универсального. При этом крышки пробирок должны быть открыты.
10. В пробирки добавить по **50 мкл ТЕ-буфера для элюции ДНК**. Перемешать на вортексе. Поместить в термостат при температуре 65 °С на 5 мин, периодически встряхивая на вортексе.
11. Процентрифугировать пробирки при 8-10 тыс об/мин (10-13 тыс об/мин при радиусе ротора 70 мм) в течение 1 мин на микроцентрифуге. Надосадочная жидкость содержит очищенную ДНК. Пробы готовы к постановке ПЦР.

Очищенную ДНК можно хранить в течение 1 нед при температуре от 2 до 8 °С и в течение года при температуре не выше минус 16 °С.

<u>ЭТАП 2.</u> ПРОВЕДЕНИЕ ПЦР-АМПЛИФИКАЦИИ И ДЕТЕКЦИИ ПРОДУКТОВ ПЦР-АМПЛИФИКАЦИИ.

(проводится в ЗОНЕ 2 - помещении для проведения ПЦР-амплификации).

Общий объем реакции - 25 мкл, объем ДНК-пробы - 10 мкл.

В комплекте реагентов применяется «горячий старт», который обеспечивается разделением нуклеотидов и Taq-полимеразы прослойкой воска. Плавление воска и перемешивание реакционных компонентов происходит только при температуре 95 °С, что значительно снижает количество неспецифически затравленных реакций.

<u>Вариант FEP.</u>

Порядок работы.

А. Подготовка пробирок для проведения ПЦР.

1. Отобрать необходимое количество пробирок с **ПЦР-смесью-1-FEP/FRT *Brucella* spp.** для амплификации ДНК исследуемых и контрольных проб.
2. На поверхность воска внести по **7 мкл ПЦР-смеси-2-FL**, при этом она не должна проваливаться под воск и смешиваться с **ПЦР-смесью-1-FEP/FRT *Brucella* spp**.
3. Сверху добавить по капле **минерального масла для ПЦР** (примерно 25 мкл).
4. Приготовить 2 образца **«Фон»**. Для этого в две пробирки с **ПЦР-смесью-1-FEP/FRT *Brucella* spp.** на поверхность воска внести **17 мкл ПЦР-смеси-Фон**, при этом она не должна проваливаться под воск и смешиваться с **ПЦР-смесью-1-FEP/FRT *Brucella* spp.** Сверху добавить по капле **минерального масла для ПЦР**.

Б. Проведение амплификации.

1. В подготовленные для ПЦР пробирки внести по **10 мкл ДНК-проб**, выделенных из исследуемых или контрольных проб этапа выделения ДНК.
2. Поставить **контрольные реакции амплификации**:
   а) **отрицательный контроль (К-) –** вместо ДНК-пробы внести в пробирку **10 мкл ДНК-буфера**.
   б) **положительный контроль (К+)** – внести в пробирку **10 мкл ПКО ДНК *Brucella*.**

3. Запустить на амплификаторе нужную программу (см. табл. 1). Когда температура в ячейках достигнет 95 °С (режим паузы), поместить пробирки в ячейки амплификатора, закрыть крышку прибора и снять программу с паузы.

Рекомендуется перед постановкой в амплификатор осадить капли со стенок пробирок кратким центрифугированием на вортексе (1-3 с).

Таблица 1.

Программа амплификации ДНК *Brucella* spp.

	Амплификаторы с активным регулированием (по раствору в пробирке):						Амплификаторы с матричным регулированием температуры: **«Uno-2» («Biometra»), «MiniCycler», «PTC-100» («MJ Research»)**		
	«GeneAmp PCR System 2400» («Applied Biosystems»), «Терцик» (точный алгоритм регулирования) («ДНК-технология»)			**«GeneAmp PCR System 2700» («Applied Biosystems»), «Gradient Palm Cycler» («Corbett Research»)**					
цикл	температура	время	циклы	температура	время	циклы	температура	время	циклы
0	**95 °С**	пауза		**93 °С**	пауза		**95 °С**	пауза	
1	**95 °С**	2 мин	1	**93 °С**	2 мин	1	**95 °С**	2 мин	1
2	**95 °С**	10 с	10	**93 °С**	10 с	10	**95 °С**	25 с	10
	65 °С	25 с		**65 °С**	25 с		**65 °С**	40 с	
	72 °С	10 с		**72 °С**	25 с		**72 °С**	25 с	
3	**95 °С**	10 с	35	**93 °С**	10 с	35	**95 °С**	25 с	35
	56 °С	25 с		**56 °С**	25 с		**56 °С**	40 с	
	72 °С	10 с		**72 °С**	25 с		**72 °С**	25 с	
4	**10 °С**	хранение		**10 °С**	хранение		**10 °С**	хранение	

4. По окончании выполнения программы амплификации приступить к детекции.

В. Детекция с помощью флуоресцентного ПЦР детектора «АЛА-1/4».

Установка параметров теста «Brucella».

1. Запустить программу «ALA_1» на компьютере, присоединенном к прибору.
2. В главном меню программы выбрать «Настройки» → «Тест».
3. Нажать кнопку «Новый» (в верхнем правом углу).
4. В открывшемся меню задать название теста «Brucella», нажать кнопку ОК.
5. В группе параметров «Каналы» отметить галочкой все задействованные в тесте каналы (FAM, HEX), в группе «ВКО» отметить канал, который используется для внутреннего контроля (FAM).

6. В полях «п-» и «п+» установить пороговые значения для отношения сигнал/фон по каналу для детекции специфической ДНК:
   HEX: «п-» = 2.5, «п+» = 3.0;
   В поле «ВКО/фон» задать пороговое значение отношения сигнала по каналу для детекции ВКО к фону:
   «ВКО/фон» = 2.5.
7. В группе параметров «Уровень фона» установить значения флуоресценции, допустимые для фоновых пробирок:
   FAM: = 100;
   HEX: = 50.
8. Ввести названия мишеней в блок параметров «Привязка каналов» и соотнести их с каналами детекции. Для этого напечатать название мишени в свободное поле и нажать клавишу «Добавить», при этом новая мишень появится в столбце уже существующих в памяти прибора мишеней. Название мишени в столбце «Привязка каналов» выделить курсором и нажать соответствующую ей кнопку канала для детекции:
   Brucella= HEX
9. Блокировать функцию «Доверительный интервал», установив в поле «Доверительный интервал», значение 555 %.
10. Нажать кнопку «Сохранить».

Измерение флуоресцентного сигнала.

1. Включить прибор и запустить программу «ALA_1» на компьютере, присоединенном к прибору.
2. Задать протокол измерения. Для этого в главном меню выбрать «Протокол» → «Создать новый» или «Открыть», чтобы открыть созданный ранее протокол.
3. В окне протокола необходимо выбрать тип используемого ротора *36 x 0,5* или *48 x 0,2*, ввести номер протокола, выбрать нужный тест («Brucella») в меню-вкладке «Тест» и ввести последовательность детектируемых образцов (в колонке «Образец»).
4. Обозначить образцы, которые являются фоновыми для данной группы образцов, как «фон» (используя сочетание клавиш «Ctrl» и «F»). В качестве образцов, обозначенных

«**ФОН**» использовать пробирки с образцами «**ФОН**».

5. Закрыть окно редактирования протокола, нажав на кнопку «Exit» в верхнем левом углу панели. Протокол сохранить.
6. Поставить пробирки в ячейки ротора в соответствии с заданной последовательностью и запустить детекцию, выбрав в меню «Протокол» → «Детекция» или значок «Детекция по протоколу» на панели инструментов (вверху экрана).

Учет результатов.

1. Полученные данные интерпретируются автоматически с помощью программы «ALA_1». Результаты в таблице представляются с помощью следующих обозначений:
   «обнаружено» – положительный результат;
   «не обнаружено» – отрицательный результат;
   «сомнительно» – результат, который нельзя однозначно интерпретировать (сигнал по каналу, отведенному для детекции специфической ДНК, превышает пороговое значение, допустимое для отрицательных образцов, но не превышает пороговое значение для положительных образцов (сигнал в так называемой «серой зоне»);
   «нд» – недостоверный результат (в образце не детектируется (не превышает заданного порогового значения) ни специфический сигнал, ни сигнал ВКО).
2. Результат считается достоверным только в случае прохождения положительных и отрицательных контролей амплификации и отрицательного контроля выделения ДНК (см. табл. 2).

Таблица 2.

Результаты постановки контролей различных этапов ПЦР-анализа

Контроль	Контролируемый этап ПЦР-анализа	Результат автоматической интерпретации	
		канал FAM	канал HEX
«ОК»	Выделение ДНК	ВКО+	«*Brucella* - не обнаружено»
«К-»	ПЦР	ВКО-	«*Brucella* - нд»
«К+»	ПЦР	ВКО-	«*Brucella* – обнаружено»

3. Образцы, для которых получен результат «**нд**» (кроме К-), требуют повторного проведения ПЦР и детекции. В случае если повторно получен результат «**нд**», требуется повторить

анализ образца, начиная с этапа выделения. Для образца «К-» результат «нд» является нормой.

4. Образцы, для которых получен результат «**сомнительно**», требуют повторного проведения ПЦР и детекции. В случае повторения аналогичного результата образцы считать положительными.
5. Отсутствие положительного сигнала в пробе с положительным контролем ПЦР может свидетельствовать о неправильно выбранной программе амплификации и о других ошибках, допущенных на этапе постановки ПЦР. В таком случае необходимо провести ПЦР еще раз.
6. Если в отрицательном контроле (ОК или К-) детектируется положительный сигнал**,** значит, произошла контаминация реактивов или проб. В этом случае результаты анализа по всем пробам считаются недействительными. Требуется повторить анализ проб, а также предпринять меры по выявлению источника контаминации.

Вариант FRT.

Порядок работы.

А. Подготовка пробирок для проведения ПЦР.

1. Отобрать необходимое количество пробирок с **ПЦР-смесью-1-FEP/FRT *Brucella* spp.** для амплификации ДНК исследуемых и контрольных проб.
2. На поверхность воска внести по **7 мкл ПЦР-смеси-2-FL**, при этом она не должна проваливаться под воск и смешиваться с **ПЦР-смесью-1-FEP/FRT *Brucella* spp.**

Б. Проведение амплификации.

1. В подготовленные для ПЦР пробирки внести по **10 мкл ДНК-проб**, выделенных из исследуемых или контрольных проб этапа выделения ДНК.
2. Поставить **контрольные реакции амплификации**:
   а) **отрицательный контроль (К-) –** вместо ДНК-пробы внести в пробирку **10 мкл ДНК-буфера**.
   б) **положительный контроль (К+)** – внести в пробирку **10 мкл ПКО ДНК *Brucella*.**
   в) **положительный контроль (ВК+) –** в подготовленные для ПЦР пробирки внести **10 мкл ПКО STI**.

В. Программирование амплификатора:

Для работы с прибором «Rotor-Gene» 3000 следует использовать программу Rotor-Gene версии 6, с прибором «Rotor-Gene» 6000- программу Rotor-Gene 6000 версии 1.7 (build 67) или выше.

Далее по тексту термины, соответствующие разным версиям приборов и программного обеспечения указаны в следующем порядке: для прибора «Rotor-Gene» 3000 / для англоязычной версии программы «Rotor-Gene» 6000 / для русскоязычной версии программы «Rotor-Gene» 6000.

1. Нажать кнопку «New»/«Новый» в основном меню программы.
2. В открывшемся окне выбрать меню «Advanced»/«Детальный мастер» и шаблон запуска эксперимента «Dual Labeled Probe»/«Hydrolysis probes»/«Флуоресцентные зонды (TaqMan)». Нажать кнопку «New»/«Новый».
3. Выбрать тип ротора «36-Well Rotor»/«36-луночный ротор». Поставить отметку в окне рядом с надписью «No Domed 0.2 ml Tubes»/«Locking ring attached»/«Кольцо закреплено».
4. Нажать кнопку «Next»/«Далее».
5. Выбрать объем реакционной смеси: Reaction volume/Объем реакции – 25 мкл. Для прибора «Rotor-Gene» 6000 должно быть активно (отмечено галочкой) окно «15 μl oil layer volume»/«15 μL объем масла/воска». (Если галочка не стоит в окне по умолчанию, поставить ее с помощью мышки).
6. Нажать кнопку «Next»/«Далее».
7. В верхней части окна нажать кнопку «Edit profile»/«Редактор профиля».
8. Задать следующие параметры эксперимента:
   1. Hold/Удерж. темп-ры 95 °C – 5 мин
   2. Cycling/Циклирование 95 °C – 10 с
      65 °C – 25 с
      72 °C -10 с
      Cycle repeats/Цикл повторить – 10 times/раз.
   3. Cycling2/Циклирование2 95 °C – 10 с
      56 °C – 25 с – Детекция
      72 °C -10 с

Cycle repeats/Цикл повторить – 35 times/раз.

4. Флюоресценцию измеряют при **56 °C** (во втором блоке циклирования) по каналам **FAM/Green, JOE/Yellow.**
5. Нажать кнопку «OK»/«Да».

9. В нижней части окна нажать кнопку «Calibrate»/«Gain Optimisation…»/«Опт.уровня сигн.». В открывшемся окне нажать кнопку «Calibrate Acquiring»/«Optimise Acquiring»/ «Опт.детек-мых». Для обоих красителей нужно указать в графе **Min Reading/Миним. Сигнал** значение **5**, а в графе **Max Reading/Максим. Сигнал** значение **10.** В графе «Tube position/Позиция Пробирки» указан номер пробирки, по которой будет автоматически выбран параметр «*gain*»/«усиление сигнала», по умолчанию это 1-я пробирка в роторе. Поэтому в 1-ой позиции в роторе должна ставиться пробирка с реакционной смесью. Пометить галочкой бокс в строке «Perform Calibration Before 1st Acquisition»/«Perform Optimisation Before 1st Acquisition»/«Выполнить оптимизацию при 1-м шаге детекции». Закрыть окно «Auto Gain Calibration Setup/Авто-оптимизация уровня сигнала», нажав кнопку «**Close»/«Закрыть»**. Нажать кнопку **«Next»/«Далее»**.
10. Поместить предварительно подготовленные пробирки в амплификатор. Запустить амплификацию кнопкой **«Start run»/«Старт»**.
11. Дать название эксперимента и сохранить его на диске (в этом файле будут автоматически сохранены результаты данного эксперимента).

В процессе работы амплификатора или по окончании его работы необходимо запрограммировать положение пробирок в карусели. Для этого надо использовать кнопку **«Edit samples»/«Правка образцов»** (в нижней правой части основного окна). Все пробы и контроли обозначить в меню **Samples/Образцы** как **Unknown/Образец**.

АНАЛИЗ РЕЗУЛЬТАТОВ.

Анализ результатов амплификации ВКО.

1. Нажать в меню кнопку «Analysis»/«Анализ», выбрать режим анализа «Quantitation»/«Количественный», нажать кнопку «Cycling A. FAM»/«Cycling A. Green», «Show»/«Показать».

2. Отменить автоматический выбор Threshold/Порог.
3. Выбрать линейную шкалу графического изображения результатов, нажав кнопку **Linear scale** в нижней части окна справа (если эта шкала активна по умолчанию, вместо кнопки Linear scale видна кнопка Log scale).
4. В меню основного окна (Quantitation analysis/Количественный анализ) должна быть нажата кнопка «Dynamic tube»/«Динамич.фон».
5. В меню основного окна «More settings»/«Outlier Removal»/«Устранение выбросов» установить значение NTC threshold/Порог Фона – ПФ (NTC) 0 %.
6. В меню «CT Calculation»/«Вычисление CT» (в правой части окна) выставить Threshold/Порог = 0.1.
7. В таблице результатов (окно «Quant. Results»/«Количественные Результаты») появятся значения Ct, которые должны быть не более 31 для исследуемых образцов и контролей.

Анализ результатов амплификации ДНК *Brucella*.

