

Product List – CE Marked

Certified by

ISO 13485:2016

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

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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 simplex 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	Corynebacterium 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 simplex 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	Corynebacterium 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

Certificate

**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

TÜVRheinland

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.

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A. Fechner



Dipl.-Ing. A. Fechner

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NovaLisa®

Ascaris lumbricoides IgG

ELISA

CE

Only for in-vitro diagnostic use

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Product Number: ASCG0020 (96 Determinations)

ENGLISH

1. INTRODUCTION

Ascaridae are big nematodes. The male individuals are up to 25 cm, the female ones are up to 40 cm long.

Is among the Ascaridae the species with the highest importance for human medicine, because it is the only one with humans as main host.

The sexually mature roundworm lives in the small intestine. The females produce up to 200 000 eggs daily, which attain to the environment by faeces. Infectious larvae develop inside the eggs and after oral ingestion they hatch in the upper part of the small intestine. They penetrate the wall of the intestine and get into the venous blood with which they get into liver and lung, where they leave the vessels and skin in the aveoles. The larvae migrate into the trachea and through the pharynx after swallowing back to the small intestine where the maturation to the adult worm takes place. Ca. 10-12 weeks after infestation the roundworms will be excreted with faeces. The adult worm lives for around 18 months.

Ascaris lumbricoides is one of the most abundant exciter of infectious diseases worldwide. Main endemic areas are Eastern Asia, Africa and Middle and South America. Children are more often affected than adults. The infestation leads to Ascariasis mostly with latent progression. The migrating larvae can lead to inflammatory, eosinophile infiltration of the lung and cause cough, dyspnoea and light fever. Conglomerates of the worms can cause intestinal blockage. If the worms migrate into gall, pancreas or stomach the corresponding clinical symptoms result.

Species	Disease	Symptoms (e.g.)	Transmission route
<i>Ascaris lumbricoides</i>	Ascariasis	Adult worms cause no symptoms in general. Conglomerates of worms can cause abdominal pain and ileus. Infection of gall, stomach or pancreas leads to corresponding symptoms. Migrating larvae are able to cause pulmonary symptoms like cough and dyspnoea.	Ingestion of infectious Ascaridae eggs (classical way of infestation is the consumption of insufficient washed salad)

Infection or presence of pathogen may be identified by:

- Microscopy: Detection of eggs in faeces
- Serology: Detection of antibodies by ELISA

2. INTENDED USE

The *Ascaris lumbricoides* IgG ELISA is intended for the qualitative determination of IgG class antibodies against *Ascaris lumbricoides* 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 *Ascaris lumbricoides* antigens; in resealable aluminium foil.
- **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; $\leq 0.0015\%$ (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 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; $\leq 0.02\%$ (v/v) MIT.
- **Cut-off Control:** 1 vial containing 3 mL control; coloured yellow; ready to use; green cap; $\leq 0.02\%$ (v/v) MIT.
- **Negative Control:** 1 vial containing 2 mL control; coloured yellow; ready to use; blue cap; $\leq 0.0015\%$ (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 μ L
- 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 *Ascaris lumbricoides* 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 μ L 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 $\pm$ 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 $< \text{Cut-off}$
- **Cut-off Control:** Absorbance value **0.150 – 1.300**
- **Positive Control:** Absorbance value $> \text{Cut-off}$

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]

Sample (mean) absorbance value x 10 = [NovaTec Units = NTU]
Cut-off

Example: $\frac{1.591 \times 10}{0.43} = 37 \text{ NTU}$

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	0.295	3.54
#2	24	0.539	4.55
#3	24	0.657	6.16
Interassay	n	Mean (NTU)	CV (%)
#1	12	18.48	2.77
#2	12	6.35	8.02
#3	12	22.58	4.03

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 95.0 % (95% confidence interval: 87.69% - 98.62%).

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: 47.82% - 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 with antibodies against *Toxocara canis*, *Trichinella*, *Fasciola*, *Filaria* and *Strongyloides* 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.: ASCG0020

Ascaris lumbricoides IgG ELISA (96 Determinations)

NovaLisa®

Bordetella pertussis IgM

ELISA

CE

Only for in-vitro diagnostic use

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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
<i>Bordetella pertussis</i>	Pertussis whooping cough	<u>1. Stadium catarrhale</u> : symptoms of a cold with slight fever (1-2 weeks) <u>2. Stadium convulsivum</u> : severe, spasmodic coughing; after deep inspiration follows a coughing staccato (2-6 weeks) <u>3. Stadium decrementi</u> : 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; $\leq 0.0015\%$ (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; $\leq 0.02\%$ (v/v) MIT.
- **Cut-off Control:** 1 vial containing 3 mL control; coloured yellow; ready to use; green cap; $\leq 0.02\%$ (v/v) MIT.
- **Negative Control:** 1 vial containing 2 mL control; coloured yellow; ready to use; blue cap; $\leq 0.0015\%$ (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 μ L
- 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 μ L 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 $\pm$ 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 $< \text{Cut-off}$
- **Cut-off Control:** Absorbance value **0.150 – 1.300**
- **Positive Control:** Absorbance value $> \text{Cut-off}$

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]

Sample (mean) absorbance value x 10 = [NovaTec Units = NTU]
Cut-off

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)

NovaLisa®

Bordetella pertussis IgG

ELISA

CE

Only for in-vitro diagnostic use

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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
<i>Bordetella pertussis</i>	Pertussis whooping cough	<u>1. Stadium catarrhale</u> : symptoms of a cold with slight fever (1-2 weeks) <u>2. Stadium convulsivum</u> : severe, spasmodic coughing; after deep inspiration follows a coughing staccato (2-6 weeks) <u>3. Stadium decrementi</u> : 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; $\leq 0.0015\%$ (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; $\leq 0.02\%$ (v/v) MIT.
- **Cut-off Control:** 1 vial containing 3 mL control; coloured yellow; ready to use; green cap; $\leq 0.02\%$ (v/v) MIT.
- **Negative Control:** 1 vial containing 2 mL control; coloured yellow; ready to use; blue cap; $\leq 0.0015\%$ (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 μ L
- 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 $\pm$ 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]

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

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

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)

NovaLisa®

Corynebacterium diphtheriae toxin IgG

ELISA

CE

Only for in-vitro diagnostic use

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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
<i>Corynebacterium diphtheriae</i>	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; $\leq 0.0015\%$ (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; $\leq 0.02\%$ (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 μ L
- 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.

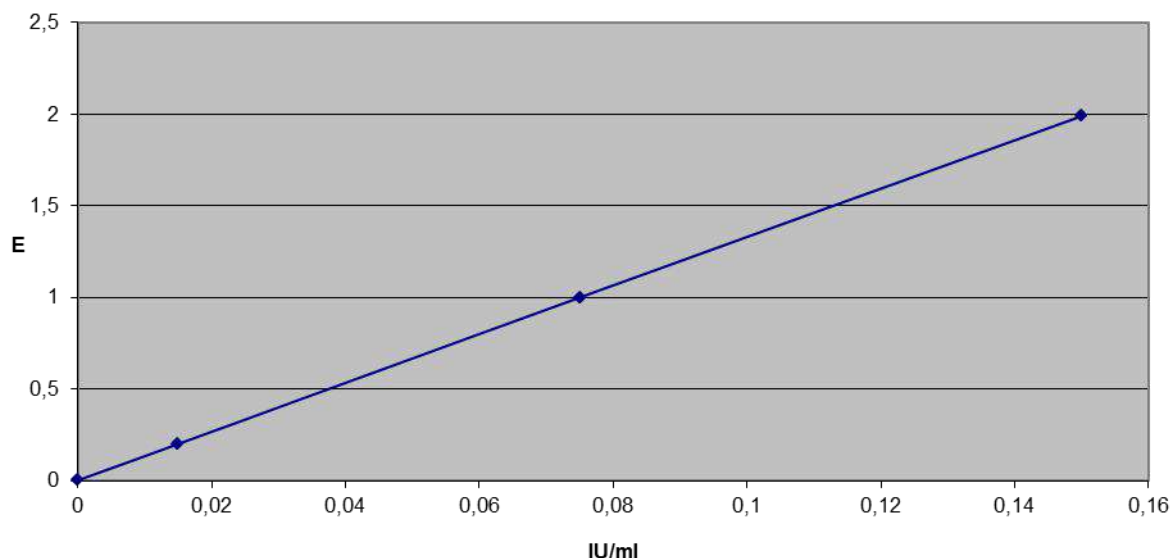
9.2. Calculation of Results

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)

NovaLisa®

Echinococcus IgG

ELISA

CE

Only for in-vitro diagnostic use

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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 (Hydatidosis, or hydatid 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
<i>E. granulosus</i>	Cystic echinococcosis (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.
<i>E. multilocularis</i>	Alveolar Echinococcosis (Alveolar Hydatid Disease, AHD)	Lungs: Thoracic pains, cough, expectoration, dyspnea, etc. CNS: Neurological symptoms	e.g.: contaminated wild berries.

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; $\leq 0.0015\%$ (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; $\leq 0.02\%$ (v/v) MIT.
- **Cut-off Control:** 1 vial containing 3 mL control; coloured yellow; ready to use; green cap; $\leq 0.02\%$ (v/v) MIT.
- **Negative Control:** 1 vial containing 2 mL control; coloured yellow; ready to use; blue cap; $\leq 0.0015\%$ (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 $\pm$ 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 $< \text{Cut-off}$
- **Cut-off Control:** Absorbance value **0.150 – 1.300**
- **Positive Control:** Absorbance value $> \text{Cut-off}$

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]

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

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

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)

Instructions For Use



Serazym® Giardia

Enzyme immunoassay for detection of *Giardia lamblia* CWP-1 in faecal samples

REF E-106-A  96  *In-vitro*-diagnostic medical device 



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Introduction

Giardia lamblia (syn. *Lamblia intestinalis*) is one of the most common intestinal protozoan pathogens worldwide. The incidence strongly depends on the geographic region and reaches 2 - 7% in central Europe and exceeds 50% in tropical countries (1, 2).

The clinical picture of a *Giardia lamblia* infection ranges from the asymptomatic carrier state to acute diarrhea often accompanied by abdominal pain and flatulence. Chronic giardiasis may cause severe malabsorption syndrome. After the incubation time of about 5 days symptoms last for about 5 days in acute cases and up to 2 months in chronic courses of the disease.

The life cycle of *Giardia lamblia* is characterized by two stages: the trophozoite and the cyst stage. The trophozoite is the motile dividing stage and inhabits the upper small intestine. Ascending infections of the gallbladder may also occur. The cyst is the infective form of the parasite. It develops in the intestine and is excreted with the faeces. Cysts are transmitted via contaminated food or drinking water but also from person to person. The ability of infecting humans as well as different mammalian species explains the zoonotic potential of this parasite (1, 2).

External factors (pH-value, composition of bile salts) induce the differentiation from trophozoites to cysts (3, 4, 5). During the process of encystation which takes about 12 to 48 hours proteins and glycopolymeric substances needed for the cyst wall formation are produced by the trophozoites. These substances are stored in encystation specific vesicles (ESV) within the cytoplasm of the trophozoites, transported to the cell surface, excreted by exocytosis and incorporated into the cyst wall.

The cyst wall proteins CWP-1 and CWP-2 as the main components of the cyst wall have a molecular weight of 26 and 29 kDa resp. (2, 4, 7), and they form heterodimers of 65 kDa which are also known as Giardia Specific Antigen, GSA-65 (6). Encystation is a continuous and often incomplete process leading to the release of cyst wall proteins into the intestine during the progeny of the parasite (2). The immunological detection of Giardia specific cyst wall proteins by enzyme immunoassay for the diagnosis of acute Giardia infections is established (10). In contrast to microscopy the detection of soluble parasite antigens has the advantage that morphologically intact cysts or trophozoites are not essentially needed (8, 9).

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Last update 14 Feb 2013

Intended use

Serazym® Giardia is an *in-vitro*-diagnostic medical device for direct detection of *Giardia lamblia* cyst wall protein 1 (CWP-1) in faecal samples.

Principle of the test

The Serazym® Giardia is based on polyclonal antibodies to *Giardia lamblia* cyst wall protein 1 (CWP-1). Within the first incubation step (60 min, room temperature) diluted stool specimens as well as positive and negative controls react with the solid-phase adsorbed polyclonal antibodies. Unbound components are removed by a subsequent washing step. Within the second incubation step (30 min, room temperature) solid-phase bound immune complexes react with the horseradish (HRP)-labelled polyclonal antibodies of the conjugate. Unbound reagents are separated from the solid-phase antibody-antigen-antibody immune complexes by a further washing step. HRP converts the subsequently added colourless chromogenic substrate solution into a blue product. The enzyme reaction is terminated by sulphuric acid dispensed into the wells after 10 min incubation at room temperature 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 *Giardia lamblia* cyst wall protein. Referring to the cut-off value results are interpreted as positive or negative.

Test components

			For 96 Wells
1	WELLS	Microtitration plate coated with polyclonal anti-Giardia CWP-1 antibodies (sheep)	12 single breakable 8 well strips colour coding white vacuum-sealed with desiccant
2	WASHBUF CONC 10x	Wash buffer 10-fold	100 ml concentrate for 1000 ml solution white cap
3	DIL	Sample diluent	100 ml · ready to use coloured yellow black cap
4	CONTROL +	Positive control Recombinant <i>Giardia lamblia</i> Cyst wall proteins	2.0 ml · ready to use coloured blue red cap
5	CONTROL -	Negative control <i>Giardia lamblia</i> negative sample	2.0 ml · ready to use coloured blue green cap
6	CONJ HRP	HRP-conjugate HRP-labelled polyclonal anti-Giardia CWP-1 antibodies (sheep)	15 ml · ready to use coloured green violet cap
7	SUBSTR TMB	Substrate 3,3',5,5'-Tetramethylbenzidine and hydrogen peroxide	15 ml · ready to use blue cap
8	STOP	Stop solution 0.25 M sulphuric acid	15 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 over 72 h at 2...8°C before testing. Faecal samples that are already diluted in transportation media (PARA-PAK PLUS, PARA-PAK PLUS SAF) may cause decreased OD values in the *Serazym*® Giardia in comparison to untreated samples. In case of samples with low antigen concentration OD values may decrease beyond the detection limit of the test and therefore it is recommended to use untreated samples. If untreated samples are not available the already prediluted sample should be further diluted with *Serazym*® sample diluent as stated below.

Preparation

Fresh, untreated samples

Quickly thaw frozen samples. Warm samples to room temperature and mix well. 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 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.

Conserved samples, prediluted in transportation media

Mix sample thoroughly. Pipette 250 µl sample diluent into a clean tube, transfer 1000 µl of the prediluted sample to this tube and mix well.

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 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 two 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.

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 μ l faeces + 1.0 ml 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 residual 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. Pipette: 100 μ l **CONTROL +** positive control (4)
100 μ l **CONTROL -** negative control (5)
100 μ l **diluted sample**.
3. Cover plate and incubate for 60 min at RT.
4. Decant, then wash each well 5x with 300 μ l wash solution (diluted from (2)) and tap dry onto absorbent paper if necessary.
5. 3 drops (or 100 μ l) **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 μ l wash solution (diluted from (2)) and tap dry onto absorbent paper if necessary.
8. 3 drops (or 100 μ l) **SUBSTR TMB** substrate (7) per well.
9. Incubate for 10 min at RT protected from light.
10. 3 drops (or 100 μ l) **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 equal with or higher than the cut-off are considered positive, samples with OD values below the cut-off are considered negative for *Giardia lamblia* antigen.

Reference values

Serazym® Giardia	
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: ≥ 0.80

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 the absorbance of a sample 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 diluted sample can cause false negative as well as false positive results.

A negative test result not necessarily excludes a *Giardia lamblia* infection. Inhomogeneous antigen distribution in the sample can cause false negative results. Therefore in case of a negative test result but clinical suspicion of an infection a further sample of the respective person should be investigated. A final interpretation of the test result should always consider clinical findings as well.

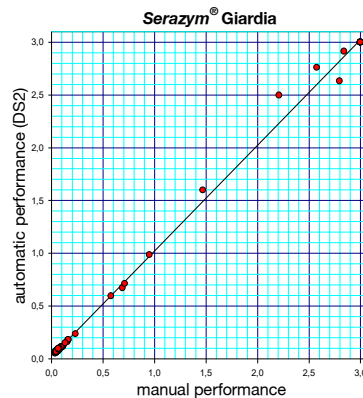
Automatic processing

Performing the Serazym® Giardia 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 90 faecal specimens was investigated in parallel by manual and automatic processing method (DS2, Dynex Technologies) resp. The correlation was calculated with 0.999.



Performance characteristics

Precision

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

sample	mean OD	standard deviation	CV (%)
1	2.182	0.098	4.79
2	1.175	0.043	3.92
3	0.563	0.027	5.08
4	0.179	0.006	3.59

Inter-Assay coefficient of variation (CV) in the *Serazym®* Giardia in 8 different test runs from twofold determinations of samples:

sample	mean OD	standard deviation	CV (%)
1	1.947	0.144	7.42
2	1.732	0.149	8.63
3	0.760	0.058	7.69
4	0.217	0.020	9.04

Lower detection limit

The lower detection limit of Giardia antigen in the *Serazym®* Giardia was determined by titration of cysts, trophozoites and recombinant CWP-1, diluted in a negative stool sample:
1.6 x 10³ cysts, 6.3 x 10⁴ trophozoites and 3.1 ng CWP-1 per ml stool suspension.

Specificity and sensitivity

Specificity: In all n = 320 stool samples from routine microbiology laboratory were investigated by the *Serazym®* Giardia: Negative: n = 317, Positive: n = 3, Specificity: 99.1%.

The 3 ELISA positive samples were reinvestigated by direct immunofluorescence and confirmed as true positive. Specificity amended: 100%.

Sensitivity: Human stool samples designated Giardia positive were tested in the *Serazym®* Giardia in comparison to 2 commercially available ELISAs.

n = 33 Human stool samples	ELISA 1	ELISA 1
	positive	negative
<i>Serazym®</i> ELISA positive	30	0
<i>Serazym®</i> ELISA negative	1	2

Sensitivity compared to ELISA 1: 96.8%

n = 23 Human stool samples	ELISA 2	ELISA 2
	positive	negative
<i>Serazym®</i> ELISA positive	19	2*
<i>Serazym®</i> ELISA negative	0	2

Sensitivity compared to ELISA 2: 100%

* Both samples with a positive test result in the *Serazym*[®] Giardia and a negative test result in ELISA 2 were confirmed true positive by direct immunofluorescence and ELISA 1.
Faecal samples from animals belonging to 7 different species were tested by *Serazym*[®] Giardia in comparison to 2 commercially available assays.

n = 60 animal faecal samples	ELISA 1 positive	ELISA 1 negative
<i>Serazym</i> [®] ELISA positive	48	0
<i>Serazym</i> [®] ELISA negative	4	8

Sensitivity compared to ELISA 1: 92.3%

n = 54 animal faecal samples	ELISA 2 positive	ELISA 2 negative
<i>Serazym</i> [®] ELISA positive	40	7*
<i>Serazym</i> [®] ELISA negative	1**	6

Sensitivity compared to ELISA 2: 97.6%

* The 7 samples with negative test result in ELISA 2 but positive in the *Serazym*[®] ELISA were confirmed true positive by direct immunofluorescence and ELISA 1.

** The *Serazym*[®] ELISA negative but ELISA 2 positive sample was tested negative by direct immunofluorescence and ELISA 1.

In all 88 samples (human and animal samples) designated Giardia positive were tested in parallel by *Serazym*[®] Giardia and direct immuno fluorescence.

n = 88 faecal and stool samples	IFT positive	IFT negative
<i>Serazym</i> [®] ELISA positive	78	1*
<i>Serazym</i> [®] ELISA negative	1	8

Sensitivity compared to IFT: 98.7%

* The ELISA positive, IFT negative sample reacted positive in both comparative ELISAs 1 and 2 as well.

Cross reactivity

Stool samples positive for one of the subsequent pathogens have been tested with the *Serazym*[®] Giardia and showed no cross reactivity:

Adenovirus (n = 2), *Rotavirus* (n = 6), *Astrovirus* (n = 4), *Norovirus* (n = 6), *Clostridium difficile* (n = 2), *Campylobacter jejuni* (n = 6), *Helicobacter pylori* (n = 7), *Salmonella ssp.* (n = 9), *Ancylostoma duodenale* (n = 1), *Ascaris lumbricoides* (n = 2), *Blastocystis hominis* (n = 1), *Cryptosporidium parvum* (n = 4), *Entamoeba dispar/histolytica* (n = 7).