1. Нажать в меню кнопку «Analysis»/«Анализ», выбрать режим анализа «Quantitation»/«Количественный», нажать кнопку «Cycling A. JOE»/«Cycling A. Yellow», «Show»/«Показать».
2. Отменить автоматический выбор Threshold/Порог.
3. Выбрать линейную шкалу графического изображения результатов, нажав кнопку **Linear scale** в нижней части окна справа (если эта шкала активна по умолчанию, вместо кнопки Linear scale видна кнопка Log scale).
4. В меню основного окна (Quantitation analysis/Количественный анализ) должна быть нажата кнопка «Dynamic tube»/«Динамич.фон».
5. В меню основного окна «More settings»/«Outlier Removal»/«Устранение выбросов» установить значение NTC threshold/Порог Фона – ПФ (NTC) 10 %.
6. В меню «CT Calculation»/«Вычисление CT» выставить Threshold/Порог = 0.1.
7. В таблице результатов (окно «Quant. Results»/«Количественные Результаты») появятся значения Ct.

УЧЕТ РЕЗУЛЬТАТОВ.

Результаты интерпретируются на основании наличия (или отсутствия) пересечения кривой флуоресценции с установленной на соответствующем уровне пороговой линией (что соответствует наличию (или отсутствию) значения порогового цикла «Ct» в соответствующей графе в таблице результатов).

Результат считается достоверным только в случае прохождения положительных и отрицательных контролей амплификации и отрицательного контроля выделения ДНК (см. табл. 3).

Таблица 3.

Результаты постановки контролей различных этапов ПЦР-анализа

Контроль	Контролируемый этап ПЦР-анализа	Значение Ct по каналу	
		FAM/Green	JOE/Yellow
ОК	Выделение ДНК	< 31	Нет значений
К-	ПЦР	Нет значений	Нет значений
К+	ПЦР	Нет значений	< 33
ВК+	ПЦР	< 31	Нет значений

1. **Образец считают положительным**, если значение Ct на канале JOE/Yellow менее 33.
2. **Образец считают отрицательным**, если по каналу JOE/Yellow для него значение Ct отсутствует, а по каналу FAM/Green для него определено значение Ct, не превышающее 31.

Результаты не подлежат учету:

1. Отсутствие положительного сигнала в пробах с положительными контролями ПЦР может свидетельствовать о неправильно выбранной программе амплификации и о других ошибках, допущенных на этапе постановки ПЦР. В таком случае необходимо провести ПЦР еще раз.
2. Если значение Ct на канале JOE/Yellow больше 33, а значение Ct по каналу FAM/Green не превышает 31, требуется повторить ПЦР и считать его положительным в случае повторения результата или получения значения Ct на канале JOE/Yellow менее 33.

3. Образцы, для которых отсутствует значение Ct как по каналу JOE/Yellow, так и по каналу FAM/Green, или получено значение Ct по каналу FAM/Green более 31 требуют повторного проведения ПЦР и детекции. В случае, если повторно получен аналогичный результат, требуется повторить анализ образца, начиная с этапа выделения.
4. Появление любого значения Ct в таблице результатов для отрицательного контроля (на канале JOE/Yellow) и для отрицательного контроля ПЦР (ДНК-буфер) (на любом из каналов) свидетельствует о наличии контаминации реактивов или образцов. В этом случае результаты анализа по всем пробам считаются недействительными. Требуется повторить анализ всех проб, а также предпринять меры по выявлению и ликвидации источника контаминации.

ОБЕЗЗАРАЖИВАНИЕ.

1. Обеззараживание биоматериалов и реагентов проводят для каждой стадии отдельно, помещая одноразовую пластиковую посуду (пробирки, наконечники), колбы-ловушки вакуумных отсосов на 20-24 ч в специальные контейнеры, содержащие дезинфицирующий 0,2 % раствор ДП-2Т.

СРОК ГОДНОСТИ. УСЛОВИЯ ТРАНСПОРТИРОВАНИЯ И ХРАНЕНИЯ.

Срок годности. 6 мес. Набор реагентов с истекшим сроком годности применению не подлежит.

Транспортирование. Набор реагентов транспортировать при температуре от 2 до 8 °С не более 5 сут. При получении разукомплектовать в соответствии с указанными температурами хранения.

Хранение. Комплект реагентов «ДНК-сорб-В» хранить при температуре от 2 до 25 °С. Комплект реагентов «ПЦР-комплект» хранить при температуре от 2 до 8 °С в защищенном от света месте.

Условия отпуска. Для лечебно-профилактических и санитарно-профилактических учреждений.

Рекламации на качество набора реагентов **«АмплиСенс®** ***Brucella*** **spp.-FL»** направлять в адрес ФГУН ГИСК им. Л.А. Тарасевича Роспотребнадзора (119002, г. Москва, пер. Сивцев Вражек, д. 41, тел. (499) 241-39-22, факс (499) 241-92-38), в адрес предприятия-изготовителя ФГУН ЦНИИЭ Роспотребнадзора (111123, г. Москва, ул. Новогиреевская, д. 3а, тел. (495) 305-39-39, факс (495) 305-54-23) и в адрес официального дилера – компанию ООО «ИнтерЛабСервис» (тел. (495) 925-05-54, факс (495) 916-18-18, e-mail: products@pcr.ru).

Руководитель Производства
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Директор ФГУЗ Российского научно-исследовательского противочумного института «Микроб» Роспотребнадзора — Кутырев В.В.

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Зав. лабораторией препаратов против чумы и других особо опасных инфекций
ФГУН ГИСК им. Л.А.Тарасевича Роспотребнадзора — Саяпина Л.В.

«УТВЕРЖДАЮ»

Зам. директора Федерального бюджетного учреждения науки «Центральный научно-исследовательский институт эпидемиологии» Федеральной службы по надзору в сфере защиты прав потребителей и благополучия человека

_____ А.В. Горелов

«25» марта 2019 г.

ИНСТРУКЦИЯ

по применению набора реагентов для выявления ДНК *Legionella pneumophila* в биологическом материале и объектах окружающей среды методом полимеразной цепной реакции (ПЦР) с гибридизационно-флуоресцентной детекцией

«АмплиСенс® *Legionella pneumophila*-FL»

АмплиСенс®

ФБУН ЦНИИ Эпидемиологии
Роспотребнадзора,
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IVD

ОГЛАВЛЕНИЕ

ВАРИАНТЫ И ФОРМЫ ВЫПУСКА НАБОРА РЕАГЕНТОВ

Набор реагентов выпускается в двух вариантах

Вариант FEP

Для ПЦР-амплификации используется амплификатор, например, «Терцик» («ДНК-Технология», Россия); «Gradient Palm Cycler» («Corbett Research», Австралия).
Для детекции используется флуоресцентный ПЦР-детектор «АЛА-1/4» («BioSan», Латвия).

Набор реагентов выпускается в 4 формах комплектации:

Форма 1 включает комплекты реагентов «ДНК-сорб-В» вариант 50, «ПЦР-комплект» вариант FEP (пробирки 0,5 мл);

Форма 2 включает комплекты реагентов «ДНК-сорб-В» вариант 50, «ПЦР-комплект» вариант FEP (пробирки 0,2 мл);

Форма 3 включает комплект реагентов «ПЦР-комплект» вариант FEP (пробирки 0,5 мл);

Форма 4 включает комплект реагентов «ПЦР-комплект» вариант FEP (пробирки 0,2 мл).

ВНИМАНИЕ! Заявленные аналитические характеристики набора реагентов при работе с формами **3 и 4** гарантируются только в случае применения дополнительного комплекта реагентов «ДНК-сорб-В» производства ФБУН ЦНИИ Эпидемиологии Роспотребнадзора.

Вариант FRT

Для ПЦР-анализа используется амплификатор с системой детекции флуоресцентного сигнала в режиме «реального времени», например, «Rotor-Gene» 3000/6000 («Corbett Research», Австралия).

Набор реагентов выпускается в 2 формах комплектации:

Форма 1 включает комплекты реагентов «ДНК-сорб-В» вариант 50, «ПЦР-комплект» вариант скрин-титр-FRT;

Форма 2 включает комплект реагентов «ПЦР-комплект» вариант скрин-титр-FRT.

ВНИМАНИЕ! Заявленные аналитические характеристики набора реагентов при работе с формой **2** гарантируются только в случае применения дополнительного комплекта реагентов «ДНК-сорб-В» производства ФБУН ЦНИИ Эпидемиологии Роспотребнадзора.

СОСТАВ

Комплект реагентов «ДНК-сорб-В» вариант 50 – комплект реагентов для выделения ДНК из клинического материала **включает:**

Реактив	*Описание*	*Объем*	*Кол-во*
Лизирующий раствор	Прозрачная бесцветная жидкость[1]	15	1 флакон
Раствор для отмывки 1	Прозрачная бесцветная жидкость[1]	15	1 флакон
Раствор для отмывки 2	Прозрачная бесцветная жидкость	50	1 флакон
Сорбент универсальный	Суспензия белого цвета	1,25	1 пробирка
ТЕ-буфер для элюции ДНК	Прозрачная бесцветная жидкость	5,0	1 пробирка

Комплект реагентов рассчитан на выделение ДНК из 50 проб, включая контроли.

Комплект реагентов «ПЦР-комплект» вариант FEP – комплект реагентов для ПЦР-амплификации ДНК *Legionella pneumophila* с гибридизационно-флуоресцентной детекцией по «конечной точке» **включает:**

[1] При хранении лизирующего раствора и раствора для отмывки 1 при температуре от 2 до 8 °С возможно образование осадка в виде кристаллов.

Реактив	*Описание*	*Объем*	*Кол-во*
ПЦР-смесь-1-FEP/FRT *Legionella pneumophila*	Прозрачная бесцветная жидкость	0,008	55 пробирок объемом 0,5 или 0,2 мл
ПЦР-смесь-2-FL	Прозрачная бесцветная жидкость	0,77	1 пробирка
ПЦР-смесь-Фон	Прозрачная бесцветная жидкость	0,5	1 пробирка
Минеральное масло для ПЦР	Бесцветная вязкая жидкость	4,0	1 флакон
LS3	Прозрачная бесцветная жидкость	0,06	2 пробирки
ДНК-буфер	Прозрачная бесцветная жидкость	0,5	1 пробирка

Комплект реагентов рассчитан на проведение 55 реакций амплификации, включая контроли.

К комплекту реагентов прилагаются контрольные образцы этапа выделения:

Реактив	*Описание*	*Объем*	*Кол-во*
ОКО	Прозрачная бесцветная жидкость	1,6	2 пробирки
ВКО STI-338	Прозрачная бесцветная жидкость	0,5	1 пробирка
ПКО ДНК *Legionella pneumophila*	Прозрачная бесцветная жидкость	0,5	1 пробирка

Комплект реагентов «ПЦР-комплект» вариант скрин-титр-FRT – комплект реагентов для ПЦР-амплификации и количественного определения ДНК *Legionella pneumophila* с гибридизационно-флуоресцентной детекцией в режиме «реального времени» **включает:**

Реактив		*Описание*	*Объем*	*Кол-во*
ПЦР-смесь-1-FEP/FRT *Legionella pneumophila*		Прозрачная бесцветная жидкость	0,008	70 пробирок объемом 0,2 мл
ПЦР-смесь-2-FL		Прозрачная бесцветная жидкость	0,77	1 пробирка
ДНК-буфер		Прозрачная бесцветная жидкость	0,5	1 пробирка
ДНК-калибраторы	**LS1**	Прозрачная бесцветная жидкость	0,06	1 пробирка
	LS2	Прозрачная бесцветная жидкость	0,06	1 пробирка
	LS3	Прозрачная бесцветная жидкость	0,06	1 пробирка

Комплект реагентов рассчитан на проведение 70 реакций амплификации, включая контроли и калибраторы.

К комплекту реагентов прилагаются контрольные образцы этапа выделения:

Реактив	*Описание*	*Объем*	*Кол-во*
ОКО	Прозрачная бесцветная жидкость	1,6	2 пробирки
ВКО STI-338	Прозрачная бесцветная жидкость	0,5	1 пробирка
ПКО ДНК *Legionella pneumophila*	Прозрачная бесцветная жидкость	0,5	1 пробирка

К комплекту реагентов «ПЦР-комплект» вариант скрин-титр-FRT прилагается на цифровом носителе или находится на официальном сайте Изготовителя программное обеспечение в формате Microsoft® Excel для автоматической обработки результатов.

НАЗНАЧЕНИЕ

Настоящая инструкция распространяется на набор реагентов **«АмплиСенс® *Legionella pneumophila*-FL»**, предназначенный для выявления ДНК *Legionella pneumophila* в биологическом материале и объектах окружающей среды методом полимеразной цепной реакции (ПЦР) с гибридизационно-флуоресцентной детекцией.

Набор реагентов позволяет обнаруживать ДНК *Legionella pneumophila* в концентрации 1×10^3 копий/мл тестируемого биологического материала и концентратов воды.

ПРИНЦИП МЕТОДА

Метод качественного выявления ДНК *Legionella pneumophila* в клиническом материале основан на одновременной амплификации участка ДНК гена mip *Legionella pneumophila* и участка гена протромбина человека (эндогенный внутренний контроль). Результат амплификации ДНК *Legionella pneumophila* регистрируется по каналу флуоресценции JOE/Yellow/HEX, результат амплификации ДНК внутреннего контроля регистрируется по каналу FAM/Green. ДНК-мишень, выбранная в качестве внутреннего контроля, является участком генома человека и должна всегда присутствовать в образце в достаточном количестве, эквивалентном количеству клеток в образце (не менее 10^3 геномов), учитывая, что искомый возбудитель является внутриклеточным патогеном. Таким образом, эндогенный внутренний контроль позволяет не только контролировать этапы ПЦР-анализа (выделение ДНК и проведение ПЦР), но и оценивать адекватность забора материала и его хранения. В случаях, если в образце присутствует недостаточное количество клеток, сигнал гена протромбина будет слабым или совсем отсутствовать.

Метод качественного выявления ДНК *Legionella pneumophila* в объектах окружающей среды основан на одновременной амплификации участка ДНК гена mip *Legionella pneumophila* и участка ДНК неконкурентного внутреннего контроля (ВКО STI-338). Результат амплификации ДНК *Legionella pneumophila* регистрируется по каналу флуоресценции JOE/Yellow/HEX, результат амплификации ДНК внутреннего контроля регистрируется по каналу FAM/Green.

Метод количественного выявления ДНК *Legionella pneumophila* в воде (при использовании комплекта реагентов «ПЦР-комплект» вариант скрин-титр-FRT) основан на одновременной амплификации в режиме «реального времени» участка ДНК гена mip *Legionella pneumophila* и участка ДНК неконкурентного количественно охарактеризованного и стандартизованного внутреннего контрольного образца (ВКО STI-338). Результат амплификации ДНК *Legionella pneumophila* регистрируется по каналу флуоресценции JOE/Yellow, результат амплификации

ДНК внутреннего контрольного образца (ВКО STI-338) регистрируется по каналу FAM/Green.

Для определения количества копий ДНК *Legionella pneumophila* и ДНК внутреннего контрольного образца (ВКО STI-338) в реакционной пробирке используются количественно охарактеризованные калибраторы.

Учет потерь ДНК внутреннего контрольного образца позволяет рассчитать реальную концентрацию ДНК *Legionella pneumophila* в каждом исследуемом образце воды.

При выполнении расчетов **учитывается степень концентрирования воды**, в связи с этим **предварительную подготовку воды проводить строго по инструкции** к данному набору реагентов.

Расчет концентрации ДНК *Legionella pneumophila* (**С $_{ДНК\ Lp}$**) в 1 л воды проводится по формуле:

С $_{ДНК\ Lp}$ (копий / л) **= К $_{ДНК\ Lp}$ / К $_{ВКО\ STI-338}$* С $_{ВКО\ STI-338}$* 2, где**

С $_{ДНК\ Lp}$ (копий / л) = Количество копий ДНК *Legionella pneumophila* в 1 л образца воды,

К $_{ДНК\ Lp}$ (копий / мл) = Расчетное количество копий ДНК *Legionella pneumophila* в 1 мл тестируемой пробы,

К $_{ВКО\ STI-338}$ (копий / мл) = Расчетное количество копий ДНК $_{ВКО\ STI-338}$ в 1 мл внутреннего контрольного образца в тестируемой пробе,

С $_{ВКО\ STI-338}$ (копий / мл) = Количество копий ДНК ВКО STI-338 в 1 мл внутреннего контрольного образца (значение указано во вкладыше к набору реагентов),

2 – коэффициент пересчёта, учитывающий изменение объёмов при фильтрации.