Negative stool samples have been spiked with $\geq 10^3$ colony forming units per ml of the following microorganisms and were tested negative with the *Serazym*[®] Giardia (OD 450 / 620 nm < Cut-Off):

<i>Aeromonas hydrophila</i>	(ATCC 7966)
<i>Bacillus cereus</i>	(ATCC 11778)
<i>Bacillus subtilis</i>	(ATCC 6633)
<i>Bacteroides fragilis</i>	(ATCC 25285)
<i>Candida albicans</i>	(ATCC 10231)
<i>Campylobacter coli</i>	(ATCC 33559)
<i>Campylobacter jejuni</i>	(ATCC 33291)
<i>Citrobacter freundii</i>	(ATCC 8090)
<i>Clostridium sordellii</i>	(ATCC 9714)
<i>Enterobacter aerogenes</i>	(ATCC 13048)
<i>Enterobacter cloacae</i>	(ATCC 13047)
<i>Enterococcus faecalis</i>	(ATCC 29212)
<i>Escherichia coli</i>	(ATCC 25922)
<i>Klebsiella pneumoniae</i>	(ATCC 13883)

<i>Peptostreptococcus anaerobius</i>	(ATCC 27337)
<i>Proteus vulgaris</i>	(ATCC 8427)
<i>Pseudomonas aeruginosa</i>	(ATCC 10145)
<i>Salmonella enterica</i> Serovar <i>enteritidis</i>	(ATCC 13076)
<i>Salmonella enterica</i> Serovar <i>typhimurium</i>	(ATCC 14028)
<i>Shigella flexneri</i>	(ATCC 12022)
<i>Shigella sonnei</i>	(ATCC 25931)
<i>Staphylococcus aureus</i>	(ATCC 25923)
<i>Staphylococcus epidermidis</i>	(ATCC 12228)
<i>Vibrio parahaemolyticus</i>	(ATCC 17802)
<i>Vibrio cholerae</i>	Clinical isolate
<i>Yersinia enterocolitica</i> Serotyp <i>O3, O9</i>	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 (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
2020-10-19	Intended use	Correction
	Assay procedure	Update
	Cross reactivity	Extension
	Common Advices and Precautions	Update
	History of Changes	New section "History of Changes"

Incubation Scheme Serazym® Giardia (E-106)

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

 Manufacturer	 Date of manufacture	 Use by	LOT Batch code	REF Catalog number
 Keep away from sunlight	 Temperature limits	 Biological risks	 Do not reuse	
 Consult instructions for use	 Caution	IVD In-vitro-diagnostic medical device	 Contains sufficient for <n> tests	

NovaLisa®

Measles Virus IgG

ELISA

CE

Only for in-vitro diagnostic use

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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 contagiousness 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 $\pm$ 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 $< \text{Cut-off}$
- **Cut-off Control:** Absorbance value **0.150 – 1.300**
- **Positive Control:** Absorbance value $> \text{Cut-off}$

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]

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

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

9.3. Interpretation of Results

	NTU	mIU/mL (3 rd 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

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

<u>Intraassay</u>	<u>n</u>	<u>Mean (E)</u>	<u>CV (%)</u>
#1	24	0.314	6.93
#2	24	0.931	3.70
#3	24	0.718	9.98
<u>Interassay</u>	<u>n</u>	<u>Mean (NTU)</u>	<u>CV (%)</u>
#1	12	33.93	2.76
#2	12	28.09	7.46
#3	12	3.85	12.11

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: 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%).

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.: MEAG0330 Measles Virus IgG ELISA (96 Determinations)

For avidity testing:

AMEA7330 Avidity Measles Virus IgG ELISA (48 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. Dadurch kommt es zu einer 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 (Aufreten 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)	Masern	Fieber, Malaise, produktiver 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
(auf English Measles Virus)	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 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 immunoenzymatische 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.

NovaLisa®

Avidity Measles Virus IgG

ELISA Supplementary Instruction



Only for in-vitro diagnostic use

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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; $\leq 0.02\%$ (v/v) MIT.
- **Control High:** 1 vial containing 2 mL control; coloured yellow; ready to use; red cap; $\leq 0.02\%$ (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 $\pm$ 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 immunoenzymatische 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 Waschschrift 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 Waschschrift 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; $\leq 0,02\%$ (v/v) MIT.
- **Kontrolle Hoch:** 1 Fläschchen mit 2 mL Kontrolle; gelb gefärbt; gebrauchsfertig; rote Verschlusskappe; $\leq 0,02\%$ (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.

NovaLisa®

Mumps Virus IgG

ELISA

CE

Only for in-vitro diagnostic use

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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 spike 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 occurring 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

The presence of pathogen or infection 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.

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

- **Mumps Virus Coated Microplate (IgG):** 12 break-apart 8-well snap-off strips coated with Mumps Virus 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.
- **Mumps Virus 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.
- **Mumps Virus IgG Positive Control:** 1 vial containing 2 ml control (human serum or plasma); coloured yellow; ready to use; red cap.
- **Mumps Virus IgG Cut-off Control:** 1 vial containing 3 ml control (human serum or plasma); coloured yellow; ready to use; green cap.
- **Mumps Virus 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 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. 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 $\pm$ 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 $< \text{Cut-off}$
- **Cut-off Control:** Absorbance value **0.150 – 1.300**
- **Positive Control:** Absorbance value $> \text{Cut-off}$

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]

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

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

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

- 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.: MUMG0340 Mumps Virus IgG ELISA (96 Determinations)

DEUTSCH

1. EINLEITUNG

Mumpsviren sind Einzelstrang-RNA-Viren der Familie der Paramyxoviridae. Sie haben eine lipidhaltige Hülle, einen Durchmesser von etwa 150-250 nm und eine pleomorphe Gestalt. Das Mumpsvirus ist der Erreger der Parotitis epidemica (Mumps).

Mumps ist weltweit verbreitet und tritt meist epidemisch im Kindesalter auf, bevorzugt in der kalten Jahreszeit. Bei etwa 30% der Infizierten verläuft die Erkrankung inapparent. Die Übertragung erfolgt aerogen durch Tröpfchen, selten durch Schmierinfektion, direkt von Mensch zu Mensch und hinterlässt lebenslange Immunität.

Das Mumpsvirus infiziert primär die Epithelien des oberen Respirationstraktes, des Gastrointestinaltraktes oder der Augen. Nach initialer Replikation kommt es zur Aussaat in regionale Lymphknoten, von wo aus nach weiteren Replikationsschritten eine erste Virämie zur Infektion weiterer Organe wie Speicheldrüsen, Brustdrüsen, Testes, Ovarien, ZNS und Pankreas führt. Beim symptomatischen Verlauf treten nach der Inkubationszeit (ca. 7-21 Tage) Fieber, Kopfschmerzen und ein allgemeines Krankheitsgefühl auf. Leitsymptom der Parotitis epidemica ist die schmerzhafte Schwellung erst der einen, nach ein bis zwei Tagen auch der anderen Ohrspeicheldrüse. Komplikationen der Infektion sind:

- Orchitis und Epididymitis bei etwa 20% der Infizierten (beim doppelseitigen Befall führt sie in 80% zur Sterilität)
- Meningitis bei etwa 20% der Patienten etwa um den 18. Tag nach Krankheitsbeginn (in der Regel rasche, folgenlose Rückbildung)
- Meningoenzephalitis (sehr viel seltener (<1%) als die reine Meningitis, aber mit höherer Letalität (5%); Ausheilung mit Residuen möglich (5%))
- Myokarditis, Pankreatitis, Adnexitis bei Frauen (seltene Komplikationen)

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

Spezies	Erkrankung	Symptome (z.B.)	Infektionsweg
Mumps Virus	Parotitis epidemica (Mumps, Ziegenpeter)	Fieber, Kopfschmerzen, Krankheitsgefühl, ein- oder beidseitige Parotisschwellung; Glandulae sublinguales und submandibulares häufig mitbetroffen Komplikationen: Orchitis, Epididymitis, Meningitis, Meningoenzephalitis, Myokarditis, Pankreatitis, Adnexitis	Aerogen durch Tröpfchen selten durch Schmierinfektion

Nachweis des Erregers bzw. der Infektion durch:

- PCR
- Serologie: z.B. ELISA

2. VERWENDUNGSZWECK

Der Mumps Virus IgG ELISA ist für den qualitativen Nachweis spezifischer IgG-Antikörper gegen Mumps Virus 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 Waschschriff 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

- **Mumps Virus beschichtete Mikrotiterplatte (IgG):** 12 teilbare 8er-Streifen, beschichtet mit Mumps Virus Antigenen; in wieder verschließbarem Aluminiumbeutel.
- **IgG-Probenverdünnungspuffer:** 1 Flasche mit 100 ml Phosphatpuffer (10 mM) zur Probenverdünnung; pH 7,2 ± 0,2; gelb gefärbt; gebrauchsfertig; weiße Verschlusskappe.
- **Stopplösung:** 1 Flasche mit 15 ml Schwefelsäure, 0,2 mol/l; gebrauchsfertig; rote Verschlusskappe.
- **Waschpuffer (20x konz.):** 1 Flasche mit 50 ml eines 20-fach konzentrierten Phosphatpuffers (0,2 M), zum Waschen der Kavitäten; pH 7,2 ± 0,2; weiße Verschlusskappe.
- **Mumps Virus anti-IgG Konjugat:** 1 Flasche mit 20 ml Peroxidase-konjugierten Antikörpern gegen humanes IgG in Phosphatpuffer (10 mM); blau gefärbt; gebrauchsfertig; schwarze Verschlusskappe.
- **TMB-Substratlösung:** 1 Flasche mit 15 ml 3,3',5,5'-Tetramethylbenzidin (TMB), < 0,1 %; gebrauchsfertig; gelbe Verschlusskappe; < 5% NMP.
- **Mumps Virus IgG Positivkontrolle:** 1 Fläschchen mit 2 ml Kontrolle (humanes Serum oder Plasma); gelb gefärbt; rote Verschlusskappe; gebrauchsfertig.
- **Mumps Virus IgG Cut-off Kontrolle:** 1 Fläschchen mit 3 ml Kontrolle (humanes Serum oder Plasma); gelb gefärbt; grüne Verschlusskappe; gebrauchsfertig.
- **Mumps Virus IgG Negativkontrolle:** 1 Fläschchen mit 2 ml Kontrolle (humanes Serum oder Plasma); gelb gefärbt; blaue Verschlusskappe; gebrauchsfertig.

Für potenzielle Gefahrstoffe überprüfen Sie bitte das Sicherheitsdatenblatt.

4.2. Mitgeliefertes Zubehör

- 1 selbstklebende Abdeckfolie
- 1 Arbeitsanleitung
- 1 Plattenlayout

4.3. Erforderliche Materialien und Geräte

- Mikrotiterplatten-Photometer mit Filtern 450/620 nm
- Inkubator 37 °C
- Manuelle oder automatische Waschvorrichtung
- Mikropipetten (10 - 1000 µl)
- Vortex-Mischer
- Destilliertes Wasser
- Plastikröhrchen für den einmaligen Gebrauch

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. Beschichtete Mikrotiterplatte

Die abbrechbaren Streifen sind mit Mumps Virus Antigenen beschichtet. Nicht verbrauchte Vertiefungen im Aluminiumbeutel zusammen mit dem Trockenmittel sofort wieder verschließen und bei 2...8 °C lagern.

6.2. Waschpuffer (20x konz.)

Der Waschpuffer ist im Verhältnis 1 + 19 zu verdünnen; z.B. 10 ml Waschpuffer + 190 ml destilliertes Wasser.

Der verdünnte Puffer ist bei Raumtemperatur (20...25 °C) 5 Tage haltbar. Sollten Kristalle im Konzentrat auftreten, die Lösung z.B. in einem Wasserbad auf 37 °C erwärmen und vor dem Verdünnen gut mischen.

6.3. TMB-Substratlösung

Die gebrauchsfertige Lösung ist bei 2...8 °C vor Licht geschützt aufzubewahren. Die Lösung ist farblos, kann aber auch leicht hellblau sein. Sollte die TMB-Substratlösung blau sein, ist sie kontaminiert und kann nicht im Test verwendet werden.

7. ENTNAHME UND VORBEREITUNG DER PROBEN

Es sollten humane Serum- oder Plasmaproben (Citrat, Heparin) verwendet werden. Für Liquor ist die Arbeitsanleitung ABVL0001 zu verwenden. Werden die Bestimmungen innerhalb von 5 Tagen nach Blutentnahme durchgeführt, können die Proben bei 2...8 °C aufbewahrt werden, sonst aliquotieren und tiefgefrieren (-70...-20 °C). Wieder aufgetaute Proben vor dem Verdünnen gut schütteln. Wiederholtes Tiefgefrieren und Auftauen vermeiden! Hitzeeinaktivierung der Proben wird nicht empfohlen.

7.1. Probenverdünnung

Proben vor Testbeginn im Verhältnis 1 + 100 mit IgG-Probenverdünnungspuffer verdünnen, z. B. 10 µl Probe und 1 ml IgG-Probenverdünnungspuffer in die entsprechenden Röhrchen pipettieren, um eine Verdünnung von 1 + 100 zu erhalten; gut mischen (Vortex).

8. TESTDURCHFÜHRUNG

8.1. Testvorbereitung

Arbeitsanleitung **vor** Durchführung des Tests sorgfältig lesen. Für die Zuverlässigkeit der Ergebnisse ist es notwendig, die Arbeitsanleitung genau zu befolgen. Die folgende Testdurchführung ist für die manuelle Methode validiert. Beim Arbeiten mit ELISA Automaten empfehlen wir, um Wascheffekte auszuschließen, die Zahl der Waschschrte von drei auf fünf und das Volumen des Waschpuffers von 300 µl auf 350 µl zu erhöhen. Kapitel 12 beachten. Vor Testbeginn auf dem mitgelieferten Plattenlayout die Verteilung bzw. Position der Proben und der Standards/Kontrollen (Doppelbestimmung empfohlen) genau festlegen. Die benötigte Anzahl von Mikrotiterstreifen (Kavitäten) in den Streifenhalter einsetzen.

Den Test in der angegebenen Reihenfolge und ohne Verzögerung durchführen.

Für jeden Pipettierschritt der Standards/Kontrollen und Proben saubere Einmalspitzen verwenden.

Den Inkubator auf 37 ± 1 °C einstellen.

1. Je 100 µl Standards/Kontrollen und vorverdünnte Proben in die entsprechenden Vertiefungen pipettieren. Vertiefung A1 ist für den Substratleerwert vorgesehen.
2. Die Streifen mit der mitgelieferten Abdeckfolie bedecken.
3. **1 h $\pm$ 5 min bei 37 °C inkubieren.**
4. Am Ende der Inkubationszeit Abdeckfolie entfernen und die Inkubationsflüssigkeit aus den Teststreifen absaugen. Anschließend dreimal mit 300 µl Waschpuffer waschen. Überfließen von Flüssigkeit aus den Vertiefungen vermeiden. Das Intervall zwischen Waschen und Absaugen sollte > 5 sec betragen. Nach dem Waschen die Teststreifen auf Fließpapier ausklopfen, um die restliche Flüssigkeit zu entfernen.
Beachte: Der Waschvorgang ist wichtig, da unzureichendes Waschen zu schlechter Präzision und falschen Messergebnissen führt!
5. 100 µl Konjugat in alle Vertiefungen, mit Ausnahme der für die Berechnung des Leerwertes A1 vorgesehenen, pipettieren.
6. **30 min bei Raumtemperatur (20...25 °C) inkubieren.** Nicht dem direkten Sonnenlicht aussetzen.
7. Waschvorgang gemäß Punkt 4 wiederholen.
8. 100 µl TMB-Substratlösung in alle Vertiefungen pipettieren.
9. **Genau 15 min im Dunkeln bei Raumtemperatur (20...25 °C) inkubieren.** Bei enzymatischer Reaktion findet eine Blaufärbung statt.
10. In alle Vertiefungen 100 µl Stopplösung in der gleichen Reihenfolge und mit den gleichen Zeitintervallen wie bei Zugabe der TMB-Substratlösung pipettieren, dadurch erfolgt ein Farbwechsel von blau nach gelb.
11. Die Extinktion der Lösung in jeder Vertiefung bei 450/620 nm innerhalb von 30 min nach Zugabe der Stopplösung messen.

8.2. Messung

Mit Hilfe des Substratleerwertes den **Nullabgleich** des Mikrotiterplatten-Photometers vornehmen.

Falls diese Eichung aus technischen Gründen nicht möglich ist, muss nach der Messung der Extinktionswert des Substratleerwertes von allen anderen Extinktionswerten subtrahiert werden, um einwandfreie Ergebnisse zu erzielen!

Extinktion aller Kavitäten bei **450 nm** messen und die Messwerte der Standards/Kontrollen und Proben in das Plattenlayout eintragen.

Eine **bichromatische** Messung mit der Referenzwellenlänge 620 nm wird empfohlen.

Falls Doppel- oder Mehrfachbestimmungen durchgeführt wurden, den **Mittelwert der Extinktionswerte** berechnen.

9. BERECHNUNG DER ERGEBNISSE

9.1. Testgültigkeitskriterien

Der Test wurde richtig durchgeführt, wenn er folgende Kriterien erfüllt:

- **Substrat-Leerwert:** Extinktionswert < **0,100**
- **Negativkontrolle:** Extinktionswert < **0,200** und < **Cut-off**
- **Cut-off Kontrolle:** Extinktionswert **0,150 – 1,300**
- **Positivkontrolle:** Extinktionswert > **Cut-off**

Sind diese Kriterien nicht erfüllt, ist der Testlauf ungültig und muss wiederholt werden.

9.2. Messwertberechnung

Der Cut-off ergibt sich aus dem Mittelwert der gemessenen Extinktionen der Cut-off Kontrolle.

Beispiel: 0,44 OD Cut-off Kontrolle + 0,42 OD Cut-off Kontrolle = 0,86: 2 = 0,43

Cut-off = 0,43

9.2.1. Ergebnisse in Einheiten [NTU]

$$\frac{\text{Mittlere Extinktion der Probe} \times 10}{\text{Cut-off}} = [\text{NovaTec Einheiten} = \text{NTU}]$$

Beispiel:
$$\frac{1,591 \times 10}{0,43} = 37 \text{ NTU}$$

9.3. Interpretation der Ergebnisse

Cut-off	10 NTU	-
Positiv	> 11 NTU	Es liegen Antikörper gegen den Erreger vor. Ein Kontakt mit dem Antigen (Erreger bzw. Impfstoff) hat stattgefunden.
Grenzwertig	9 – 11 NTU	Antikörper gegen den Erreger können nicht eindeutig nachgewiesen werden. Es wird empfohlen den Test nach 2 bis 4 Wochen mit einer frischen Patientenprobe zu wiederholen. Finden sich die Ergebnisse erneut im grenzwertigen Bereich, gilt die Probe als negativ .
Negativ	< 9 NTU	Es liegen keine Antikörper gegen den Erreger vor. Ein vorausgegangener Kontakt mit dem Antigen (Erreger bzw. Impfstoff) ist unwahrscheinlich.
Die Diagnose einer Infektionskrankheit darf nicht allein auf der Basis des Ergebnisses einer Bestimmung gestellt werden. Die anamnestischen Daten sowie die Symptomatologie des Patienten müssen zusätzlich zu den serologischen Ergebnissen in Betracht gezogen werden. Bei Immunsupprimierten und Neugeborenen besitzen die Ergebnisse serologischer Tests nur einen begrenzten Wert.		

9.3.1. Antikörper-Isotypen und Infektionsstatus

Serologie	Bedeutung
IgM	Typisch für Primärantwort Hoher IgM-Titer bei gleichzeitig niedrigem IgG-Titer: → Hinweis auf relativ frische Infektion Selten: → persistierendes IgM
IgG	Typisch für Sekundärantwort Können auch noch nach Jahren nachweisbar sein Hoher IgG-Titer bei gleichzeitig niedrigem IgM-Titer: → wahrscheinlich länger zurückliegende Infektion

10. TESTMERKMALE

Die Ergebnisse beziehen sich auf die untersuchten Probenkollektive; es handelt sich nicht um garantierte Spezifikationen. Für weitere Informationen zu den Testmerkmalen kontaktieren Sie bitte NovaTec Immundiagnostica GmbH.