ВНИМАНИЕ! Для проведения количественного исследования каждый образец воды необходимо тестировать в двух повторах (начиная с этапа экстракции ДНК), при этом результат выдаётся как среднее из двух полученных значений.

ОТБОР И ХРАНЕНИЕ ОБРАЗЦОВ

Для проведения анализа используется следующий материал

Клинический материал:

- Мокрота (индуцированная мокрота) или аспират из трахеи в одноразовых контейнерах после предварительной подготовки;
- Мазки со слизистой носоглотки и ротоглотки (в «Транспортной среде для хранения и транспортировки респираторных мазков» (РУ № ФСР 2009/05011)) без предварительной подготовки;
- Промывные воды бронхов (бронхоальвеолярный лаваж) в одноразовых контейнерах после предварительной подготовки;
- Секционный материал (фрагменты пораженной части легких) после предварительной подготовки.

Взятие материала со слизистой носоглотки.

Исследуемый материал из носоглотки берут сухими стерильными зондами с ватными тампонами. Зонд с ватным тампоном вводят легким движением по наружной стенке носа на глубину 2-3 см до нижней раковины. Затем зонд слегка опускают книзу, вводят в нижний носовой ход под нижнюю носовую раковину, делают вращательное движение и удаляют вдоль наружной стенки носа.

После забора материала тампон (рабочую часть зонда с ватным тампоном) помещают в стерильную одноразовую пробирку с 500 мкл транспортной среды для хранения и транспортировки респираторных мазков (либо стерильного 0,9 % раствора натрия хлорида или 0,01 М калий-фосфатного буфера, pH 7,0 (состав: $K_2HPO_4x3H_2O$ – 3,2 г, KH_2PO_4 – 1,4 г, вода дистиллированная – 1 л)). Конец зонда отламывают или отрезают, с расчетом, чтобы он позволил плотно закрыть крышку пробирки. Пробирку с раствором и рабочей частью зонда закрывают, и используют 50 мкл содержимого для последующего анализа.

Взятие материала со слизистой ротоглотки.

Исследуемый материал из ротоглотки берут сухими стерильными зондами с ватными тампонами вращательными движениями с поверхности миндалин, небных дужек и задней стенки ротоглотки после предварительного полоскания полости рта водой.

После забора материала тампон (рабочую часть зонда с ватным тампоном) помещают в стерильную одноразовую пробирку с 500 мкл транспортной среды для хранения и транспортировки респираторных мазков (либо стерильного 0,9 % раствора натрия хлорида или 0,01 М калий-фосфатного буфера, pH 7,0). Конец зонда отламывают или отрезают, с расчетом, чтобы он позволил плотно закрыть крышку пробирки. Пробирку с раствором и рабочей частью зонда закрывают, и используют 50 мкл содержимого для последующего анализа.

ВНИМАНИЕ! Рекомендуется совмещать исследуемый материал из носоглотки и ротоглотки в одной пробирке. Для этого рабочие концы зондов после взятия мазков у пациента помещаются в одну пробирку с 500 мкл транспортной среде для хранения и транспортировки респираторных мазков и исследуются как один образец.

Мазки со слизистой носоглотки и ротоглотки используются для исследования при легионеллезной инфекции, протекающей в форме ОРЗ (лихорадка Понтиак).

Культуры микроорганизмов, подозрительные на *Legionella* spp:

- Колонии ресуспендировать в 1 мл 0,9 % раствора натрия хлорида или 0,01 М калий-фосфатном-буфере, pH 7,0. Центрифугируют при 12000 об/мин в течение 15 мин. Надосадочную жидкость удаляют в емкость с дезинфицирующим раствором, осадок ресуспендируют в 50 мкл 0,9 % раствора натрия хлорида. Для исследования используют 50 мкл суспензии.

Допускается **хранение** вышеперечисленного **материала** до проведения исследования **в течение 1 сут** при температуре **от 2 до 8 °С, 1 мес** при температуре **не выше минус 16 °С**. Дальнейшее хранение материала возможно в течение года при температуре не выше минус 68 °С. Допускается однократное

замораживание-оттаивание материала.

Объекты окружающей среды:

Отбор проб осуществляют в соответствии с требованиями ГОСТ Р 51592-2000 «Вода. Общие требования к отбору проб» и ГОСТ Р 51593-2000 «Вода питьевая. Отбор проб»; МУК 4.2.1018-01 «Методические указания по санитарно-микробиологическому анализу питьевой воды»; МУК 4.2.1884-04 «Санитарно-микробиологический и санитарно-паразитологический анализ воды поверхностных водных объектов», **МУК 4.2.2217-07 «Выявление бактерий *Legionella pneumophila* в объектах окружающей среды».**

- **Вода** (сточная, из водоема, питьевая) в объеме 0,5 л после предварительной подготовки;
- **Смывы с объектов окружающей среды** берут зондами с тампонами, смоченными стерильным 0,9 % раствором натрия хлорида. Рабочую часть зонда с тампоном помещают в пробирку объёмом 1,5 мл с 0,5 мл стерильного 0,9 % раствора натрия хлорида, остальную часть зонда отламывают и удаляют. Для исследования используется 50 мкл раствора;
- **Соскобы биопленок** с внутренней поверхности водопроводного, промышленного и иного оборудования (например, из поддонов внутри кондиционеров). Соскобы влажных биопленок с поверхности, находящейся под водой или на границе соприкосновения воды и воздуха, берут сухим стерильным зондом (рабочая часть зонда с тампоном помещается в пробирку объёмом 1,5 мл с 0,5 мл стерильного 0,9 % раствора натрия хлорида, остальная часть зонда отламывается и удаляется). Для исследования используется 50 мкл раствора. Соскобы биопленок с высохшей поверхности берут тампонами, смоченными стерильным 0,9 % раствором натрия хлорида. Рабочая часть зонда с тампоном помещается в пробирку объёмом 1,5 мл с 0,5 мл стерильного 0,9 % раствора натрия хлорида, остальная часть зонда отламывается и удаляется. Для исследования используется 50 мкл раствора;

– **Почва** в количестве 100 г берётся в местах предполагаемого обсеменения и используется после предварительной подготовки.

Допускается **хранение** вышеперечисленного **материала** до проведения исследования **в течение 1 нед** при температуре не выше плюс 20 °С, **1 мес** при температуре **не выше минус 16 °С**. Допускается однократное замораживание-оттаивание материала. Температурный режим транспортирования не ограничен.

ПОДГОТОВКА ИССЛЕДУЕМОГО МАТЕРИАЛА

Все манипуляции, связанные с подготовкой проб, проводятся с использованием стерильных ступок, пестиков, инструментов (ножниц, пинцетов, скальпелей), дозаторов переменных объемов, одноразовых полипропиленовых пробирок на 1,5 мл и наконечников с фильтром. Одноразовая пластиковая посуда (пробирки, наконечники) должна сбрасываться в специальный контейнер, содержащий дезинфицирующий 0,2 % раствор ДП-2Т, и утилизироваться в соответствии с вышеуказанными документами.

Промывные воды бронхов (бронхоальвеолярный лаваж). Образец перемешивают переворачиванием в исходной емкости. Автоматическим дозатором, используя наконечник с фильтром, отбирают 1 мл образца и переносят в пробирку объемом 1,5 мл для проведения центрифугирования при 10000 об/мин в течение 10 мин. Надосадочную жидкость аккуратно отбирают, используя наконечник с фильтром, оставляя над осадком 100 мкл жидкости, в которой ресуспендируют осадок. Полученную суспензию (50 мкл) используют для последующей работы.

Мокрота. Дополнительно требуется реагент «Муколизин» производства ФБУН ЦНИИ Эпидемиологии Роспотребнадзора. Сбор и предобработка материала выполняется по инструкции к реагенту «Муколизин». Подготовленную мокроту (50 мкл) используют для последующей работы. При необходимости повторного проведения анализа остаток мокроты замораживают при температуре **не выше минус 16 °С**.

Секционный материал гомогенизируют с использованием стерильных фарфоровых ступок и пестиков, затем готовят 10 % суспензию на стерильном 0,9 % растворе натрия хлорида или 0,01 М калий-фосфатном буфере, pH 7,0. Суспензию переносят в пробирку на 1,5 мл и позволяют отстояться осадку в течение 1-3 мин. Надосадочную жидкость (50 мкл) используют для последующей работы. При необходимости повторного проведения анализа остаток суспензии замораживают при температуре **не выше минус 16 °С**.

Подготовка проб объектов окружающей среды проводится согласно **МУК 4.2.2217-07 «Выявление бактерий *Legionella pneumophila* в объектах окружающей среды».**

Подготовка образцов воды:

Воду (0,5 л) предварительно фильтруют с помощью стеклянной воронки через бумажный фильтр. После предварительной фильтрации воду пропускают через мембранный фильтр с диаметром пор не более 0,45 мкм. После окончания фильтрации мембранный фильтр измельчают стерильными (обожжёнными в пламени горелки) ножницами (в одноразовую чашку Петри) и помещают стерильным (обожжённым в пламени горелки) пинцетом в пробирки объемом 1,5 мл с 1 мл 0,9 % раствора натрия хлорида. Пробирку инкубируют при комнатной температуре в течение 15-20 мин, периодически перемешивая на вортексе, чтобы обеспечить переход микрофлоры в раствор. Раствор (50 мкл) используют для последующей работы. Фильтрат хранят при температуре **от 2 до 8 °С в течение 1 нед. При необходимости более длительного хранения фильтрат замораживают при температуре не выше минус 16 °С**.

Подготовка проб почвы:

В пробирки на 5 мл с плотно закрывающейся (завинчивающейся) крышкой отдельным шпателем (или одноразовыми лопатками) внести по 0,4–1,0 г (около 1,0 мл) земли. В каждую пробирку внести по 3 мл 0,9 % раствора натрия хлорида, тщательно перемешивают и декантируют 5 мин. Надосадочная жидкость (50 мкл) используется для последующей работы.

ОБЕЗЗАРАЖИВАНИЕ МАТЕРИАЛА

Проводят согласно МУ 1.3.2569-09 «Организация работы лабораторий, использующих методы амплификации нуклеиновых кислот при работе с материалом, содержащим микроорганизмы I–IV групп патогенности».

1. Лизирующий раствор из комплекта реагентов «ДНК-сорб-В» (если он хранился при температуре от 2 до 8 °С) прогреть при температуре от 60 до 65 °С до полного растворения кристаллов.
2. К 50 мкл подготовленных образцов (см. раздел «Подготовка исследуемого материала») добавляют 50 мкл ОКО (отрицательного контрольного образца), тщательно перемешивают и добавляют в них отдельным наконечником 300 мкл лизирующего раствора, прогревают при температуре (65±1) °С в течение 15 мин.

Дальнейшие исследования проб проводить как с обеззараженным материалом по порядку процедур, описанных в разделе «Выделение ДНК из исследуемого материала».

МЕРЫ ПРЕДОСТОРОЖНОСТИ И СВЕДЕНИЯ ОБ УТИЛИЗАЦИИ

Работа должна проводиться в лаборатории, выполняющей молекулярно-биологические (ПЦР) исследования клинического материала на наличие возбудителей инфекционных болезней, с соблюдением санитарно-эпидемиологических правил СП 1.3.2322-08 «Безопасность работы с микроорганизмами III - IV групп патогенности (опасности) и возбудителями паразитарных болезней», СанПиН 2.1.7.2790-10 «Санитарно-эпидемиологические требования к обращению с медицинскими отходами» и методических указаний МУ 1.3.2569-09 «Организация работы лабораторий, использующих методы амплификации нуклеиновых кислот при работе с материалом, содержащим микроорганизмы I–IV групп патогенности».

При работе необходимо всегда выполнять следующие требования:

1. Температура в помещении лаборатории от 20 до 28 °С, относительная влажность от 15 до 75%.

2. Рассматривать исследуемые образцы как инфекционно-опасные, организовывать работу и хранение в соответствии с СП 1.3.2322-08 «Безопасность работы с микроорганизмами III–IV групп патогенности (опасности) и возбудителями паразитарных болезней».
3. Убирать и дезинфицировать разлитые образцы, используя дезинфицирующие средства в соответствии с СП 1.3.2322-08 «Безопасность работы с микроорганизмами III–IV групп патогенности (опасности) и возбудителями паразитарных болезней».
4. Лабораторный процесс должен быть однонаправленным. Анализ проводится в отдельных помещениях (зонах). Работу следует начинать в Зоне Экстракции, продолжать в Зоне Амплификации и Детекции. Не возвращать образцы, оборудование и реагенты в зону, в которой была проведена предыдущая стадия процесса.
5. Неиспользованные реагенты, реагенты с истекшим сроком годности, а также использованные реагенты, упаковку[2], биологический материал, включая материалы, инструменты и предметы, загрязненные биологическим материалом, следует удалять в соответствии с требованиями СанПиН 2.1.7.2790-10 «Санитарно-эпидемиологические требования к обращению с медицинскими отходами»

ВНИМАНИЕ! При удалении отходов после амплификации (пробирок, содержащих продукты ПЦР) недопустимо открывание пробирок и разбрызгивание содержимого, поскольку это может привести к контаминации продуктами ПЦР лабораторной зоны, оборудования и реагентов.

6. Использовать и менять при каждой операции одноразовые наконечники для автоматических дозаторов с фильтром[3]. Одноразовую пластиковую посуду (пробирки, наконечники) необходимо сбрасывать в специальный контейнер, содержащий дезинфицирующее средство, которое может быть использовано для обеззараживания медицинских отходов.

[2] Неиспользованные реагенты, реагенты с истекшим сроком годности, использованные реагенты, упаковка относятся к классу опасности медицинских отходов Г.

[3] Для удаления надосадочной жидкости с помощью вакуумного отсасывателя используются одноразовые наконечники без фильтра.

7. Поверхности столов, а также помещения, в которых проводится постановка ПЦР, до начала и после завершения работ необходимо подвергать ультрафиолетовому облучению в течение 30 мин.
8. Набор реагентов предназначен для одноразового применения для проведения ПЦР-исследования указанного количества проб (см. раздел «Состав»).
9. Набор реагентов готов к применению согласно данной инструкции. Применять набор реагентов строго по назначению.
10. К работе с набором реагентов допускается только персонал, обученный методам молекулярной диагностики и правилам работы в клинико-диагностической лаборатории в установленном порядке (СП 1.3.2322-08 «Безопасность работы с микроорганизмами III–IV групп патогенности (опасности) и возбудителями паразитарных болезней»).
11. Не использовать набор реагентов, если нарушена внутренняя упаковка или внешний вид реагента не соответствует описанию.
12. Не использовать набор реагентов, если не соблюдались условия транспортирования и хранения согласно инструкции.
13. Не использовать набор реагентов по истечении срока годности.
14. Использовать одноразовые неопудренные перчатки, лабораторные халаты, защищать глаза во время работы с образцами и реагентами. Тщательно вымыть руки по окончании работы. Все операции проводятся только в перчатках для исключения контакта с организмом человека.
15. Избегать вдыхания паров, контакта с кожей, глазами и слизистой оболочкой. Вреден при проглатывании. При контакте немедленно промыть пораженное место водой, при необходимости обратиться за медицинской помощью.
16. При соблюдении условий транспортировки, эксплуатации и хранения риски взрыва и возгорания отсутствуют.
17. Листы безопасности реагентов (SDS – safety data sheet) доступны по запросу.

Оценка вероятных событий, в результате наступления которых могут произойти отрицательные последствия для организма человека (для форм комплектации, не включающих комплект реагентов «ДНК-сорб-В»).

При использовании по назначению и соблюдении вышеперечисленных мер предосторожности набор реагентов безопасен.

Оценка вероятных событий, в результате наступления которых могут произойти отрицательные последствия для организма человека (для формы комплектации, включающей комплект реагентов «ДНК-сорб-В»).

При использовании по назначению и соблюдении вышеперечисленных мер предосторожности контакт с организмом человека исключен. При аварийных ситуациях возможно следующее:

- раздражение слизистой оболочки глаз у чувствительных лиц,
- раздражение кожи у чувствительных лиц,
- аллергическая реакция,
- вред при вдыхании,
- вред при приеме внутрь.

Специфические воздействия набора реагентов на организм человека (для всех форм комплектации):

- Мутагенное действие отсутствует.
- Репродуктивная токсичность отсутствует.

ДОПОЛНИТЕЛЬНЫЕ МАТЕРИАЛЫ И ОБОРУДОВАНИЕ, ТРЕБУЕМЫЕ ДЛЯ ПРОВЕДЕНИЯ ПЦР-АНАЛИЗА (с указанием фирм изготовителей/поставщиков):

ЗОНА 1

Для выделения ДНК из исследуемого материала требуются:

1. Стерильный ламинарный шкаф (например, «БАВп-01-«Ламинар-С»-1,2», «Ламинарные системы», Россия).
2. Термостат для пробирок типа «Эппендорф» от 25 до 100 °С (например, «ТЕРМО 24-15», «Биоком», Россия).
3. Микроцентрифуга для пробирок типа «Эппендорф» до 16 тыс об/мин (например, «MiniSpin», «Eppendorf», Германия).
4. Вакуумный отсасыватель медицинский с колбой-ловушкой для удаления надосадочной жидкости (например, «ОМ-1», г.

Ульяновск, Россия).
5. Вортекс (например «ТЭТА-2», «Биоком», Россия).
6. Отдельный набор автоматических дозаторов переменного объема (например, «Ленпипет», Россия).
7. Одноразовые полипропиленовые завинчивающиеся или плотно закрывающиеся микропробирки объемом 1,5 мл (например, «Axygen», США).
8. Штативы для микропробирок объемом 1,5 мл (например, «ИнтерЛабСервис», Россия) и наконечников (например, «Axygen», США).
9. Одноразовые наконечники с фильтром для дозаторов переменного объема до 200 мкл и до 1000 мкл (например, «Axygen», США).
10. Одноразовые наконечники для дозаторов переменного объема до 200 мкл (например, «Axygen», США).
11. Холодильник от 2 до 8 °С с морозильной камерой не выше минус 16 °С.
12. Отдельный халат и одноразовые перчатки.
13. Емкость с дезинфицирующим раствором.

ЗОНА 2

Для проведения ПЦР-амплификации и гибридизационно-флуоресцентной детекции продуктов ПЦР-амплификации требуются:

(с указанием фирм изготовителей / поставщиков):

Вариант FEP: амплификатор для микропробирок 0,5 мл (например, «Терцик», «ДНК-Технология», Россия или эквивалентный); для микропробирок 0,2 мл (например, «Gradient Palm Cycler», «Corbett Research», Австралия или эквивалентный). Флуоресцентный ПЦР-детектор, например, «АЛА-1/4» («BioSan», Латвия), «Джин» («ДНК-Технология», Россия) или эквивалентный.

Вариант FRT: амплификатор роторного типа, например, «Rotor-Gene» 3000/6000 («Corbett Research», Австралия) или эквивалентный.

1. ПЦР-бокс (например, «БАВ-ПЦР-«Ламинар-С», «Ламинарные системы», Россия).
2. Для приборов для ПЦР в режиме «реального времени» с детекцией через дно пробирки (например, «Rotor-Gene»).

Одноразовые полипропиленовые микропробирки для ПЦР на 0,2 мл (плоская крышка, нестрипованные), (например, «Axygen», США) для постановки в ротор на 36 пробирок.
Для амплификаторов, адаптированных для ПЦР-пробирок 0,2 мл («Gradient Palm Cycler», «GeneAmp PCR System 2700» и др.). Одноразовые полипропиленовые микропробирки для ПЦР на 0,2 мл (плоская крышка, нестрипованные) (например, «Axygen», США).
Для амплификаторов, адаптированных для ПЦР-пробирок 0,5 мл («Терцик» и др.). Одноразовые полипропиленовые микропробирки для ПЦР на 0,5 мл (плоская крышка, нестрипованные) (например, «Axygen», США).

3. ПЦР-бокс (например, «БАВ-ПЦР-«Ламинар-С», «Ламинарные системы», Россия).
4. Вортекс (например, «ТЭТА-2», «Биоком», Россия).
5. Отдельный набор автоматических дозаторов переменного объема (например, «Ленпипет», Россия).
6. Одноразовые наконечники с фильтром до 200 мкл (например, «Axygen», США).
7. Штативы для наконечников (например, «Axygen», США) и микропробирок на 0,2 (0,5) мл (например, «ИнтерЛабСервис», Россия).
8. Холодильник от 2 до 8 °С с морозильной камерой не выше минус 16 °С.
9. Отдельный халат и одноразовые перчатки.
10. Емкость с дезинфицирующим раствором.

ПРОВЕДЕНИЕ ПЦР-АНАЛИЗА

ЭТАП 1. ВЫДЕЛЕНИЕ ДНК ИЗ ИССЛЕДУЕМОГО МАТЕРИАЛА (проводится в ЗОНЕ 1 - помещении для обработки исследуемого материала)

Порядок работы

1. Подготовить пробирку **отрицательный контроль выделения ДНК (ОК).** В пробирку объемом 1,5 мл внести **300 мкл лизирующего раствора и 100 мкл ОКО** и **10 мкл ВКО STI-338.**
2. Подготовить пробирку **положительный контроль выделения ДНК (ПК)**. В пробирку объемом 1,5 мл внести

300 мкл лизирующего раствора, **50 мкл ОКО, 10 мкл ВКО STI-338** и **50 мкл ПКО ДНК *Legionella pneumophila***.

3. В подготовленные пробы из объектов окружающей среды и культур микроорганизмов (см. раздел «Обеззараживание материала») отдельными наконечниками с фильтром внести по **10 мкл ВКО-STI-338.** В подготовленные пробы клинического материала (см. раздел «Обеззараживание материала») реагент ВКО не добавлять. Промаркировать пробирки. Перемешивают содержимое микропробирок на вортексе и центрифугируют при 2000-3000 об/мин, чтобы удалить капли с крышек микропробирок.
4. Пробы тщательно перемешать на вортексе, прогреть 5 мин при 65 °С, осадить на вортексе 5 с. Если в пробирках находятся взвешенные частицы (не растворившийся полностью материал) то необходимо процентрифугировать пробирку на микроцентрифуге 5 мин при 8-10 тыс об/мин (10-13 тыс об/мин при радиусе ротора 70 мм) и использовать для выделения ДНК надосадочную жидкость, перенеся ее в новую пробирку.
5. Тщательно ресуспендировать **сорбент универсальный** на вортексе. В каждую пробирку отдельным наконечником добавить по **25 мкл** ресуспендированного **сорбента универсального**. Перемешать на вортексе, поставить в штатив на 5 мин, еще раз перемешать и оставить в штативе на 5 мин.
6. Осадить сорбент универсальный в пробирках центрифугированием при 8-10 тыс об/мин (10-13 тыс об/мин при радиусе ротора 70 мм) в течение 30 с. Удалить надосадочную жидкость, используя вакуумный отсасыватель и отдельный наконечник для каждой пробы.
7. Добавить в пробы по **300 мкл раствора для отмывки 1**, перемешать на вортексе до полного ресуспендирования сорбента, процентрифугировать 30 с при 8-10 тыс об/мин (10-13 тыс об/мин при радиусе ротора 70 мм) на микроцентрифуге. Удалить надосадочную жидкость, используя вакуумный отсасыватель и отдельный наконечник для каждой пробы.
8. Добавить в пробы по **500 мкл раствора для отмывки 2**,

перемешать на вортексе до полного ресуспендирования сорбента универсального, процентрифугировать 30 с при 8-10 тыс об/мин (10-13 тыс об/мин при радиусе ротора 70 мм) на микроцентрифуге. Удалить надосадочную жидкость, используя вакуумный отсасыватель и отдельный наконечник для каждой пробы.

9. Повторить отмывку еще раз, следуя пункту **8**, удалить надосадочную жидкость полностью.
10. Поместить пробирки в термостат при температуре 65 °С на 5-10 мин для подсушивания сорбента универсального. При этом крышки пробирок должны быть открыты.
11. В пробирки добавить по **50 мкл ТЕ-буфера для элюции ДНК**. Перемешать на вортексе. Поместить в термостат при температуре 65 °С на 5 мин, периодически встряхивая на вортексе.
12. Процентрифугировать пробирки при 8-10 тыс об/мин (10-13 тыс об/мин при радиусе ротора 70 мм) в течение 1 мин на микроцентрифуге. Надосадочная жидкость содержит очищенную ДНК. Пробы готовы к постановке ПЦР.

Очищенную ДНК можно хранить в течение 1 нед при температуре от 2 до 8 °С и в течение года при температуре не выше минус 16 °С.

ЭТАП 2. ПРОВЕДЕНИЕ ПЦР-АМПЛИФИКАЦИИ И ДЕТЕКЦИИ ПРОДУКТОВ ПЦР-АМПЛИФИКАЦИИ

(проводится в ЗОНЕ 2 - помещении для проведения ПЦР-амплификации)

Общий объем реакции - 25 мкл, объем ДНК-пробы - 10 мкл.

ВНИМАНИЕ! Перед работой необходимо ознакомиться с инструкциями к соответствующим приборам и «Методическими Рекомендациями по применению набора реагентов для выявления ДНК *Legionella pneumophila* в биологическом материале и объектах окружающей среды методом полимеразной цепной реакции (ПЦР) с гибридизационно-флуоресцентной детекцией «АмплиСенс® *Legionella pneumophila*-FL», утвержденные директором ФБУН ЦНИИ Эпидемиологии Роспотребнадзора».

В комплекте реагентов применяется «горячий старт», который обеспечивается разделением нуклеотидов и Taq-полимеразы прослойкой воска. Плавление воска и перемешивание реакционных компонентов происходит только при 95 °С, что значительно снижает количество неспецифически затравленных реакций.

Вариант FEP

Порядок работы

А. Подготовка пробирок для проведения ПЦР

1. Отобрать необходимое количество пробирок с **ПЦР-смесью-1-FEP/FRT *Legionella pneumophila*** для амплификации ДНК исследуемых и контрольных проб.

ВНИМАНИЕ! Выбор пробирок для амплификации зависит от используемых приборов:

- пробирки объемом **0,2 мл** для амплификаторов «GeneAmp PCR System 2700» («Applied Biosystems»), «Gradient Palm Cycler» («Corbett Research»), «Uno-2» («Biometra»).
- пробирки объемом **0,5 мл** для амплификаторов «Терцик» («ДНК-Технология»), «Uno-2» («Biometra»).

2. На поверхность воска внести по **7 мкл ПЦР-смеси-2-FL**, при этом она не должна проваливаться под воск и смешиваться с ПЦР-смесью-1-FEP/FRT *Legionella pneumophila*.
3. Сверху добавить по капле **минерального масла для ПЦР**[4] (примерно 25 мкл).
4. Приготовить 2 контрольных образца **«Фон»**. Для этого в две пробирки с **ПЦР-смесью-1-FEP/FRT *Legionella pneumophila*** на поверхность воска внести **17 мкл ПЦР-смеси-Фон**, при этом она не должна проваливаться под воск и смешиваться с ПЦР-смесью-1-FEP/FRT *Legionella pneumophila*. Сверху добавить каплю **минерального масла для ПЦР**.

Б. Проведение амплификации

1. В подготовленные для ПЦР пробирки внести отдельными наконечниками с фильтром по **10 мкл ДНК-проб**, выделенных из исследуемых или контрольных проб этапа

[4] При использовании амплификатора с термостатируемой крышкой минеральное масло для ПЦР можно не применять.

выделения ДНК.

2. Поставить **контрольные реакции амплификации**:
   а) **отрицательный контроль (К-) –** в подготовленную для ПЦР пробирку внести **10 мкл ДНК-буфера.**
   б) **положительный контроль (К+)** – в подготовленную для ПЦР пробирку внести **10 мкл LS3**.
3. Запустить на амплификаторе нужную программу (см. табл. 1).

Программы амплификации для приборов разных изготовителей см. также в «Методических Рекомендациях по применению набора реагентов для выявления ДНК *Legionella pneumophila* в биологическом материале и объектах окружающей среды методом полимеразной цепной реакции (ПЦР) с гибридизационно-флуоресцентной детекцией «АмплиСенс® *Legionella pneumophila*-FL», утвержденными директором ФБУН ЦНИИ Эпидемиологии Роспотребнадзора», в которых уточняются параметры программирования соответствующего амплификатора и проведения детекции с использованием программного обеспечения флуоресцентного ПЦР-детектора.

4. Когда температура в ячейках достигнет 95 °С (режим паузы) поставить пробирки в ячейки амплификатора и нажать кнопку продолжения программы. Рекомендуется перед постановкой в амплификатор осадить капли со стенок пробирок кратким центрифугированием на вортексе (1-3 с).

Таблица 1

Программа амплификации ДНК *Legionella pneumophila*

	Амплификатор с активным регулированием (по раствору в пробирке)[5]		
цикл	температура,°С	время	циклы
0	**95**	пауза	
1	**95**	2 мин	1
2	**95**	10 с	10
	60	20 с	
	72	10 с	
3	**95**	10 с	35
	56	20 с	
	72	10 с	
4	**10**	хранение	

[5] Например, «Терцик» («ДНК-Технология»).

Вариант FRT

Порядок работы

А. Подготовка пробирок для проведения ПЦР

1. Отобрать необходимое количество пробирок с **ПЦР-смесью-1-FEP/FRT *Legionella pneumophila*** для амплификации ДНК исследуемых, контрольных проб и калибраторов.
2. На поверхность воска внести по **7 мкл ПЦР-смеси-2-FL**, при этом она не должна проваливаться под воск и смешиваться с ПЦР-смесью-1-FEP/FRT *Legionella pneumophila*.

Б. Проведение амплификации

1. В подготовленные для ПЦР пробирки внести по **10 мкл ДНК-проб**, выделенных из исследуемых или контрольных проб этапа выделения ДНК.
2. Поставить **контрольные реакции амплификации**:

   При проведении качественного анализа:

   а) отрицательный контроль (К-) – в подготовленную для ПЦР пробирку внести **10 мкл ДНК-буфера**.

   б) положительный контроль (К+) – в подготовленную для ПЦР пробирку внести **10 мкл LS3**.

   При проведении количественного анализа:

   а) отрицательный контроль (К-) – в подготовленную для ПЦР пробирку внести **10 мкл ДНК-буфера**.

   б) калибраторы – в подготовленные для ПЦР три пробирки внести по **10 мкл** каждого ДНК-калибратора **(LS1**, **LS2** или **LS3)**.
3. Запрограммировать прибор для выполнения соответствующей программы амплификации и детекции флуоресцентного сигнала согласно описанию для данного прибора (см. табл. 2).

Программирование амплификатора см. также в «Методических Рекомендациях по применению набора реагентов для выявления ДНК *Legionella pneumophila* в биологическом материале и объектах окружающей среды методом полимеразной цепной реакции (ПЦР) с гибридизационно-флуоресцентной детекцией «АмплиСенс® *Legionella pneumophila*-FL» ФБУН ЦНИИ Эпидемиологии Роспотребнадзора».

Таблица 2

Программа амплификации для приборов роторного типа[6]

Этап	Температура, °С	Продолжительность этапа	Измерение флуоресценции	Количество циклов
1	95	5 мин	–	1
2	95	10 с	–	10
	60	20 с	–	
	72	10 с	–	
3	95	10 с	–	35
	56	20 с	FAM/Green, JOE/Yellow	
	72	10 с	–	

ИНТЕРПРЕТАЦИЯ РЕЗУЛЬТАТОВ

Результаты интерпретируются в соответствии с «Методическими Рекомендациями по применению набора реагентов для выявления ДНК *Legionella pneumophila* в биологическом материале и объектах окружающей среды методом полимеразной цепной реакции (ПЦР) с гибридизационно-флуоресцентной детекцией «АмплиСенс® *Legionella pneumophila*-FL» ФБУН ЦНИИ Эпидемиологии Роспотребнадзора».

Качественный анализ (вариант FEP)

Полученные результаты интерпретируют на основании данных об уровне флуоресцентного сигнала в исследуемых образцах относительно уровня сигнала в образцах «фон». Интерпретация происходит автоматически с помощью программного обеспечения используемого флуоресцентного ПЦР-детектора. Для интерпретации результата по каждому каналу детекции используются установленные пороговые значения. Детекция ДНК *Legionella pneumophila* проводится по каналу HEX (или аналогичному, в зависимости от модели прибора). Детекция ДНК ВКО проводится по каналу FAM (или аналогичному, в зависимости от модели прибора). Результат считается достоверным только в случае прохождения положительных и отрицательных контролей выделения ДНК и амплификации.