10.1. Präzision

Intraassay	n	Mittelwert (E)	Vk (%)
#1	24	0,411	4,14
#2	24	1,173	4,32
#3	24	1,338	1,41
Interassay	n	Mittelwert (NTU)	Vk (%)
#1	12	30,07	4,58
#2	12	31,66	5,52
#3	12	2,87	9,27

10.2. Diagnostische Spezifität

Die diagnostische Spezifität ist definiert als die Wahrscheinlichkeit des Tests, ein negatives Ergebnis bei Fehlen des spezifischen Analyten zu liefern. Sie beträgt 95,83% (95% Konfidenzintervall: 85,75% - 99,49%).

10.3. Diagnostische Sensitivität

Die diagnostische Sensitivität ist definiert als die Wahrscheinlichkeit des Tests, ein positives Ergebnis bei Vorhandensein des spezifischen Analyten zu liefern. Sie ist 93,55% (95% Konfidenzintervall: 89,41% - 96,43%).

10.4. Interferenzen

Hämolytische, lipämische und ikterische Proben ergaben bis zu einer Konzentration von 10 mg/ml für Hämoglobin, von 5 mg/ml Triglyceride und von 0,5 mg/ml für Bilirubin keine Interferenzen im vorliegenden ELISA.

10.5. Kreuzreaktivität

Die Untersuchung eines Probenpanels mit Antikörperaktivitäten gegen potenziell kreuzreagierende Parameter ließ keine Anzeichen von falsch-positiven Ergebnissen aufgrund von Kreuzreaktivitäten erkennen.

11. GRENZEN DES VERFAHRENS

Kontamination der Proben durch Bakterien oder wiederholtes Einfrieren und Auftauen können zu einer Veränderung der Messwerte führen.

12. SICHERHEITSMASSNAHMEN UND WARNHINWEISE

- Gemäß Art. 1 Abs. 2b der EU-Richtlinie 98/79/EG legt der Hersteller die Zweckbestimmung von In-vitro-Diagnostika fest, um deren Eignung, Leistung und Sicherheit sicherzustellen. Daher sind die Testdurchführung, die Information, die Sicherheitsmaßnahmen und Warnhinweise in der Arbeitsanleitung strikt zu befolgen. Bei Anwendung des Testkits auf Diagnostika-Geräten ist die Testmethode zu validieren. Jede Änderung am Aussehen, der Zusammensetzung und der Testdurchführung sowie jede Verwendung in Kombination mit anderen Produkten, die der Hersteller nicht autorisiert hat, ist nicht zulässig; der Anwender ist für solche Änderungen selbst verantwortlich. Der Hersteller haftet für falsche Ergebnisse und Vorkommnisse aus solchen Gründen nicht. Auch für falsche Ergebnisse aufgrund von visueller Auswertung wird keine Haftung übernommen.
- Nur für in-vitro-Diagnostik.
- Alle Materialien menschlichen oder tierischen Ursprungs sind als potentiell infektiös anzusehen und entsprechend zu behandeln.
- Alle verwendeten Bestandteile menschlichen Ursprungs sind auf Anti-HIV-AK, Anti-HCV-AK und HBsAg nicht-reaktiv getestet.
- Reagenzien und Mikrotiterplatten unterschiedlicher Chargen nicht untereinander austauschen.
- Keine Reagenzien anderer Hersteller zusammen mit den Reagenzien dieses Testkits verwenden.
- Nicht nach Ablauf des Verfallsdatums verwenden.
- Nur saubere Pipettenspitzen, Dispenser und Labormaterialien verwenden.
- Verschlusskappen der einzelnen Reagenzien nicht untereinander vertauschen, um Kreuzkontaminationen zu vermeiden.
- Flaschen sofort nach Gebrauch fest verschließen, um Verdunstung und mikrobielle Kontamination zu vermeiden.
- Nach dem ersten Öffnen Konjugat und Standards/Kontrollen vor weiterem Gebrauch auf mikrobielle Kontamination prüfen.
- Zur Vermeidung von Kreuzkontamination und falsch erhöhten Resultaten, Reagenzien sorgfältig in die Kavitäten pipettieren.
- Der ELISA ist nur für die Anwendung durch Fachpersonal vorgesehen, welches die Arbeitstechniken einwandfrei beherrscht.

12.1. Entsorgungshinweise

Chemikalien und Zubereitungen sind in der Regel Sonderabfälle. Deren Beseitigung unterliegt den nationalen abfallrechtlichen Gesetzen und Verordnungen. Die zuständige Behörde informiert über die Entsorgung von Sonderabfällen.

13. BESTELLINFORMATIONEN

Produktnummer: MUMG0340 Mumps Virus IgG ELISA (96 Bestimmungen)

FRANÇAIS

1. INTRODUCTION

Les virus des oreillons sont des virus ARN de la famille Paramyxoviridae. Les virions sont des particules sphériques de 150-250 nm de diamètre qui se composent d'une ribonucléoprotéine avec une symétrie hélicoïdale et qui sont enveloppées par une protéine matrice et une double couche lipidique qui contient deux autres protéines: hemagglutinin viral (H) et neuraminidase virale (N). Le virus des oreillons atteint surtout la parotide et les glandes salivaires reliées; cependant l'infection peut mener à une maladie du SNC et à l'accumulation du virus dans le liquide céphalo-rachidien. Les oreillons (parotidite épidémique) sont une maladie virale contagieuse aiguë qui se produit surtout chez les enfants. Presque 50% de toutes les infections sont subcliniques. Le plus haute incidence des manifestations cliniques est trouvée dans la catégorie d'âge de 4 à 15 ans. Les infections secondaires sont rares à cause de l'immunité durable. 10 à 35 % des cas d'oreillons développent l'orchite qui se produit presque toujours après la puberté. Pour la plupart du temps, le processus est unilatéral et d'habitude le pronostic est bon. Le virus des oreillons a été l'une des causes les plus importantes de la maladie virale du SNC (méningite et encéphalite) aux Etats-Unis; l'administration des vaccinations a considérablement réduit cette maladie.

Espèce	La maladie	Symptômes (p.ex.)	Modes de transmission
Virus des oreillons (en Anglais Mumps Virus)	Oreillons	Fièvre et gonflement unilatéral ou bilatéral de la glande parotide; les glandes sublingual peuvent également être impliquées Complications: Orchite, Méningo-encéphalite, Pancréatites	La transmission du virus se produit par l'infection de gouttelettes; La transmission du virus se produit par une infection gouttelette;

L'identification de l'agent pathogène ou d'une infection par:

- PCR
- Sérologie: p.ex. ELISA

2. INDICATION D'UTILISATION

La trousse Mumps Virus IgG ELISA est prévue pour la détection qualitative des anticorps IgG anti-Mumps Virus (**Virus des Oreillons**) dans le sérum humain ou plasma (citrate, héparine).

3. PRINCIPE DU TEST

La détermination immunoenzymatique qualitative des anticorps spécifiques est basée sur la technique ELISA (du anglais, Enzyme-Linked Immunosorbent Assay).

Microplaques sont recouvertes d'antigènes spécifiques pour lier les anticorps correspondants de l'échantillon. Après le lavage des puits pour éliminer l'échantillon détaché, le conjugué peroxydase de raifort (HRP) est ajouté. Ce conjugué se lie aux anticorps capturés. Dans une deuxième étape de lavage, le conjugué non lié est éliminé. Le complexe immun formé par le conjugué lié est visualisé par l'addition tétraméthylbenzidine (TMB) qui donne un produit de réaction bleu.

L'intensité de ce produit est proportionnelle à la quantité d'anticorps spécifiques dans l'échantillon. L'acide sulfurique est ajouté pour arrêter la réaction. Cela produit un changement du bleu au jaune. L'absorbance à 450/620 nm est lue en utilisant un lecteur de microplaques ELISA.

4. MATERIEL

4.1. Réactifs fournis

- **Mumps Virus IgG microplaque revêtus:** 12 barrettes de 8 puits sécables revêtus d'antigène d'Virus des Mumps; en sachets d'aluminium refermables.
- **Diluant pour échantillon IgG:** 1 flacon contenant 100 ml de tampon phosphaté (10 mM) pour la dilution de l'échantillon; pH $7,2 \pm 0,2$; prêt à l'emploi; couleur jaune; bouchon blanc.
- **Solution d'arrêt:** 1 flacon contenant 15 ml d'acide sulfurique, 0,2 mol/l; prêt à l'emploi; bouchon rouge.
- **Tampon de lavage (concentré x 20):** 1 flacon contenant 50 ml d'un tampon phosphaté (0,2 M) concentré 20 fois (pH $7,2 \pm 0,2$) pour laver les puits; bouchon blanc.
- **Conjugué Mumps Virus anti-IgG:** 1 flacon contenant 20 ml d'anticorps IgG anti-humaines conjuguées à de la peroxydase du raifort dans le tampon phosphaté (10 mM); prêt à l'emploi; couleur bleue, bouchon noir.
- **Solution de substrat TMB:** 1 flacon contenant 15 ml de 3,3',5,5'-tétraméthylbenzidine (TMB), < 0,1 %; prêt à l'emploi; bouchon jaune; < 5 % NMP.
- **Contrôle positif Mumps Virus IgG:** 1 flacon contenant 2 ml contrôle (sérum humain ou plasma); prêt à l'emploi; couleur jaune; bouchon rouge.
- **Contrôle cut-off Mumps Virus IgG:** 1 flacon contenant 3 ml contrôle (sérum humain ou plasma); prêt à l'emploi; couleur jaune; bouchon vert.
- **Contrôle négatif Mumps Virus IgG:** 1 flacon contenant 2 ml contrôle (sérum humain ou plasma); prêt à l'emploi; couleur jaune; bouchon bleu.

Pour les substances potentiellement dangereuses s'il vous plaît vérifiez la fiche de données de sécurité.

4.2. Matériel fourni

- 1 couvercle autocollante
- 1 instructions d'utilisation
- 1 présentation de la plaque

4.3. Matériel et équipement requis

- Lecteur de microplaques ELISA, pour mesurer l'absorbance à 450/620 nm
- Incubateur 37 °C
- Laveur manuel ou automatique pour le lavage des puits
- Pipettes pour utilisation entre 10 et 1000 µl
- Mélangeur Vortex
- Eau distillée
- Tubes jetables

5. STABILITE ET CONSERVATION

Conserver le kit à 2...8 °C. Les réactifs ouverts sont stables jusqu'à la date de péremption indiquée sur l'étiquette lorsqu'il est conservé à 2...8 °C.