[6] Например, «Rotor-Gene» 3000 и «Rotor-Gene» 6000 («Corbett Research», Австралия).

Образцы, для которых получен отрицательный результат по всем каналам (кроме К-), требуют повторного проведения ПЦР и детекции. В случае если данный результат получен повторно, требуется повторить анализ образца, начиная с этапа выделения. Для образца «К-» отрицательный результат по всем каналам является нормой.

Образцы, для которых ВКО положительный, а значения специфического сигнала лежат в диапазоне значений выше отрицательного порога, но не превышают положительного порога для данного канала, требуют повторного проведения ПЦР и детекции. В случае повторения аналогичного результата образцы считать положительными. При получении при повторной постановке отрицательного результата образец считать сомнительным и рекомендовать повторный забор материала для анализа.

Отсутствие положительного сигнала в пробе с положительным контролем ПЦР может свидетельствовать о неправильно выбранной программе амплификации и о других ошибках, допущенных на этапе постановки ПЦР. В таком случае необходимо провести ПЦР еще раз.

Если в отрицательном контроле (ОК или К-) детектируется положительный сигнал, значит, произошла контаминация реактивов или проб. В этом случае результаты анализа по всем пробам считаются недействительными. Требуется повторить анализ проб, а также предпринять меры по выявлению источника контаминации.

Качественный анализ (вариант FRT)

Полученные результаты интерпретируются на основании наличия (или отсутствия) пересечения кривой флуоресценции с установленной на соответствующем уровне пороговой линией (что соответствует наличию (или отсутствию) значения порогового цикла (*Ct*) в соответствующей графе в таблице результатов).

Детекция ДНК *Legionella pneumophila* проводится по каналу Yellow (JOE или аналогичному, в зависимости от модели прибора). Детекция ДНК ВКО проводится по каналу Green (FAM или аналогичному, в зависимости от модели прибора).

Результат считается достоверным только в случае

прохождения положительных и отрицательных контролей выделения ДНК и амплификации. Результаты анализа не подлежат учету, если в таблице результатов для отрицательного контроля выделения на канале Yellow и для отрицательного контроля ПЦР на любом из каналов появляется любое значение (*Ct*), что свидетельствует о наличии контаминации реактивов или образцов. В этом случае результаты анализа по всем пробам считаются недействительными. Требуется повторить анализ всех проб, а также предпринять меры по выявлению и ликвидации источника контаминации.

Количественный анализ (вариант FRT)

При проведении количественного анализа используют значения концентраций калибраторов ДНК *Legionella pneumophila* и калибраторов ДНК ВКО-STI-338 указанные во вкладыше к набору реагентов. Расчет количества копий ДНК *Legionella pneumophila* в 1 мл тестируемой пробы проводится автоматически программой прибора по заданным значениям калибраторов, и полученное значение появляется в соответствующей графе в таблице результатов.

Расчет концентрации ДНК *Legionella pneumophila* в 1 л воды (С $_{\text{ДНК Lp}}$ (копий / л)) проводят вручную или с использованием программного обеспечения, прилагающегося к комплекту реагентов, по следующей формуле:

С $_{\text{ДНК Lp}}$ (копий / л) **= К $_{\text{ДНК Lp}}$ / К $_{\text{ВКО-STI-338}}$** * **С $_{\text{ВКО-STI-338}}$** * **2,** где

К $_{\text{ДНК Lp}}$ (копий / мл) = Расчетное количество копий ДНК *Legionella pneumophila* в 1 мл тестируемой пробы,

К $_{\text{ВКО-STI-338}}$ (копий / мл) = Расчетное количество копий ДНК ВКО-STI-338 в 1 мл внутреннего контрольного образца в тестируемой пробе,

С $_{\text{ВКО-STI-338}}$ (копий / мл) = Количество копий ДНК ВКО-STI-338 в 1 мл внутреннего контрольного образца (значение указано во вкладыше к набору реагентов),

2 – коэффициент пересчёта.

Количественный анализ является достоверным, если полученное значение расчетной концентрации контрольной пробы «ПК» *Legionella pneumophila*, укладывается в пределы диапазона заданного во вкладыше к набору реагентов.

Результаты анализа образца являются недействительными, если количество копий ДНК ВКО-STI-338 в 1 мл тестируемой пробы, ниже среднего значения концентрации ДНК препарата ВКО-STI-338, указанного во вкладыше к набору реагентов, более чем в 5 раз. Это свидетельствует о низкой эффективности выделения ДНК из данного образца или о неэффективной очистке от ингибиторов – необходимо протестировать образец повторно, начиная с этапа выделения ДНК.

СРОК ГОДНОСТИ. УСЛОВИЯ ТРАНСПОРТИРОВАНИЯ И ХРАНЕНИЯ

Срок годности. 9 мес. Набор реагентов с истекшим сроком годности применению не подлежит.

Транспортирование. Набор реагентов транспортировать при температуре от 2 до 8 °С не более 5 сут. При получении разукомплектовать в соответствии с указанными температурами хранения.

Хранение. Комплект реагентов «ДНК-сорб-В» хранить при температуре от 2 до 25 °С. Комплект реагентов «ПЦР-комплект» хранить при температуре от 2 до 8 °С в защищенном от света месте.

ГАРАНТИЙНЫЕ ОБЯЗАТЕЛЬСТВА ИЗГОТОВИТЕЛЯ

Изготовитель гарантирует соответствие основных параметров и характеристик набора реагентов требованиям, указанным в технической и эксплуатационной документации, в течение указанного срока годности при соблюдении всех условий транспортирования, хранения и применения.

Медицинское изделие техническому обслуживаю и ремонту не подлежит.

Рекламации на качество набора реагентов направлять по адресу 111123, г. Москва, ул. Новогиреевская, дом 3А, e-mail: cs@pcr.ru[7].

При выявлении побочных действий, не указанных в инструкции по применению набора реагентов, нежелательных реакций при его использовании, фактов и обстоятельств, создающих угрозу жизни и здоровью граждан и медицинских работников при применении и эксплуатации набора реагентов, рекомендуется направить сообщение по адресу, указанному выше, и в уполномоченную государственную регулирующую организацию (в РФ – Федеральная служба по надзору в сфере здравоохранения) в соответствии с действующим законодательством.

Заведующий НПЛ ОМДиЭ
ФБУН ЦНИИ Эпидемиологии
Роспотребнадзора — Е.Н. Родионова

Главный врач
ФГБУЗ ГЦГ и Э ФМБА России — С.А. Богдан

[7] Отзывы и предложения о продукции «АмплиСенс» вы можете оставить, заполнив анкету потребителя на сайте: www.amplisens.ru.

«УТВЕРЖДАЮ»
Зам. директора Федерального бюджетного учреждения науки «Центральный научно-исследовательский институт эпидемиологии» Федеральной службы по надзору в сфере защиты прав потребителей и благополучия человека

_____ А.В. Горелов

«12» декабря 2019 г.

ИНСТРУКЦИЯ ПО ПРИМЕНЕНИЮ

набора реагентов

АмплиСенс® MDR A.b.-OXA-FL

АмплиСенс®

ФБУН ЦНИИ Эпидемиологии
Роспотребнадзора,
Российская Федерация, 111123,
город Москва, улица Новогиреевская, дом 3А

IVD

ОГЛАВЛЕНИЕ

СПИСОК СОКРАЩЕНИЙ

В настоящей инструкции применяются следующие сокращения и обозначения:

БАЛ	- бронхо-альвеолярный лаваж
ВКО	- экзогенный внутренний контрольный образец
ДНК	- дезоксирибонуклеиновая кислота
дНТФ	- дезоксирибонуклеозидтрифосфат
К–	- отрицательный контроль ПЦР
К+	- положительный контроль ПЦР
ОК	- отрицательный контроль экстракции
ОКО	- отрицательный контрольный образец
ПКО	- положительный контрольный образец
ПЦР	- полимеразная цепная реакция
УДГ	- урацил-ДНК-гликозилаза
ФБУН ЦНИИ Эпидемиологии Роспотребнадзора	- Федеральное бюджетное учреждение науки «Центральный научно-исследовательский институт эпидемиологии» Федеральной службы по надзору в сфере защиты прав потребителей и благополучия человека
FRT	- флуоресцентная детекция в режиме «реального времени»
MDR	- Полирезистентность (Multidrug-resistance)

НАЗНАЧЕНИЕ

Набор реагентов АмплиСенс® MDR A.b.-OXA-FL предназначен для качественного определения генов OXA-карбапенемаз групп OXA-23-подобных, OXA-58-подобных и OXA-40-подобных (характерных для ацинетобактеров) и генов-маркеров *Acinetobacter baumannii* (генов OXA-51-подобных карбапенемаз) в образцах бактериальных культур, полученных путем посева биологического материала (аспират из трахеи, БАЛ, кровь, ликвор, мокрота, моча, раневое отделяемое) на плотную или жидкую питательную среду, методом ПЦР с гибридизационно-флуоресцентной детекцией продуктов амплификации. Детекцию генов OXA-карбапенемаз указанных групп проводят с целью выявления штаммов ацинетобактеров, резистентных к β-лактамным антибиотикам группы карбапенемов. Выявление генов-маркеров *Acinetobacter baumannii* (генов OXA-51-подобных карбапенемаз) служит для обнаружения данного микроорганизма. Материалом для проведения ПЦР служат пробы ДНК, экстрагированные из исследуемого материала.

В соответствии с федеральным законом от 21.11.2011 № 323-ФЗ «Об основах охраны здоровья граждан в Российской Федерации» ПЦР-исследование является одним из методов

всестороннего обследования пациента, на основании которых лечащий врач устанавливает диагноз и выбирает мероприятия по лечению пациента.

Показания и противопоказания к применению набора реагентов

Набор реагентов используется в клинической лабораторной диагностике для исследования образцов бактериальных культур, полученных путем посева биологического материала, взятого от лиц с клиническими и/или лабораторными признаками инфекций различной локализации, в первую очередь, инфекций, вызванных *A.baumannii* и связанных с оказанием медицинской помощи.

Противопоказания отсутствуют, за исключением случаев, когда забор материала не может быть осуществлен по медицинским показаниям.

Потенциальные пользователи медицинского изделия

К работе с набором реагентов допускаются только медицинские работники, обученные методам молекулярной диагностики и правилам работы в клинико-диагностической лаборатории в установленном порядке (СП 1.3.2322-08 «Безопасность работы с микроорганизмами III–IV групп патогенности (опасности) и возбудителями паразитарных болезней»).

ПРИНЦИП МЕТОДА

Принцип тестирования основывается на экстракции ДНК из образцов исследуемого материала совместно с экзогенным внутренним контрольным образцом (ВКО[1]) и одновременной амплификации участков генов OXA-карбапенемаз ацинетобактеров и ДНК ВКО с гибридизационно-флуоресцентной детекцией. ВКО позволяет контролировать все этапы ПЦР-исследования для каждого образца и оценивать влияние ингибиторов на результаты ПЦР-исследования.

С полученными на этапе экстракции пробами ДНК проводится реакция амплификации участка ДНК при помощи специфичных к этому участку праймеров и фермента Taq-полимеразы. В составе реакционной смеси присутствуют

[1] ВКО входит в состав реагента ГК-экспресс.

флуоресцентно-меченые олигонуклеотиды, которые гибридизуются с комплементарным участком амплифицируемой ДНК-мишени, в результате чего происходит нарастание интенсивности флуоресценции. Это позволяет регистрировать накопление специфического продукта амплификации путем измерения интенсивности флуоресцентного сигнала с помощью амплификатора с системой детекции флуоресцентного сигнала в режиме «реального времени».

Набор реагентов содержит систему защиты от контаминации ампликонами за счет применения фермента урацил-ДНК-гликозилазы (УДГ) и дезоксиуридинтрифосфата. Фермент УДГ распознает и катализирует разрушение цепей ДНК, содержащих дезоксиуридин, но не ДНК, содержащей дезокситимидин. Дезоксиуридин отсутствует в природной ДНК, но всегда присутствует в ампликонах, поскольку дезоксиуридинтрифосфат входит в состав смеси дНТФ в реагентах для амплификации. Дезоксиуридин делает контаминирующие ампликоны восприимчивыми к разрушению ферментом УДГ до начала амплификации ДНК-мишени, и, следовательно, они не могут быть в дальнейшем амплифицированы.

Фермент УДГ термолабилен и инактивируется при нагревании выше 50 °С и поэтому не разрушает ампликоны мишени, нарабатываемые в процессе ПЦР.

На этапе амплификации одновременно в одной пробирке проводятся 5 реакций – амплификация ДНК генов-маркеров *A. baumannii* (генов OXA-51-подобных карбапенемаз), генов OXA-карбапенемаз ацинетобактеров групп OXA-23-подобных, OXA-58-подобных и OXA-40-подобных, а также амплификация последовательности ВКО. Результаты амплификации регистрируются по пяти различным каналам флуоресцентной детекции:

Таблица 1

Канал для флуорофора	FAM	JOE	ROX	Cy5	Cy5.5
ДНК-мишень (группа генов карбапенемаз)	ДНК *A.baumannii*	OXA-23-подобные	OXA-58-подобные	OXA-40-подобные	ДНК ВКО
Область амплификации	участок гена группы *blaOXA-51-like*	участок гена группы *blaOXA-23-like*	участок гена группы *blaOXA-58-like*	участок гена группы *blaOXA-40-like*	искусственная нуклеотидная последовательность

ФОРМЫ КОМПЛЕКТАЦИИ

Форма 1: ГК-экспресс, «ПЦР-комплект» вариант FRT-100 F
Форма 2: ГК-экспресс, «ПЦР-комплект» вариант FRT-L

Формы 1, 2 предназначены для проведения исследования образцов бактериальных культур, полученных при посеве различных видов нативного биоматериала на жидкую или плотную питательную среду.

Форма 1 рассчитана на проведение 110 реакций амплификации, включая контроли. Форма 2 рассчитана на проведение 96 реакций амплификации, включая контроли.

АНАЛИТИЧЕСКИЕ ХАРАКТЕРИСТИКИ

Для данного набора реагентов применимы следующие характеристики:

Аналитическая чувствительность (предел обнаружения)

Таблица 2

Вид исследуемого материала	Объем образца для экстракции, мкл	Комплект для экстракции ДНК	Комплект для амплификации	Аналитическая чувствительность (предел обнаружения), копий/мл
Бактериальные культуры, полученные путем посева биологического материала на жидкую или плотную[2] питательную среду	согласно Приложению 1	ГК-экспресс	«ПЦР-комплект» вариант FRT-100 F, FRT-L	$5x10^5$

[2] Для бактериальных культур, полученных путем посева биологического материала на плотную питательную среду, указана чувствительность в отношении суспензии бактериальных клеток в реагенте ГК-экспресс.

Данный предел обнаружения достигается при соблюдении правил, указанных в разделах «Взятие, транспортирование и хранение исследуемого материала» и «Подготовка исследуемого материала к экстракции ДНК».

Аналитическая специфичность

Набор реагентов обнаруживает участки ДНК заявленных микроорганизмов. Аналитическая специфичность набора реагентов доказана при исследовании ДНК следующих микроорганизмов, а также геномной ДНК человека:

1. Штаммы из коллекции ATCC® (American Type Culture Collection, США) в концентрации не менее $1x10^7$ ГЭ/мл: *Acinetobacter lwoffii* ATCC® 17925™, *Escherichia coli* ATCC® 25922™, *Klebsiella oxytoca* ATCC® 49131™, *Klebsiella pneumoniae* ATCC® 27736™, *Enterobacter cloaceae* ATCC® 13047™, *Enterobacter aerogenes* ATCC® 13048™, *Pseudomonas aeruginosa* ATCC® 15442™, *Pseudomonas stutzeri* ATCC® 39524™, *Pseudomonas putida* ATCC® 47054™, *Stenotrophomonas maltophilia* ATCC® 13637™, *Proteus mirabilis* ATCC® 12453™, *Proteus vulgaris* ATCC® 6380™, *Citrobacter freundii* ATCC® 8090™, *Serratia marcescens* ATCC® 14756™, *Staphylococcus aureus* ATCC® 6538P™, *Staphylococcus saprophyticus* ATCC® 49907™, *Staphylococcus epidermidis* ATCC® 12228™, *Streptococcus anginosus* ATCC® 9895™, *Streptococcus pneumoniae* ATCC® 49619™, *Enterococcus faecium* ATCC® 35667™, *Bordetella bronchiseptica* ATCC® 10580™, *Moraxella catarrhalis* ATCC® 25238™, *Rhodococcus equi* ATCC® 6939™, *Neisseria lactamica* ATCC® 23970™, *Neisseria gonorrhoeae* ATCC® 19424™, *Neisseria meningitides* ATCC® 13102™, *Corynebacterium minutissimum* ATCC® 23348™, *Corynebacterium jeiklium* ATCC® 43734™, *Corynebacterium xerosis* ATCC® 373™, *Hemophilis parainfluensae* ATCC® 7901™.
2. ДНК человека в концентрации 1 мг/мл.