6. PREPARATION DES REACTIFS

Il est très important porter tous les réactifs et échantillons à température ambiante (20... 25 °C) et les mélanger avant de commencer le test!

6.1. Microplaque revêtues

Les barrettes sécables sont revêtues d'antigène des Mumps. Immédiatement après avoir prélevé les barrettes nécessaires, les barrette restantes doivent être scellés le vide dans de feuille d'aluminium avec le sac de silicium (le déshydratant) fourni et emmagasiner à 2...8 °C.

6.2. Tampon de lavage (conc. x 20)

Diluer le Tampon de lavage 1+19; par exemple 10 ml du Tampon de lavage + 190 ml d'eau distillée. L'échantillon de tampon diluée est stable pendant 5 jours à la température ambiante (20...25 °C). Cas apparaissent des cristaux dans le concentré, chauffer la solution à 37 °C par exemple dans un bain-marie mélangez bien avant dilution.

6.3. Solution de substrat TMB

La solution est prête à utiliser et doit être emmagasiné à 2...8 °C, à l'abri de la lumière. La solution doit être incolore ou pourrait avoir une légère couleur bleu clair. Si le substrat devient bleu, il peut avoir été contaminé et ne peut pas être utilisé dans le test.

7. PRELEVEMENT ET PREPARATION DES ECHANTILLONS

Utiliser des échantillons humain de sérum ou plasma (citrate, héparine) pour ce test. Les instructions d'utilisation ABVL0001 doit être utilisé pour le LCS. Si le test est réalisé dans les 5 jours après le prélèvement, les échantillons doivent être conservés à 2...8 °C; autrement ils doivent être aliquotés et conservés surgelés (-70...-20 °C). Si les échantillons sont conservés congelés, bien mélanger les échantillons décongelés avant le test. Éviter les cycles répétés de congélation et décongélation. L'inactivation par la chaleur des échantillons n'est pas recommandée.

7.1. Dilution de l'échantillon

Avant du test, tous les échantillons doivent être dilués 1 + 100 avec diluant de l'échantillon IgG. Diluer 10 µl d'échantillon avec 1 ml I diluant de l'échantillon IgG dans des tubes pour obtenir une dilution 1 + 100 et mélanger soigneusement sur un Vortex.

8. PROCEDE DE TEST

8.1. Préparation du test

Lire attentivement les instructions d'utilisation **avant de** réaliser le test. La fiabilité des résultats dépend du suivi strict d'utilisation comme décrit. La technique de test suivante a été validée uniquement pour une procédure manuelle. Si le test doit être effectué sur un systèmes automatiques pour ELISA, nous conseillons d'augmenter le nombre d'étapes de lavage de trois à cinq et le volume du Tampon de lavage de 300 à 350 µl. Faites attention au chapitre 12. Avant de commencer le test, le plan de distribution et d'identification de tous les échantillons et les étalons/contrôles (il est recommandé déterminer en double) doivent être soigneusement établi sur la feuille présentation de la plaque prévue dans le conseil de kit. Sélectionner le nombre de barrettes ou de puits nécessaires et les placer sur le support.

Réaliser toutes les étapes du test dans l'ordre donné et sans délai.

Un embout de pipette propre et jetable doit être utilisé pour distribuer chaque étalon/contrôle et échantillon.

Régler l'incubateur à 37 ± 1 °C.

1. Pipeter 100 µl de étalons/contrôles et d'échantillons dilués dans leurs puits respectifs. Garder le puits A1 pour le blanc substrat.
2. Couvrir les puits avec le couvercle, fourni dans le kit.
3. **Incuber pendant 1 heure ± 5 minutes à 37 ± 1 °C.**
4. A la fin de l'incubation, enlever le couvercle, aspirer le contenu des puits et laver chaque puits trois fois avec 300 µl de Tampon de lavage. Éviter les débordements des puits de réaction. L'intervalle entre le cycle de lavage et l'aspiration doit être > 5 sec. À la fin, enlever soigneusement le liquide restant en tapotant les barrettes sur du papier absorbant avant la prochaine étape.
Note: L'étape de lavage est très importante! Un lavage insuffisant peut conduire à une précision faible et de faux résultats!
5. Pipeter 100 µl du conjugué dans tous les puits sauf le puits Blanc A1.
6. **Incuber pendant 30 minutes à température ambiante (20...25°C).** N'exposer pas à la lumière directe du soleil.
7. Répéter l'étape numéro 4.
8. Pipeter 100 µl de la solution de substrat TMB dans tous les puits.
9. **Incuber pendant exactement 15 minutes à température ambiante (20...25°C) dans l'obscurité.** Une couleur bleue se produit en raison d'une réaction enzymatique.
10. Pipeter 100 µl de la solution d'arrêt dans tous les puits dans le même ordre et à la même vitesse que pour la solution de substrat TMB, ainsi, il y a un changement du bleu au jaune.
11. Mesurer l'absorbance à 450/620 nm dans les 30 minutes après l'addition de la solution d'arrêt.

8.2. Mesure

Réglez le lecteur de microplaques ELISA à **zéro** en utilisant le **Blanc substrat**.

Si - pour des raisons techniques - le lecteur de microplaques ELISA ne peut pas être ajusté à zéro en utilisant le Blanc substrat, la valeur d'absorbance de cette doit être soustraire la valeur d'absorbance de toutes les autres valeurs d'absorbance mesurées afin d'obtenir des résultats fiables!

Mesurer l'absorbance de tous les puits à **450 nm** et enregistrer les valeurs d'absorbance pour chaque étalon/contrôle et échantillon dans la présentation de la plaque.

Il est recommandé d'effectuer la mesure **dichromatique** utilisant 620 nm comme longueur d'onde de référence.

Si doubles déterminations ont été effectuées, calculer **les valeurs moyennes d'absorbance**.

9. RESULTATS

9.1. Critères de validation

Afin de valider le test, les critères suivants doivent être respectés:

- **Blanc Substrat:** Valeur d'absorbance < **0,100**
- **Contrôle négatif:** Valeur d'absorbance < **0,200** et < **Cut-off**
- **Contrôle cut-off:** Valeur d'absorbance **0,150 – 1,300**
- **Contrôle positif:** Valeur d'absorbance > **Contrôle cut-off**

Lorsque ces critères ne sont pas remplis, le test n'est pas valide et doit être recommencé.

9.2. Calcul des résultats

La valeur seuil correspond à la moyenne des valeurs d'absorbance du Contrôle cut-off.

Exemple: $0,44 \text{ DO Contrôle cut-off} + 0,42 \text{ DO Contrôle cut-off} = 0,86 : 2 = 0,43$
Cut-off = 0,43

9.2.1. Résultats en unités [NTU]

$\text{Valeur (moyenne) d'absorbance de l'échantillon} \times \frac{10}{\text{Cut-off}} = [\text{unités NovaTec} = \text{NTU}]$

Exemple: $\frac{1,591 \times 10}{0,43} = 37 \text{ NTU}$

9.3. Interprétation des résultats

Cut-off	10 NTU	-
Positif	> 11 NTU	Les anticorps dirigés contre l'agent pathogène sont présents. Il ya eu un contact avec l'antigène (pathogène resp. vaccin).
Zone grise	9 – 11 NTU	Les anticorps dirigés contre l'agent pathogène ne pouvaient pas être détectés clairement. Il est recommandé de répéter le test avec un échantillon frais dans 2 à 4 semaines. Si le résultat est encore dans la zone grise l'échantillon est jugé négatif .
Négatif	< 9 NTU	L'échantillon ne contient pas d'anticorps contre l'agent pathogène. Un contact préalable avec l'antigène (pathogène resp. vaccin) est peu probable.

Le diagnostic d'une maladie infectieuse ne devrait pas être établi sur la base du résultat d'une seule analyse. Un diagnostic précis devrait prendre en considération l'histoire clinique, la symptomatologie ainsi que les données sérologiques. Les données sérologiques sont de valeur limitée dans le cas des patients immunodéprimés et des nouveaux-nés.

9.3.1. Isotypes d'anticorps et l'Etat de l'infection

Sérologie	Signification
IgM	Caractéristique de la réponse primaire du anticorps Titre élevé d'IgM avec une faible titre d'IgG: → suggère une infection très récente ou aiguë Rare: → persistante IgM
IgG	Caractéristique de la réponse secondaire du anticorps Peut persister pendant plusieurs années Des titres élevés d'IgG à faible titre d'IgM: → peuvent indiquer une infection ancienne

10. PERFORMANCES DU TEST

Ces résultats s'appuient sur les groupes d'échantillons étudiés; il n'agit pas de caractéristiques techniques garanties. Pour plus d'informations sur les performances du test s'il vous plaît contactez NovaTec Immundiagnostica GmbH.

10.1. Précision

Intra-essai	n	moyenne (E)	CV (%)
#1	24	0,411	4,14
#2	24	1,173	4,32
#3	24	1,338	1,41
Inter-essai	n	moyenne (NTU)	CV (%)
#1	12	30,07	4,58
#2	12	31,66	5,52
#3	12	2,87	9,27

10.2. Spécificité diagnostique

La spécificité diagnostique est définie comme la probabilité d'obtenir un résultat négatif en l'absence d'un analyte spécifique. Elle est 95,83% (95% Intervalle de confiance: 85,75% - 99,49%).

10.3. Sensibilité diagnostique

La sensibilité diagnostique est définie comme la probabilité d'obtenir un résultat positif en présence d'un analyte spécifique. Elle est 93,55% (95% Intervalle de confiance: 89,41% - 96,43%).

10.4. Interférences

Des échantillons hémolytiques ou lipémiques ou ictériques n'ont pas montré d'interférences, avec des concentrations jusqu'à 10 mg/ml de hémoglobine, 5 mg/ml de triglycérides et 0,5 mg/ml de bilirubine.

10.5. Réaction croisée

L'étude d'un panel d'échantillons avec des anticorps dirigés contre différents paramètres interférents n'a pas révélé de preuves de résultats faussement positifs dus à des réactions croisées.

11. LIMITES DE LA TECHNIQUE

Une contamination bactérienne ou des cycles de congélation/décongélation répétés de l'échantillon peuvent affecter les valeurs d'absorption.