При тестировании образцов ДНК вышеперечисленных микроорганизмов и ДНК человека неспецифических реакций выявлено не было.

При тестировании изолятов *Acinetobacter baumannii*, у которых отсутствовали фенотипические признаки продукции карбапенемаз, неспецифических реакций выявлено не было,

т.е. был получен отрицательный результат детекции генов OXA-карбапенемаз групп OXA-23-подобных, OXA-58-подобных и OXA-40-подобных и положительный результат детекции генов-маркеров *Acinetobacter baumannii*.

Информация об интерферирующих соединениях указана в разделе «Интерферирующие вещества и ограничения по использованию проб исследуемого материала».

Повторяемость, воспроизводимость исследования

Повторяемость и воспроизводимость исследования были определены путем тестирования положительных и отрицательных модельных образцов. Положительные образцы представляли собой смесь стандартных образцов предприятия, содержащих ДНК *A.baumannii*, ДНК *blaOXA-23*, ДНК *blaOXA-40* и ДНК *blaOXA-58*, с концентрацией $1x10^6$ копий/мл каждого, в качестве отрицательного образца был использован реагент ГК-экспресс. Условия повторяемости включали в себя тестирование в одной и той же лаборатории, одним и тем же оператором, с использованием одного и того же оборудования в пределах короткого промежутка времени. Условия воспроизводимости – тестирование в разных лабораториях, разными операторами, с использованием различного оборудования. Результаты представлены в табл. 3.

Таблица 3

АмплиСенс® MDR A.b.-OXA-FL	**Тип образцов**	**Повторяемость**		**Воспроизводимость**	
		Количество образцов	**Совпадение результатов с ожидаемыми, %**	**Количество образцов**	**Совпадение результатов с ожидаемыми, %**
Форма 1	Положительные	30	100	60	100
	Отрицательные	30	100	60	100
Форма 2	Положительные	30	100	60	100
	Отрицательные	30	100	60	100

ДИАГНОСТИЧЕСКИЕ ХАРАКТЕРИСТИКИ

Таблица 4

Результаты тестирования набора реагентов АмплиСенс® MDR A.b.-OXA-FL в сравнении с референтным методом

Тип образцов	Результаты применения АмплиСенс® MDR A.b.-OXA-FL		Результаты применения референтного[3] метода	
			положи-тельных	отрица-тельных
Бактериальные культуры, полученные путем посева биологического материала на жидкую или плотную питательную среду	Всего исследовано 220 образцов	**положительных**	100	0
		отрицательных	0	120

Таблица 5

Диагностические характеристики набора реагентов АмплиСенс® MDR A.b.-OXA-FL

Тип образцов	Диагностическая чувствительность[4] (с доверительной вероятностью 95 %)	Диагностическая специфичность[5], (с доверительной вероятностью 95 %)
Бактериальные культуры, полученные путем посева биологического материала на жидкую или плотную питательную среду	100 (96,4-100) %	100 (97-100) %

МЕРЫ ПРЕДОСТОРОЖНОСТИ И СВЕДЕНИЯ ОБ УТИЛИЗАЦИИ

Работа должна проводиться в лаборатории, выполняющей молекулярно-биологические (ПЦР) исследования биологического материала на наличие возбудителей инфекционных болезней, с соблюдением санитарно-эпидемиологических правил СП 1.3.2322-08 «Безопасность работы с микроорганизмами III–IV групп патогенности (опасности) и возбудителями паразитарных болезней», СанПиН 2.1.7.2790-10 «Санитарно-эпидемиологические требования к обращению с медицинскими отходами» и

[3] В качестве референтного метода использовались культуральные методы исследования и секвенирование участков генома.
[4] Относительная чувствительность в сравнении с использованным референтным методом.
[5] Относительная специфичность в сравнении с использованным референтным методом.

методических указаний МУ 1.3.2569-09 «Организация работы лабораторий, использующих методы амплификации нуклеиновых кислот при работе с материалом, содержащим микроорганизмы I–IV групп патогенности».

При работе необходимо всегда выполнять следующие требования:

- Температура в помещении лаборатории от 20 до 28 °С, относительная влажность от 15 до 75 %.
- Рассматривать исследуемые образцы как инфекционно-опасные, организовывать работу и хранение в соответствии с СП 1.3.2322-08 «Безопасность работы с микроорганизмами III–IV групп патогенности (опасности) и возбудителями паразитарных болезней».
- Убирать и дезинфицировать разлитые образцы, используя дезинфицирующие средства в соответствии с СП 1.3.2322-08 «Безопасность работы с микроорганизмами III–IV групп патогенности (опасности) и возбудителями паразитарных болезней».
- Лабораторный процесс должен быть однонаправленным. Анализ проводится в отдельных помещениях (зонах). Работу следует начинать в Зоне Экстракции, продолжать в Зоне Амплификации и Детекции. Не возвращать образцы, оборудование и реагенты в зону, в которой была проведена предыдущая стадия процесса.
- Неиспользованные реагенты, реагенты с истекшим сроком годности, а также использованные реагенты, упаковку[6], биологический материал, включая материалы, инструменты и предметы, загрязненные биологическим материалом, следует удалять в соответствии с требованиями СанПиН 2.1.7.2790-10 «Санитарно-эпидемиологические требования к обращению с медицинскими отходами».

ВНИМАНИЕ! При удалении отходов после амплификации (пробирок, содержащих продукты ПЦР) недопустимо открывание пробирок и разбрызгивание содержимого, поскольку это может привести к контаминации продуктами ПЦР лабораторной зоны, оборудования и реагентов.

- Использовать и менять при каждой операции одноразовые

[6] Неиспользованные реагенты, реагенты с истекшим сроком годности, использованные реагенты, упаковка относятся к классу опасности медицинских отходов Г.

наконечники для автоматических дозаторов с фильтром[7]. Одноразовую пластиковую посуду (пробирки, наконечники) необходимо сбрасывать в специальный контейнер, содержащий дезинфицирующее средство, которое может быть использовано для обеззараживания медицинских отходов.

- Поверхности столов, а также помещения, в которых проводится постановка ПЦР, до начала и после завершения работ необходимо подвергать ультрафиолетовому облучению в течение 30 мин.
- Набор реагентов предназначен для одноразового применения для проведения ПЦР-исследования указанного количества проб (см. раздел «Состав»).
- Набор реагентов готов к применению согласно данной инструкции. Применять набор реагентов строго по назначению.
- К работе с набором реагентов допускается только персонал, обученный методам молекулярной диагностики и правилам работы в клинико-диагностической лаборатории в установленном порядке (СП 1.3.2322-08 «Безопасность работы с микроорганизмами III–IV групп патогенности (опасности) и возбудителями паразитарных болезней»).
- Не использовать набор реагентов, если нарушена внутренняя упаковка или внешний вид реагента не соответствует описанию.
- Не использовать набор реагентов, если не соблюдались условия транспортирования и хранения согласно инструкции.
- Не использовать набор реагентов по истечении срока годности.
- Использовать одноразовые неопудренные перчатки, лабораторные халаты, защищать глаза во время работы с образцами и реагентами. Тщательно вымыть руки по окончании работы. Все операции проводятся только в перчатках для исключения контакта с организмом человека.
- Избегать вдыхания паров, контакта с кожей, глазами и слизистой оболочкой. Вреден при проглатывании. При

[7] Для удаления надосадочной жидкости с помощью вакуумного отсасывателя используются одноразовые наконечники без фильтра.

контакте немедленно промыть пораженное место водой, при необходимости обратиться за медицинской помощью.
- При соблюдении условий транспортировки, эксплуатации и хранения риски взрыва и возгорания отсутствуют.
- Информационное письмо о безопасности набора реагентов доступно по запросу.

Оценка вероятных событий, в результате наступления которых могут произойти отрицательные последствия для организма человека

При использовании по назначению и соблюдении вышеперечисленных мер предосторожности набор реагентов безопасен.

Специфические воздействия набора реагентов на организм человека:
- Канцерогенный эффект отсутствует.
- Мутагенное действие отсутствует.
- Репродуктивная токсичность отсутствует.

ДОПОЛНИТЕЛЬНЫЕ МАТЕРИАЛЫ И ОБОРУДОВАНИЕ

Предварительная подготовка исследуемого материала

1. 0,9 % раствор натрия хлорида (стерильный физиологический раствор) или фосфатный буферный раствор (PBS) (натрия хлорид, 137 мМ; калия хлорид, 2,7 мМ; натрия монофосфат, 10 мМ; калия дифосфат, 2 мМ; pH=7,5±0,2).
2. Петли бактериологические, стерильные (например, Nuova Aptaca, Италия, или аналогичные).
3. Одноразовые полипропиленовые плотно закрывающиеся пробирки на 1,5 мл (например, Axygen, Inc. («Эксиджен, Инк»), США, или аналогичные).
4. Одноразовые наконечники для дозаторов переменного объема с фильтром до 100, до 200 и до 1000 мкл (например, Axygen, Inc. («Эксиджен, Инк»), США, или аналогичные).
5. Штативы для пробирок объемом 1,5 мл (например, Axygen, Inc. («Эксиджен, Инк»), США, или аналогичные).
6. Ламинарный бокс класс биологической безопасности II тип А (например, «БАВп-01-«Ламинар-С.»-1,2», ЗАО «Ламинарные системы», Россия, или аналогичный).
7. Автоматические дозаторы переменного объема (например,

ООО «Биохит», Россия, или аналогичные).

8. Микроцентрифуга для пробирок типа «Эппендорф» с максимальной скоростью центрифугирования не менее 12 тыс g (например, MiniSpin, Eppendorf Manufacturing Corporation («Эппендорф Мануфэктуринг Корпорэйшн»), Германия, или аналогичная).
9. Вакуумный отсасыватель медицинский с колбой-ловушкой для удаления надосадочной жидкости (например, «ОМ-1», ООО «Утес», Россия, или аналогичный).
10. Холодильник от 2 до 8 °С с морозильной камерой от минус 24 до минус 16 °С.
11. Отдельный халат, шапочки, обувь и одноразовые перчатки по МУ 1.3.2569-09.
12. Одноразовые пластиковые контейнеры для сброса и инактивации материалов.

Экстракция ДНК из исследуемых образцов

13. Одноразовые полипропиленовые плотно закрывающиеся пробирки объемом 1,5 мл (например, Axygen, Inc. («Эксиджен, Инк»), США, или аналогичные).
14. Одноразовые наконечники для дозаторов переменного объема с фильтром до 100, до 200 и до 1000 мкл (например, Axygen, Inc. («Эксиджен, Инк»), США, или аналогичные).
15. Штативы для пробирок объемом 1,5 мл (например, Axygen, Inc. («Эксиджен, Инк»), США, или аналогичные).
16. Ламинарный бокс класс биологической безопасности II тип А (например, «БАВп-01-«Ламинар-С.»-1,2», ЗАО «Ламинарные системы», Россия, или аналогичный).
17. Микроцентрифуга для пробирок типа «Эппендорф» с максимальной скоростью центрифугирования не менее 12 тыс g (например, MiniSpin, Eppendorf Manufacturing Corporation («Эппендорф Мануфэктуринг Корпорэйшн»), Германия, или аналогичная).
18. Вортекс (например, SIA Biosan, Латвия, или аналогичный).
19. Термостат для пробирок типа «Эппендорф» от 25 до 100 °С (например, SIA Biosan, Латвия, или аналогичный).
20. Автоматические дозаторы переменного объема (например, ООО «Биохит», Россия, или аналогичные).
21. Холодильник от 2 до 8 °С.
22. Отдельный халат, шапочки, обувь и одноразовые перчатки

по МУ 1.3.2569-09.

23. Одноразовые пластиковые контейнеры для сброса и инактивации материалов.

Амплификация с гибридизационно-флуоресцентной детекцией продуктов амплификации

24. Одноразовые полипропиленовые пробирки при работе с «ПЦР-комплектом» FRT-100 F:
    а) завинчивающиеся или плотно закрывающиеся пробирки объемом 1,5 мл (например, Axygen, Inc. («Эксиджен, Инк»), США, или аналогичные) для приготовления реакционной смеси;
    б) тонкостенные пробирки для ПЦР объемом 0,2 мл с выпуклой или плоской оптически прозрачной крышкой или пробирки объемом 0,2 мл в стрипах по 8 шт. с прозрачными крышками (например, Axygen, Inc. («Эксиджен, Инк»), США, или аналогичные) – при использовании прибора планшетного типа;
    в) тонкостенные пробирки для ПЦР объемом 0,2 мл с плоской крышкой (например, Axygen, Inc. («Эксиджен, Инк»), США, или аналогичные) или пробирки для ПЦР к Rotor-Gene объемом 0,1 мл в стрипах по 4 шт. с крышками (например, QIAGEN GmbH («Киаген ГмбХ»), Германия, или аналогичные) – при использовании прибора роторного типа.
25. Одноразовые наконечники для дозаторов переменного объема с фильтром до 10, до 100, до 200 и до 1000 мкл (например, Axygen, Inc. («Эксиджен, Инк»), США, или аналогичные).
26. Штативы для пробирок объемом 0,2 мл или 0,1 мл (например, Axygen, Inc. («Эксиджен, Инк»), США, или аналогичные).
27. Бокс абактериальной воздушной среды (ПЦР-бокс) (например, «БАВ-ПЦР-«Ламинар-С.», ЗАО «Ламинарные системы», Россия, или аналогичный).
28. Вортекс (например, SIA Biosan, Латвия, или аналогичный).
29. Автоматические дозаторы переменного объема (например, ООО «Биохит», Россия, или аналогичные).
30. Программируемый амплификатор с системой детекции флуоресцентного сигнала в режиме «реального времени»,

имеющий 5 или более независимых каналов флуоресцентной детекции (например, Rotor-Gene Q (QIAGEN GmbH («Киаген ГмбХ»), Германия), CFX96 (Bio-Rad Laboratories, Inc. («Био-Рад Лабораториз, Инк.»), США) и другие, рекомендованные Изготовителем).

31. Холодильник от 2 до 8 °С с морозильной камерой от минус 24 до минус 16 °С.
32. Отдельный халат, шапочки, обувь и одноразовые перчатки по МУ 1.3.2569-09.
33. Емкость для сброса наконечников.

ВЗЯТИЕ, ТРАНСПОРТИРОВАНИЕ И ХРАНЕНИЕ ИССЛЕДУЕМОГО МАТЕРИАЛА

Материалом для исследования служат образцы бактериальных культур, полученные путем посева биологического материала (аспират из трахеи, БАЛ, кровь, ликвор, мокрота, моча, раневое отделяемое) на жидкую или плотную питательную среду.

ПОДГОТОВКА ИССЛЕДУЕМОГО МАТЕРИАЛА К ЭКСТРАКЦИИ ДНК

Образцы бактериальных культур, полученных путем посева биологического материала на плотную питательную среду, не требуют предварительной подготовки.

Допускается приготовление суспензии бактериальных клеток в PBS-буфере или в 0,9 % растворе натрия хлорида. Для этого внести около 10^7-10^9 бактериальных клеток, взятых петлей или стерильным наконечником, в подготовленную пробирку с 500 мкл PBS-буфера или 0,9 % раствора натрия хлорида. Полученную суспензию использовать для дальнейшей работы.

Образцы бактериальных культур, полученных путем посева биологического материала на жидкую питательную среду, требуют предварительной подготовки.