12. PRECAUTIONS ET AVERTISSEMENTS

- En accord avec l'article 1 paragraphe 2b de la directive européenne 98/79/EC, l'utilisation des dispositifs médicaux de diagnostic in vitro est destinée par le fabricant à garantir le bien-fondé, les performances et la sécurité du produit. Par conséquent, la procédure de test, l'information, les précautions et mises en garde de la notice d'emploi, doivent être suivies de façon stricte. L'utilisation de ces trousses avec des automates ou dispositifs similaires doit être validée. Aucun changement de la conception, composition et procédure de test, ainsi que l'utilisation avec d'autres produits non approuvés par le fabricant, ne sont pas autorisés; seul l'utilisateur est responsable de tels changements. Le fabricant n'est pas responsable des faux résultats et des incidents dus à ces motifs. Le fabricant n'est pas responsable des résultats fournis par analyse visuelle des échantillons des patients.
- Uniquement pour diagnostic in vitro.
- Tous les matériaux d'origine humaine ou animale doivent être considérés et traités comme étant potentiellement infectieux.
- Tous les composants d'origine humaine utilisés pour la fabrication de ces réactifs ont été analysés et ont été trouvés non réactifs en Ag HBs, en anticorps anti-VHI 1 et 2 et en anticorps anti-VHC.
- Ne pas échanger les réactifs ou les barrettes provenant de différents lots de production.
- Ne pas utiliser de réactifs provenant d'autres fabricants avec les réactifs de cette trousse.
- Ne pas utiliser les réactifs après la date de péremption indiquée sur l'étiquette.
- Utiliser seulement des embouts de pipette, des distributeurs et du matériel de laboratoire propres.
- Ne pas échanger les bouchons des flacons, pour éviter la contamination croisée.
- Fermer soigneusement les flacons après utilisation pour éviter l'évaporation et la contamination microbienne.
- Avant une nouvelle utilisation, vérifier les flacons de conjugué et de étalon/contrôle, déjà utilisés, pour exclure une contamination microbienne.
- Pour éviter la contamination croisée et des résultats faussement élevés, introduire les échantillons de patients et les réactifs exactement au fond des puits sans éclabousser.
- Le ELISA est uniquement destinée à l'utilisation par un personnel compétent, maîtrisant parfaitement les techniques de travail.

12.1. Elimination des déchets

Les résidus des produits chimiques et des préparations sont considérés en général comme des déchets dangereux. L'élimination de ce type de déchet est réglementée par des lois et réglementations nationales et régionales. Contacter les autorités compétentes ou les sociétés de gestion des déchets pour obtenir des renseignements sur l'élimination des déchets dangereux.

13. INFORMATION POUR LES COMMANDES

Référence:	MUMG0340	Virus des Mumps ELISA (96 déterminations)

ITALIANO

1. INTRODUZIONE

Il Virus della parotite è a RNA a singola elica e appartiene alla famiglia dei paramyxoviridae. Presenta un involucro contenente lipidi e ha un diametro di ca 150-250 nm. Il virus causa la parotite epidemica (orecchioni), una malattia presente in tutto il mondo, contratta soprattutto da i bambini nel periodo freddo dell'anno. Nel 30 % circa dei casi la malattia è inapparente. Viene trasmessa attraverso goccioline e risulta con immunità durevole a vita. Il virus infetta soprattutto gli epiteli delle vie aeree superiori ma anche il tratto gastrointestinale e gli occhi. Dopo la replicazione iniziale si introduce nei linfonodi per poi infettare anche altri organi come le ghiandole salivari, le ghiandole mammarie, i testicoli, le ovaie, l'SNC e il pancreas. Dopo un periodo di incubazione di 7-21 giorni si manifesta con febbre, mal di testa e malessere in generale. Il sintomo classico della parotite è l'ingrossamento doloroso di una o delle due ghiandole parotidi. Le complicazioni dell'infezione sono: l'orchite e l'epididimite (20%) (nel 80% dei casi risulta con sterilità), meningite (20%), dopo il 18esimo giorno della infezione meningoencefalite (raramente <1% con una letalità del 5%), miocardite e pancreatite. Esiste un vaccino specifico.

Specie	Malattia	Sintomi (p.es.)	Via di trasmissione
Virus della parotite, Paramyxovirus (In inglese Mumps Virus)	Parotite epidemica (orecchioni)	La febbre e gonfiore unilaterale o bilaterale della parotite; le ghiandole sublinguali e sottomascellari possono anche essere coinvolti. Complicanze: Orchite, meningoencefalite, pancreatite	Trasmissione: aerea; contatto diretto con una persona infetta

La presenza di agenti patogeni o infezione può essere identificato mediante:

- PCR
- Sierologia: p.es. ELISA

2. USO PREVISTO

Il Mumps Virus IgG ELISA è un kit per la determinazione qualitativa degli anticorpi specifici della classe IgG per Mumps Virus (**Virus della Parotite**) nel siero o plasma (citrato, eparina) umano.

3. PRINCIPIO DEL TEST

La determinazione immunoenzimatico qualitativa degli anticorpi specifici si basa sulla tecnica ELISA (d'inglese Enzyme-linked immunosorbent assay).

Micropiastre sono rivestiti con antigeni specifici che si legano agli anticorpi presenti nel campione. Dopo aver lavato i pozzetti per rimuovere tutto il materiale campione non legato, il coniugato di perossidasi di rafano (HRP) è aggiunto. Questo coniugato si lega agli anticorpi catturati. In una seconda fase di lavaggio coniugato, non legato è rimosso. Il complesso immunitario formato dal coniugato legato sarà evidenziato aggiungendo tetrametilbenzidina (TMB) substrato che dà una colorazione blu. L'intensità di questa colorazione è direttamente proporzionale alla quantità di anticorpi specifici presenti nel campione. Acido solforico è aggiunto per bloccare la reazione. Questo produce un cambiamento di colore dal blu al giallo. Assorbanza a 450/620 nm viene letto utilizzando un lettore di micropiastre ELISA.

4. MATERIALI

4.1. Reagenti forniti

- **Mumps Virus IgG:** 12 strisce divisibili in 8 pozzetti, con adesi antigeni del Virus della Mumps; dentro una busta d'alluminio richiudibile.
- **Tampone diluente IgG:** 1 flacone contenente 100 ml di tampone fosfato (10 mM) per diluire i campioni; pH 7,2 ± 0,2; colore giallo; pronto all'uso; tappo bianco.
- **Soluzione bloccante:** 1 flacone contenente 15 ml di acido solforico, 0,2 mol/l, pronto all'uso; tappo rosso.
- **Tampone di lavaggio (20x conc.):** 1 flacone contenente 50 ml di un tampone fosfato concentrato 20 volte (0,2 M) per il lavaggio dei pozzetti; pH 7,2 ± 0,2; tappo bianco.
- **Coniugato Mumps Virus anti-IgG:** 1 flacone contenente 20 ml di anticorpi IgG anti-umani, coniugati a perossidasi in tampone fosfato (10 mM); colore azzurro; pronto all'uso; tappo nero.
- **Soluzione Substrato TMB:** 1 flacone contenente 15 ml di 3,3',5,5'-Tetrametilbenzidina (TMB), < 0,1 %; pronto all'uso; tappo giallo; < 5% NMP.
- **Controllo positivo Mumps Virus IgG:** 1 flacone da 2 ml controllo (siero o plasma umano); colore giallo; tappo rosso; pronto all'uso.
- **Controllo cut-off Mumps Virus IgG:** 1 flacone da 3 ml controllo (siero o plasma umano); colore giallo; tappo verde; pronto all'uso.
- **Controllo negativo Mumps Virus IgG:** 1 flacone da 2 ml controllo (siero o plasma umano); colore giallo; tappo blu; pronto all'uso.

Per le sostanze potenziali pericolose si prega di leggere la scheda di dati di sicurezza.

4.2. Accessori forniti

- 1 supporto per micropiastre
- 1 istruzione per l'uso
- 1 schema della piastra

4.3. Materiali e attrezzature necessari

- Fotometro per micropiastre con filtri da 450/620 nm
- Incubatrice 37 °C
- Lavatore, manuale o automatico, di micropiastre
- Micropipette per l'uso tra 10-1000 µl
- Vortex-Mixer
- Acqua distillata
- Provette monouso

5. MODALITÀ DI CONSERVAZIONE

Conservare il kit a 2...8 °C. I reagenti aperti sono stabili fino alla data di scadenza indicata sull'etichetta quando sono conservati a 2...8 °C.

6. PREPARAZIONE DEI REAGENTI

È molto importante, portare tutti i reagenti e campioni a temperatura ambiente (20...25 °C) e mescolare prima di iniziare il test.

6.1. Micropiastre rivestita

Le strisce divisibile sono rivestiti con l'antigeni del Virus della Mumps. Immediatamente dopo la rimozione degli strisce necessari, le strisce rimanenti devono essere sigillare nuovamente in un foglio di alluminio insieme con il sacchetto di gel di silice conservati a 2...8 °C.

6.2. Tampone di lavaggio (20x conc.)

Diluire il Tampone di lavaggio 1+19; per esempio: 10 ml del Tampone di lavaggio + 190 ml di acqua distillata. Il campione di tampone diluito è stabile per 5 giorni a temperatura ambiente (20...25 °C). Se cristalli appaiono nel concentrato, riscaldare la soluzione a 37 °C per esempio in un bagnomaria. Mescolare bene prima della diluizione.

6.3. Soluzione Substrato TMB

La soluzione sta pronta all'uso e deve essere conservata a 2...8 °C, al riparo dalla luce. La soluzione deve essere incolore o potrebbe avere un leggero colore blu chiaro. Se il substrato diventa blu, potrebbe essere stato contaminato e non può essere utilizzato nel test.

7. PRELIEVO E PREPARAZIONE DEI CAMPIONI

Per questo test si prega di usare campioni di siero o plasma (citrato, eparina) umano. Per il CSF, si prega di utilizzare le istruzioni per l'uso ABVL0001. Se il test è fatto entro 5 giorni dal prelievo i campioni possono essere conservati tra 2...8 °C. Altrimenti devono essere aliquotati e congelati tra (-70...-20 °C). Se i campioni sono conservati congelati, mescolare bene i campioni scongelati prima del test. Evitare cicli ripetuti di congelamento/scongelamento. L'inattivazione dei campioni per mezzo del calore non è raccomandata.