Перенести от 100 до 250 мкл культуры в жидкой питательной среде в стерильную одноразовую пробирку объемом 1,5 мл (с помощью одноразовой пастеровской пипетки или автоматического дозатора с наконечником с фильтром). Центрифугировать пробирки 10 мин при 10 тыс g (например, 12 тыс об/мин для микроцентрифуги MiniSpin, Eppendorf Manufacturing Corporation). Используя вакуумный отсасыватель,

полностью удалить надосадочную жидкость, не захватывая осадок и используя для каждого образца отдельный наконечник без фильтра. Экстракцию ДНК проводить из полученного осадка.

Допускается хранение бактериального осадка или суспензии бактериальных клеток до проведения ПЦР-исследования:

– при температуре от минус 24 до минус 16 °С – в течение 1 недели;
– при температуре не выше минус 68 °С – длительно.

ИНТЕРФЕРИРУЮЩИЕ ВЕЩЕСТВА И ОГРАНИЧЕНИЯ ПО ИСПОЛЬЗОВАНИЮ ПРОБ ИССЛЕДУЕМОГО МАТЕРИАЛА

Для контроля эффективности экстракции ДНК и реакции амплификации в наборе реагентов предусмотрено использование внутреннего контрольного образца (ВКО), который добавляется в каждый биологический образец на этапе экстракции нуклеиновых кислот. По окончании реакции амплификации наличие сигнала, свидетельствующего о накоплении фрагментов ДНК ВКО, говорит о достаточной эффективности экстракции нуклеиновых кислот и отсутствии ингибиторов ПЦР.

Потенциально интерферирующие вещества

Для оценки потенциальной интерференции были протестированы образцы бактериальных культур и смеси стандартных образцов предприятия без добавления и с добавлением 15 мг агаризованной питательной среды (кровяной агар, LB-агар) или 25 мкл жидкой питательной среды (LB) (см. табл. 6).

Для тестирования использовали бактериальные культуры *A. baumannii*, а также смеси стандартных образцов предприятия, содержащих ДНК *A.baumannii*, ДНК *blaOXA-23*, ДНК *blaOXA-40* и ДНК *blaOXA-58*, с концентрацией $5x10^5$ копий/мл каждого.

Таблица 6

Потенциальный интерферент	Содержание в образце	Наличие интерференции
Кровяной агар (blood sheep agar)	15 мг	Не обнаружено
LB-агар (LB-agar)	15 мг	Не обнаружено
Жидкая питательная среда (LB)	25 мкл	Не обнаружено

ПРОВЕДЕНИЕ ПЦР-ИССЛЕДОВАНИЯ

ПЦР-исследование состоит из следующих этапов:

– экстракция ДНК из исследуемых образцов,
– амплификация ДНК с гибридизационно-флуоресцентной детекцией в режиме «реального времени»,
– анализ и интерпретация результатов.

ЭКСТРАКЦИЯ ДНК ИЗ ИССЛЕДУЕМЫХ ОБРАЗЦОВ

Для экстракции ДНК из образцов бактериальных культур, полученных путем посева биологического материала на жидкую или плотную питательную среду, используется реагент **ГК-экспресс** в соответствии с **Приложением 1**.

ФОРМА 1 (ГК-экспресс и «ПЦР-комплект» вариант FRT-100 F)

СОСТАВ

ГК-экспресс – реагент для экстракции ДНК из образцов бактериальных культур, полученных при посеве исследуемого материала на жидкую или плотную питательную среду.

Реагент	Описание	Объем, мл	Количество
ГК-экспресс	Прозрачная бесцветная жидкость	5,0	6 пробирок

Реагент рассчитан на проведение экстракции 120 проб, включая контроли.

«ПЦР-комплект» вариант FRT-100 F – комплект реагентов для амплификации участков генов ОХА-карбапенемаз ацинетобактеров с гибридизационно-флуоресцентной детекцией в режиме «реального времени» – позволяет проводить ПЦР-исследование в качественном формате. Комплект реагентов включает:

Реагент	Описание	Объем, мл	Количество
ПЦР-смесь-FL Ab-OXA	Прозрачная жидкость от бесцветного до светло-лилового цвета	1,2	1 пробирка
ПЦР-буфер-В	Прозрачная бесцветная жидкость	0,6	1 пробирка
Полимераза (TaqF)	Прозрачная бесцветная жидкость	0,06	1 пробирка
ПКО-1 Ab-OXA	Прозрачная бесцветная жидкость	0,2	1 пробирка
К–	Прозрачная бесцветная жидкость	0,2	1 пробирка

Комплект реагентов рассчитан на проведение 110 реакций амплификации, включая контроли.

Реагенты комплекта упакованы раздельно в соответствии с температурой хранения (см. раздел «Хранение»). Комплект реагентов состоит из 2-х частей: 1) температура хранения от 2 до 8 °С; 2) температура хранения от минус 24 до минус 16 °С.

АМПЛИФИКАЦИЯ С ДЕТЕКЦИЕЙ В РЕЖИМЕ «РЕАЛЬНОГО ВРЕМЕНИ»

Выбор пробирок для амплификации зависит от используемого амплификатора с системой детекции в

режиме «реального времени».

Для внесения в пробирки реагентов, проб ДНК и контрольных образцов используются одноразовые наконечники с фильтрами.

А. Подготовка проб для амплификации

Общий объем реакционной смеси – 25 мкл, включая объем пробы ДНК – 10 мкл.

1. Рассчитать количество каждого реагента, требующееся для приготовления реакционной смеси. На одну реакцию требуется **10 мкл ПЦР-смеси-FL Ab-ОХА**, **5 мкл ПЦР-буфера-В** и **0,5 мкл полимеразы (TaqF)**. Смесь готовить на общее число исследуемых и контрольных образцов (количество контрольных образцов см. в п.7) плюс запас на несколько реакций.

ВНИМАНИЕ! Компоненты реакционной смеси следует смешивать непосредственно перед проведением ПЦР-исследования.

2. Разморозить пробирку с **ПЦР-смесью-FL Ab-ОХА**. Перемешать содержимое пробирок с **ПЦР-смесью-FL Ab-ОХА**, **ПЦР-буфером-В** и **полимеразой (TaqF)**, осадить капли на вортексе.
3. В отдельной пробирке подготовить реакционную смесь. Смешать необходимое количество **ПЦР-смеси-FL Ab-ОХА**, **ПЦР-буфера-В** и **полимеразы (TaqF)**, осадить капли на вортексе.
4. Отобрать необходимое количество пробирок или стрипов для амплификации ДНК исследуемых и контрольных проб.
5. Внести в каждую пробирку по **15 мкл** приготовленной реакционной смеси. Неиспользованные остатки реакционной смеси выбросить.
6. В подготовленные пробирки внести по **10 мкл проб ДНК**, полученных в результате экстракции из исследуемых образцов.
7. Поставить контрольные реакции:
   а) **положительный контроль ПЦР (К+)** – в пробирку с реакционной смесью внести **10 мкл ПКО-1 Ab-ОХА**.
   б) **отрицательный контроль экстракции (ОК)** – в пробирку с реакционной смесью внести **10 мкл** пробы, экстрагированной как образец ОК (см. Приложение 1).
   в) **отрицательный контроль ПЦР (К–)** – в пробирку с

реакционной смесью внести **10 мкл К–**.

Б. Проведение амплификации с детекцией в режиме «реального времени»

1. Запрограммировать амплификатор с системой детекции в режиме «реального времени» для выполнения соответствующей программы амплификации и детекции флуоресцентного сигнала (см. табл. 7).

Таблица 7

Программа амплификации и детекции флуоресцентного сигнала «АмплиСенс-В»

Цикл	Приборы роторного типа[8]			Приборы планшетного типа[9]		
	Температура, °С	**Время**	**Количество циклов**	**Температура, °С**	**Время**	**Количество циклов**
1	**95**	15 мин	1	**95**	15 мин	1
2	**95**	5 с	35	**95**	5 с	35
	60	20 с детекция флуоресц. сигнала		**60**	30 с детекция флуоресц. сигнала	
	72	15 с		**72**	15 с	

Детекция флуоресцентного сигнала назначается по каналам для флуорофоров **FAM**, **JOE**, **ROX**, **Cy5**, **Cy5.5**.

2. Установить пробирки в ячейки реакционного модуля прибора. Рекомендуется перед постановкой в амплификатор планшетного типа осадить капли со стенок пробирок на вортексе.

ВНИМАНИЕ! В случае неполной загрузки приборов планшетного типа рекомендуется дополнительно установить пустые пробирки по краям реакционного модуля амплификатора.

3. Запустить выполнение программы амплификации с детекцией флуоресцентного сигнала.
4. По окончании выполнения программы приступить к анализу и интерпретации результатов.

В. Анализ и интерпретация результатов

Анализ полученных результатов проводят с помощью программного обеспечения прибора, используемого для

[8] Например, Rotor-Gene Q (QIAGEN) и другие, рекомендованные Изготовителем
[9] Например, CFX 96 (Bio-Rad) и другие, рекомендованные Изготовителем.

проведения ПЦР с детекцией в режиме «реального времени». Анализируют кривые накопления флуоресцентного сигнала по пяти каналам:

Таблица 8

Канал для флуорофора	**FAM**	**JOE**	**ROX**	**Cy5**	**Cy5.5**
Регистрация сигнала, свидетельствующего о накоплении продукта амплификации	ДНК *A.baumannii*	гены карбапенемаз группы OXA-23-подобных	гены карбапенемаз группы OXA-58-подобных	гены карбапенемаз группы OXA-40-подобных	ДНК ВКО

Результаты интерпретируются на основании наличия (или отсутствия) пересечения кривой флуоресценции с установленной на соответствующем уровне пороговой линией, что определяет наличие (или отсутствие) для данной пробы ДНК значения порогового цикла (*Ct*) в соответствующей графе таблицы результатов. Принципы интерпретации результатов следующие:

– ДНК *A.baumannii* **обнаружена**, если для данной пробы в таблице результатов по каналу для флуорофора FAM определено значение *Ct*, не превышающее граничное значение. При этом кривая флуоресценции данной пробы должна однократно пересекать пороговую линию на участке характерного экспоненциального подъема флуоресценции;
– ДНК *A.baumannii* **не обнаружена**, если для данной пробы значение *Ct* по каналу для флуорофора FAM отсутствует или превышает граничное значение, а в таблице результатов по каналу для флуорофора Cy5.5 определено значение *Ct*, не превышающее граничное значение;
– гены приобретенных OXA-карбапенемаз ацинетобактеров групп OXA-23-подобных и/или OXA-58-подобных, и/или OXA-40-подобных **обнаружены**, если для данной пробы в таблице результатов по каналам для флуорофоров JOE и/или ROX, и/или Cy5, соответственно, определено значение *Ct*, не превышающее граничное значение. При этом кривая флуоресценции данной пробы должна однократно пересекать пороговую линию на участке характерного экспоненциального подъема флуоресценции;
– гены приобретенных OXA-карбапенемаз ацинетобактеров групп OXA-23-подобных и/или OXA-58-подобных, и/или OXA-

40-подобных **не обнаружены**, если для данной пробы в таблице результатов по каналам для флуорофоров JOE и/или ROX, и/или Cy5, соответственно, не определено (отсутствует) значение *Ct* (кривая флуоресценции не пересекает пороговую линию) или значение *Ct* превышает граничное значение, а в таблице результатов по каналу для флуорофора Cy5.5 определено значение *Ct*, не превышающее граничное значение;

– результат анализа **невалидный**, если для данной пробы по каналам для флуорофоров FAM, JOE, ROX и Cy5 не определено (отсутствует) значение *Ct* или оно превышает граничное значение, и по каналу для флуорофора Cy5.5 значение *Ct* также не определено (отсутствует) или превышает граничное значение. Необходимо провести повторное ПЦР-исследование соответствующего исследуемого образца, начиная с этапа экстракции ДНК;
– результат анализа **невалидный**, если для исследуемого образца бактериальной культуры, по результатам бактериологического анализа содержащего *A.baumannii,* отсутствуют значения пороговых циклов *Ct* по каналам для флуорофоров JOE, ROX и Cy5, и по каналу для флуорофора FAM значение *Ct* также отсутствует или превышает указанное граничное значение, а в таблице результатов по каналу для флуорофора Cy5.5 определено значение *Ct*, не превышающее граничное значение. Необходимо провести повторное ПЦР-исследование соответствующего исследуемого образца, начиная с этапа экстракции ДНК.

ВНИМАНИЕ! Граничные значения *Ct* указаны во вкладыше, прилагаемом к набору реагентов.

Результат ПЦР-исследования считается достоверным, если получены правильные результаты для контролей этапов экстракции и амплификации ДНК в соответствии с табл. 9 и вкладышем, прилагаемым к набору реагентов.

Таблица 9

Результаты для контролей различных этапов ПЦР-исследования

Контроль	Контролируемый этап ПЦР-исследования	Значение порогового цикла по каналу для флуорофора (*Ct*)				
		FAM	JOE	ROX	Cy5	Cy5.5
К+	ПЦР	определено меньше граничного	определено меньше граничного	определено меньше граничного	определено меньше граничного	определено меньше граничного
ОК	Экстракция ДНК	отсутствует	отсутствует	отсутствует	отсутствует	определено меньше граничного
К–	ПЦР	отсутствует	отсутствует	отсутствует	отсутствует	отсутствует

Возможные ошибки:

1. Для положительного контроля ПЦР (К+) значение порогового цикла (*Ct*) по любому из указанных каналов для флуорофоров (см. табл. 9) отсутствует или превышает граничное значение. Необходимо повторить амплификацию и детекцию для всех образцов.
2. Для отрицательного контроля экстракции (ОК):
   а) по каналам для флуорофоров FAM и/или JOE, и/или ROX, и/или Cy5 определено значение порогового цикла (*Ct*). Вероятна контаминация лаборатории продуктами амплификации или контаминация реагентов, исследуемых образцов на каком-либо этапе ПЦР-исследования. Необходимо предпринять меры по выявлению и ликвидации источника контаминации и повторить ПЦР-исследование для всех образцов, в которых обнаружена специфическая ДНК, начиная с этапа экстракции ДНК.
   б) по каналу для флуорофора Cy5.5 значение порогового цикла (*Ct*) отсутствует или определено больше граничного. Это означает, что ОК не выполнил функцию контроля контаминации. Требуется повторное ПЦР-исследование всех образцов, в которых обнаружена ДНК анализируемых мишеней, начиная с этапа экстракции ДНК.

3. Для отрицательного контроля ПЦР (К–) по каналам для флуорофоров FAM и/или JOE, и/или ROX, и/или Cy5, и/или Cy5.5 определено значение порогового цикла (*Ct*). Вероятна контаминация лаборатории продуктами амплификации или контаминация реагентов, исследуемых образцов на каком-либо этапе ПЦР-исследования. Необходимо предпринять меры по выявлению и ликвидации источника контаминации и повторить амплификацию и детекцию для всех образцов, в которых обнаружена специфическая ДНК.
4. Для исследуемого образца определено значение порогового цикла по одному из каналов для флуорофоров FAM и/или JOE, и/или ROX, и/или Cy5, при этом на графике флуоресценции по этому каналу отсутствует участок характерного экспоненциального подъема (график представляет собой приблизительно прямую линию). Необходимо проверить правильность выбранного уровня пороговой линии или параметров расчета базовой линии. Если результат получен при правильном уровне пороговой линии (базовой линии), требуется повторно провести амплификацию и детекцию для этого образца. При повторном анализе ожидается получение отрицательного результата по этому каналу.

ФОРМА 2 (ГК-экспресс и «ПЦР-комплект» вариант FRT-L)

СОСТАВ

ГК-экспресс – реагент для экстракции ДНК из образцов бактериальных культур, полученных при посеве исследуемого материала на жидкую или плотную питательную среду.

Реагент	Описание	Объем, мл	Количество
ГК-экспресс	Прозрачная бесцветная жидкость	5,0	6 пробирок

Реагент рассчитан на проведение экстракции 120 проб, включая контроли.

«ПЦР-комплект» вариант FRT-L – комплект реагентов для амплификации участков генов ОХА-карбапенемаз ацинетобактеров с гибридизационно-флуоресцентной детекцией в режиме «реального времени» – позволяет проводить ПЦР-исследование в качественном формате. Комплект реагентов включает:

Реагент	Описание	Объем, мл	Количество
ПЦР-смесь Ab-OXA-Lyo	Порошок белого цвета	-	96 пробирок объемом 0,2 мл
ПКО-1 Ab-OXA	Прозрачная бесцветная жидкость	0,5	1 пробирка
К–	Прозрачная бесцветная жидкость	0,5	1 пробирка

Комплект реагентов рассчитан на проведение 96 реакций амплификации, включая контроли.

АМПЛИФИКАЦИЯ С ДЕТЕКЦИЕЙ В РЕЖИМЕ «РЕАЛЬНОГО ВРЕМЕНИ»

Для внесения в пробирки реагентов, проб ДНК и контрольных образцов используются одноразовые наконечники с фильтрами.

А. Подготовка проб для амплификации

Общий объем реакционной смеси – 25 мкл, включая объем пробы ДНК – 25 мкл.

1. Отобрать необходимое количество пробирок для амплификации с готовой лиофилизированной реакционной **ПЦР-смесью Ab-OXA-Lyo** для амплификации ДНК исследуемых и контрольных образцов (количество

контрольных образцов см. в п. 3).

2. В подготовленные пробирки внести по **25 мкл проб ДНК**, полученных в результате экстракции из исследуемых образцов.
3. Поставить контрольные реакции:
   а) **положительный контроль ПЦР (К+)** – в пробирку с реакционной смесью внести **25 мкл ПКО-1 Ab-ОХА**.
   б) **отрицательный контроль экстракции (ОК) –** в пробирку с реакционной смесью внести **25 мкл** пробы, экстрагированной как образец ОК (см. Приложение 1).
   в) **отрицательный контроль ПЦР (К–)** – в пробирку с реакционной внести смесью **25 мкл К–**.

ВНИМАНИЕ! Содержимое пробирок необходимо тщательно перемешать пипетированием, не допуская появления пузырьков воздуха.

Б. Проведение амплификации с детекцией в режиме «реального времени»

1. Запрограммировать амплификатор с системой детекции в режиме «реального времени» для выполнения соответствующей программы амплификации и детекции флуоресцентного сигнала (см. табл. 10).

Таблица 10

Программа амплификации и детекции флуоресцентного сигнала «АмплиСенс-В»

Цикл	Приборы роторного типа[10]			Приборы планшетного типа[11]		
	Температура, °С	Время	Количество циклов	Температура, °С	Время	Количество циклов
1	**95**	15 мин	1	**95**	15 мин	1
2	**95**	5 с	35	**95**	5 с	35
	60	20 с детекция флуоресц. сигнала		**60**	30 с детекция флуоресц. сигнала	
	72	15 с		**72**	15 с	

Детекция флуоресцентного сигнала назначается по каналам для флуорофоров **FAM**, **JOE**, **ROX**, **Cy5**, **Cy5.5**.

[10] Например, Rotor-Gene Q (QIAGEN) и другие, рекомендованные Изготовителем
[11] Например, CFX 96 (Bio-Rad) и другие, рекомендованные Изготовителем.

2. Установить пробирки в ячейки реакционного модуля прибора. Рекомендуется перед постановкой в амплификатор планшетного типа осадить капли со стенок пробирок на вортексе.

ВНИМАНИЕ! В случае неполной загрузки приборов планшетного типа рекомендуется дополнительно установить пустые пробирки по краям реакционного модуля амплификатора.

3. Запустить выполнение программы амплификации с детекцией флуоресцентного сигнала.
4. По окончании выполнения программы приступить к анализу и интерпретации результатов.

В. Анализ и интерпретация результатов

Анализ полученных результатов проводят с помощью программного обеспечения прибора, используемого для проведения ПЦР с детекцией в режиме «реального времени». Анализируют кривые накопления флуоресцентного сигнала по пяти каналам:

Таблица 11

Канал для флуорофора	FAM	JOE	ROX	Cy5	Cy5.5
Регистрация сигнала, свидетельствующего о накоплении продукта амплификации	ДНК *A.baumannii*	гены карбапенемаз группы OXA-23-подобных	гены карбапенемаз группы OXA-58-подобных	гены карбапенемаз группы OXA-40-подобных	ДНК ВКО

Результаты интерпретируются на основании наличия (или отсутствия) пересечения кривой флуоресценции с установленной на соответствующем уровне пороговой линией, что определяет наличие (или отсутствие) для данной пробы ДНК значения порогового цикла (*Ct*) в соответствующей графе таблицы результатов. Принципы интерпретации результатов следующие:

– ДНК *A.baumannii* **обнаружена**, если для данной пробы в таблице результатов по каналу для флуорофора FAM определено значение *Ct*, не превышающее граничное значение. При этом кривая флуоресценции данной пробы должна однократно пересекать пороговую линию на участке характерного экспоненциального подъема флуоресценции;

– ДНК *A.baumannii* **не обнаружена**, если для данной пробы значение *Ct* по каналу для флуорофора FAM отсутствует или превышает граничное значение, а в таблице результатов по каналу для флуорофора Cy5.5 определено значение *Ct*, не превышающее граничное значение;
– гены приобретенных OXA-карбапенемаз ацинетобактеров групп OXA-23-подобных и/или OXA-58-подобных, и/или OXA-40-подобных **обнаружены**, если для данной пробы в таблице результатов по каналам для флуорофоров JOE и/или ROX, и/или Cy5, соответственно, определено значение *Ct*, не превышающее граничное значение. При этом кривая флуоресценции данной пробы должна однократно пересекать пороговую линию на участке характерного экспоненциального подъема флуоресценции;
– гены приобретенных OXA-карбапенемаз ацинетобактеров групп OXA-23-подобных и/или OXA-58-подобных, и/или OXA-40-подобных **не обнаружены**, если для данной пробы в таблице результатов по каналам для флуорофоров JOE и/или ROX, и/или Cy5, соответственно, не определено (отсутствует) значение *Ct* (кривая флуоресценции не пересекает пороговую линию) или значение *Ct* превышает граничное значение, а в таблице результатов по каналу для флуорофора Cy5.5 определено значение *Ct*, не превышающее граничное значение;
– результат анализа **невалидный**, если для данной пробы по каналам для флуорофоров FAM, JOE, ROX и Cy5 не определено (отсутствует) значение *Ct* или оно превышает граничное значение, и по каналу для флуорофора Cy5.5 значение *Ct* также не определено (отсутствует) или превышает граничное значение. Необходимо провести повторное ПЦР-исследование соответствующего исследуемого образца, начиная с этапа экстракции ДНК;
– результат анализа **невалидный**, если для исследуемого образца бактериальной культуры, по результатам бактериологического анализа содержащего *A.baumannii*, отсутствуют значения пороговых циклов *Ct* по каналам для флуорофоров JOE, ROX и Cy5, и по каналу для флуорофора FAM значение *Ct* также отсутствует или превышает указанное граничное значение, а в таблице

результатов по каналу для флуорофора Cy5.5 определено значение *Ct*, не превышающее граничное значение. Необходимо провести повторное ПЦР-исследование соответствующего исследуемого образца, начиная с этапа экстракции ДНК.

ВНИМАНИЕ! Граничные значения *Ct* указаны во вкладыше, прилагаемом к набору реагентов.

Результат ПЦР-исследования считается достоверным, если получены правильные результаты для контролей этапов экстракции и амплификации ДНК в соответствии с табл. 12 и вкладышем, прилагаемым к набору реагентов.

Таблица 12

Результаты для контролей различных этапов ПЦР-исследования

Контроль	Контролируемый этап ПЦР-исследования	Значение порогового цикла по каналу для флуорофора (*Ct*)				
		FAM	JOE	ROX	Cy5	Cy5.5
К+	ПЦР	определено меньше граничного	определено меньше граничного	определено меньше граничного	определено меньше граничного	определено меньше граничного
ОК	Экстракция ДНК	отсутствует	отсутствует	отсутствует	отсутствует	определено меньше граничного
К–	ПЦР	отсутствует	отсутствует	отсутствует	отсутствует	отсутствует

Возможные ошибки:

1. Для положительного контроля ПЦР (К+) значение порогового цикла (*Ct*) по любому из указанных каналов для флуорофоров (см. табл. 12) отсутствует или превышает граничное значение. Необходимо повторить амплификацию и детекцию для всех образцов.
2. Для отрицательного контроля экстракции (ОК):
   а) по каналам для флуорофоров FAM и/или JOE, и/или ROX, и/или Cy5 определено значение порогового цикла (*Ct*). Вероятна контаминация лаборатории продуктами амплификации или контаминация реагентов, исследуемых образцов на каком-либо этапе ПЦР-исследования. Необходимо предпринять меры по

выявлению и ликвидации источника контаминации и повторить ПЦР-исследование для всех образцов, в которых обнаружена специфическая ДНК, начиная с этапа экстракции ДНК.

б) по каналу для флуорофора Cy5.5 значение порогового цикла (*Ct*) отсутствует или определено больше граничного. Это означает, что ОК не выполнил функцию контроля контаминации. Требуется повторное ПЦР-исследование всех образцов, в которых обнаружена ДНК анализируемых мишеней, начиная с этапа экстракции ДНК.

3. Для отрицательного контроля ПЦР (К–) по каналам для флуорофоров FAM и/или JOE, и/или ROX, и/или Cy5, и/или Cy5.5 определено значение порогового цикла (*Ct*). Вероятна контаминация лаборатории продуктами амплификации или контаминация реагентов, исследуемых образцов на каком-либо этапе ПЦР-исследования. Необходимо предпринять меры по выявлению и ликвидации источника контаминации и повторить амплификацию и детекцию для всех образцов, в которых обнаружена специфическая ДНК.
4. Для исследуемого образца определено значение порогового цикла по одному из каналов для флуорофоров FAM и/или JOE, и/или ROX, и/или Cy5, при этом на графике флуоресценции по этому каналу отсутствует участок характерного экспоненциального подъема (график представляет собой приблизительно прямую линию). Необходимо проверить правильность выбранного уровня пороговой линии или параметров расчета базовой линии. Если результат получен при правильном уровне пороговой линии (базовой линии), требуется повторно провести амплификацию и детекцию для этого образца. При повторном анализе ожидается получение отрицательного результата по этому каналу.

СРОК ГОДНОСТИ. УСЛОВИЯ ТРАНСПОРТИРОВАНИЯ И ХРАНЕНИЯ

Срок годности. 15 мес. Набор реагентов с истекшим сроком годности применению не подлежит. Срок годности вскрытых реагентов соответствует сроку годности, указанному на этикетках для невскрытых реагентов, если в инструкции не указано иное.

Транспортирование. Набор реагентов транспортировать при температуре от 2 до 8 °С не более 5 сут в термоконтейнерах, содержащих хладоэлементы, всеми видами крытых транспортных средств. Допускается транспортирование при температуре от 2 до 25 °С не более 3 сут.

«ПЦР-комплект» вариант FRT-100 F при получении разукомплектовать в соответствии с указанными температурами хранения.

Хранение.

Форма 1. Реагент ГК-экспресс, «ПЦР-комплект» вариант FRT-100 F хранить в холодильной камере при температуре от 2 до 8 °С, кроме ПЦР-смеси-FL Ab-OXA, ПЦР-буфера-B и полимеразы (TaqF). ПЦР-смесь-FL Ab-OXA, ПЦР-буфер-B и полимеразу (TaqF) хранить в морозильной камере при температуре от минус 24 до минус 16 °С. ПЦР-смесь-FL Ab-OXA хранить в защищенном от света месте.

Форма 2. Реагент ГК-экспресс, «ПЦР-комплект» вариант FRT-L хранить в холодильной камере при температуре от 2 до 8 °С. Лиофилизированные реагенты (ПЦР-смесь Ab-OXA-Lyo) хранить в пакетах с влагопоглотителем. ПЦР-смесь Ab-OXA-Lyo хранить в защищенном от света месте.

Холодильные и морозильные камеры должны обеспечивать регламентированный температурный режим.

ГАРАНТИЙНЫЕ ОБЯЗАТЕЛЬСТВА ИЗГОТОВИТЕЛЯ

Изготовитель гарантирует соответствие основных параметров и характеристик набора реагентов требованиям, указанным в технической и эксплуатационной документации, в течение указанного срока годности при соблюдении всех условий транспортирования, хранения и применения.

Медицинское изделие техническому обслуживанию и ремонту не подлежит.

Рекламации на качество набора реагентов направлять по адресу 111123, г. Москва, ул. Новогиреевская, дом 3А, e-mail: cs@pcr.ru[12].

При выявлении побочных действий, не указанных в инструкции по применению набора реагентов, нежелательных реакций при его использовании, фактов и обстоятельств, создающих угрозу жизни и здоровью граждан и медицинских работников при применении и эксплуатации набора реагентов, рекомендуется направить сообщение по адресу, указанному выше, и в уполномоченную государственную регулирующую организацию (в РФ – Федеральная служба по надзору в сфере здравоохранения) в соответствии с действующим законодательством.

Заведующий НПЛ ОМДиЭ
ФБУН ЦНИИ Эпидемиологии
Роспотребнадзора

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Е.В. Ржевская

[12] Отзывы и предложения о продукции «АмплиСенс» вы можете оставить, заполнив анкету потребителя на сайте: www.amplisens.ru.

СИМВОЛЫ, ИСПОЛЬЗУЕМЫЕ В ПЕЧАТНОЙ ПРОДУКЦИИ

REF	Номер по каталогу		Содержимого достаточно для проведения n-количества тестов
LOT	Код партии		Использовать до
IVD	Медицинское изделие для диагностики in vitro		Обратитесь к инструкции по применению
VER	Дата изменения		Не допускать воздействия солнечного света
	Температурный диапазон		Дата изготовления
	Изготовитель		Беречь от влаги
	Осторожно! Обратитесь к инструкции по применению		

ПРИЛОЖЕНИЕ 1.
Экстракция ДНК из исследуемых образцов с использованием реагента ГК-экспресс

Порядок работы

1. Включить термостат и установить температуру **70 °С**.

<u>При анализе образцов бактериальных культур, полученных путем посева биологического материала на плотную питательную среду</u>

2. Подготовить необходимое количество пустых пробирок, включая пробирку отрицательного контроля (ОК), и промаркировать их.
3. Внести в каждую пробирку по **250 мкл** реагента **ГК-экспресс**[13].
4. В пробирку с реагентом **ГК-экспресс** внести около 10^7-10^9 бактериальных клеток, взятых петлей или стерильным наконечником.
5. При анализе образцов суспензии бактериальных клеток в PBS-буфере или в 0,9 % растворе натрия хлорида в пробирки с реагентом **ГК-экспресс** внести по **20 мкл** суспензии бактериальных клеток, используя для каждого образца отдельный наконечник с фильтром.
6. В пробирку отрицательного контроля (ОК) ничего, кроме реагента **ГК-экспресс**, не добавлять. Перейти к п.9.

<u>При анализе образцов бактериальных культур, полученных путем посева биологического материала на жидкую питательную среду</u>

7. В пробирки, содержащие осадок бактериальных клеток, внести по **250 мкл** реагента **ГК-экспресс**, используя для каждой пробирки отдельный наконечник с фильтром.
8. Промаркировать одну дополнительную пробирку отрицательного контроля (ОК) и внести в нее **250 мкл** реагента **ГК-экспресс** и **20 мкл** используемой жидкой питательной среды. Перейти к п.9.
9. Закрыть крышки и перемешать на вортексе. Осадить капли жидкости на вортексе (2-3 сек).
10. Содержимое пробирок прогреть **10 мин при 70 °С** в термостате, перемешать.

[13] ВКО входит в состав реагента ГК-экспресс.

11. После окончания инкубации перемешать и процентрифугировать пробирки на микроцентрифуге в течение **1 мин** при **12 тыс g** (например, 13400 об/мин для центрифуги MiniSpin, Eppendorf). Надосадочная жидкость содержит ДНК. Пробы готовы к постановке ПЦР.

ДНК-пробы могут храниться в течение недели при температуре от 2 до 8 °С или в течение года при температуре не выше минус 68 °С.

ВНИМАНИЕ! При повторном ПЦР-исследовании проб ДНК содержимое пробирок необходимо перемешать на вортексе и повторить центрифугирование в соответствии с п.11.