7.1. Diluizione dei campioni

Prima del test, diluire i campioni 1+100 con tampone diluente IgG. Per esempio, pipettare nelle provette 10 µl di campione + 1 ml di tampone IgG e mescolare bene (Vortex).

8. PROCEDIMENTO

8.1. Preparazione del test

Leggere bene le istruzioni per l'uso **prima** di iniziare il test. L'affidabilità dei risultati dipende dalla stretta aderenza alle istruzioni per l'uso di prova come descritto. La seguente procedura è stata validata per l'esecuzione manuale. Per un'esecuzione su strumentazione automatica si consiglia di incrementare il numero di lavaggi da 3 a 5 volte e il volume del Tampone di lavaggio da 300 a 350 µl per evitare effetti di lavaggio. Prestare attenzione al capitolo 12. Stabilire innanzitutto il piano di distribuzione e identificazione dei campioni e standards/controlli (è raccomandato determinare in duplicato) sullo schema della piastra fornito con il kit. Inserire i pozzetti necessari nel supporto.

Eseguire il test nell'ordine stabilito dalle istruzioni, senza ritardi.

Sul pipettaggio utilizzare puntali nuovi e puliti per ogni campione e standard/controllo.

Regolare l'incubatore a $37 \pm 1^\circ\text{C}$.

1. Pipettare 100 µl di standard/controllo e di campione diluito nei relativi pozzetti. Usare il pozzetto A1 per il Bianco-substrato.
2. Coprire i pozzetti con la pellicola adesiva, fornita nel kit.
3. **Incubare 1 ora $\pm$ 5 min a $37 \pm 1^\circ\text{C}$.**
4. Al termine dell'incubazione, togliere la pellicola e aspirare il liquido dai pozzetti. Successivamente lavare i pozzetti tre volte con 300 µl di tampone di lavaggio. Evitare che la soluzione trabocchi dai pozzetti. L'intervallo tra il lavaggio e l'aspirazione deve essere > 5 sec. Dopo il lavaggio picchiare delicatamente i pozzetti su una carta assorbente per togliere completamente il liquido, prima del passo successivo.
Attenzione: Il lavaggio è una fase molto importante. Da lavaggio insufficiente risulta una bassa precisione e risultati falsi!
5. Pipettare 100 µl di Coniugato in tutti i pozzetti, escludendo quello con il Bianco-substrato (Blank) A1.
6. **Incubare per 30 min a temperatura ambiente ($20...25^\circ\text{C}$).** Non esporre a fonti di luce diretta.
7. Ripetere il lavaggio secondo punto 4.
8. Pipettare 100 µl di Soluzione Substrato TMB in tutti i pozzetti.
9. **Incubare precisamente per 15 min a temperatura ambiente ($20...25^\circ\text{C}$) al buio.** Un colore blu verifica a causa della reazione enzimatica.
10. Pipettare 100 µl di Soluzione bloccante in tutti i pozzetti, nello stesso ordine della Soluzione Substrato TMB, in tal modo un cambiamento di colore dal blu al giallo avviene.
11. Misurare l'assorbanza a 450/620 nm entro 30 min dopo l'aggiunta della Soluzione bloccante.

8.2. Misurazione

Regolare il fotometro per le micropiastre ELISA **a zero** usando il substrato-Bianco (Blank).

Se, per motivi tecnici, non è possibile regolare il fotometro a zero usando il Bianco-substrato, il valore di assorbanza di questo deve essere sottratto dai valori dell'assorbanza da tutti i valori delle altre assorbanze per ottenere risultati affidabili!

Misurare l'assorbanza di tutti i pozzetti a **450 nm** e inserire tutti i valori misurati nello schema della piastra.

È raccomandato fare le misurazioni delle onde **bichrome** (due colori). Utilizzando la lunghezza d'onda di 620 nm come misura di riferimento.

Dove sono state misurate in doppio, calcolare **la media delle assorbanze**.

9. RISULTATI

9.1. Validazione del test

Il test è valido se risponde ai prossimi criteri:

- **Substrato Bianco (Blank):** Valore di assorbanza $< 0,100$
- **Controllo negativo:** Valore di assorbanza $< 0,200$ e $< \text{Cut-off}$
- **Controllo cut-off:** Valore di assorbanza $0,150 - 1,300$
- **Controllo positivo:** Valore di assorbanza $> \text{Cut-off}$

Se non sono soddisfatti questi criteri, il test non è valido e deve essere ripetuto.

9.2. Calcolo dei risultati

Il Cut-off è la media dei valori di assorbanza dei Controlli cut-off.

Esempio: Valore di assorbanza del Controllo cut-off 0,44 + valore di assorbanza del Controllo cut-off 0,42 = $0,86/2 = 0,43$
Cut-off = 0,43

9.2.1. Risultati in unità [NTU]

$$\frac{\text{Assorbanza media del campione} \times 10}{\text{Cut-off}} = [\text{unità NovaTec} = \text{NTU}]$$

Esempio:
$$\frac{1,591 \times 10}{0,43} = 37 \text{ NTU}$$

9.3. Interpretazione dei risultati

Cut-off	10 NTU	-
Positivo	> 11 NTU	Anticorpi contro il patogeno sono presenti. C'è stato un contatto con l'antigene (patogeno resp. vaccino).
zona grigia	9 – 11 NTU	Anticorpi contro il patogeno non è stato possibile rilevare chiaramente. Si consiglia di ripetere il test con un nuovo campione in 2-4 settimane. Se il risultato è nuovamente nella zona grigia, il campione viene giudicato come negativo .
Negativo	< 9 NTU	Il campione non contiene anticorpi contro il patogeno. Un precedente contatto con l'antigene (patogeno resp. vaccino) è improbabile.
La diagnosi di una malattia infettiva non deve essere fatta soltanto sulla risultanza di un unico test. È importante considerare anche l'anamnesi ed i sintomi del paziente. I risultati del test da pazienti immunosoppressi e neonati hanno un valore limitato.		

9.3.1. Isotipi degli anticorpi e Stato dell'infezione

Sierologia	Significato
IgM	Caratteristica della risposta primaria dell'anticorpo Alto titolo IgM con basso titolo IgG: → suggerisce una infezione molto recente o acuta Raro: → IgM persistente
IgG	Caratteristica della risposta secondaria dell'anticorpo Può persistere per diversi anni Alto titolo IgG con basso titolo IgM: → può indicare un'infezione passata

10. CARATTERISTICHE DEL TEST

I risultati si riferiscono al gruppo di campioni investigato; questi non sono specifiche garantite.

Per ulteriori informazioni su caratteristiche del test, si prega, di contattare NovaTec Immundiagnostica GmbH.

10.1. Precisione

Intradosaggio	n	Media (E)	CV (%)
#1	24	0,411	4,14
#2	24	1,173	4,32
#3	24	1,338	1,41
Interdosaggio	n	Media (NTU)	CV (%)
#1	12	30,07	4,58
#2	12	31,66	5,52
#3	12	2,87	9,27

10.2. Specificità diagnostica

La specificità diagnostica è la probabilità del test di fornire un risultato negativo in assenza di analiti specifici.

La specificità diagnostica è 95,83% (95% intervallo di confidenza: 85,75% - 99,49%).

10.3. Sensibilità diagnostica

La sensibilità diagnostica è la probabilità del test di fornire un risultato positivo alla presenza di analiti specifici.

La sensibilità diagnostica è 93,55% (95% intervallo di confidenza: 89,41% - 96,43%).

10.4. Possibili interferenze

Campioni emolitici, lipidici ed itterici contenenti fino a 10 mg/ml di emoglobina, 5 mg/ml di trigliceridi e 0,5 mg/ml di bilirubina non hanno presentato fenomeni d'interferenza nel presente test.

10.5. Reattività crociata

L'investigazione di un gruppo di campioni con attività di anticorpi contro parametri potenzialmente interferenti non ha rivelato alcuna evidenza di risultati falsamente positivi dovuto a reattività crociata.

11. LIMITAZIONI

Una contaminazione da microorganismi o ripetuti cicli di congelamento-scongelo possono alterare i valori delle assorbanze.

12. PRECAUZIONI E AVVERTENZE

- In ottemperanza all'articolo 1, paragrafo 2 della direttiva Europea 98/79/EC, l'uso dei diagnostici medici in vitro è inteso da parte del produttore ad assicurare la congruenza, le prestazioni e la sicurezza del prodotto. Di conseguenza la procedura analitica, le informazioni, le precauzioni e le avvertenze contenute nelle istruzioni per l'uso devono essere seguite scrupolosamente. L'uso dei kit con analizzatori e attrezzature simili deve essere previamente convalidato. Qualunque cambiamento nello scopo, nel progetto, nella composizione o struttura e nella procedura analitica, così come qualunque uso dei kit in associazione ad altri prodotti non approvati dal produttore non è autorizzato; l'utilizzatore stesso è responsabile di questi eventuali cambiamenti. Il produttore non è responsabile per falsi risultati e incidenti che possano essere causati da queste ragioni. Il produttore non è responsabile per qualunque risultato ottenuto attraverso esame visivo dei campioni dei pazienti.
- Solo per uso diagnostico in-vitro.
- Tutti i materiali di origine umana o animale devono essere considerati potenzialmente contagiosi e infettivi.
- Tutti i componenti di origine umana sono stati trovati non reattivi con Anti-HIV-Ab, Anti-HCV-Ab e HBsAg.
- Non scambiare reagenti e micropiastre di lotti diversi.
- Non utilizzare reagenti di altri produttori insieme con i reagenti di questo kit.
- Non usare dopo la data di scadenza.
- Utilizzare soltanto punte per pipette, distributori, e articoli da laboratorio puliti.
- Non scambiare i tappi dei flaconi, per evitare contaminazione incrociata.
- Richiudere i flaconi immediatamente dopo l'uso per evitare la vaporizzazione e contaminazione.
- Una volta aperti e dopo relativo stoccaggio verificare i reagenti per una loro eventuale contaminazione prima dell'uso.
- Per evitare contaminazioni crociate e risultati erroneamente alti pipettare i campioni e reagenti con molta precisione nei pozzetti senza spruzzi.
- Il ELISA è previsto soltanto per essere impiegato da parte di personale specializzato che conosce perfettamente le tecniche di lavoro.

12.1. Smaltimento

In genere tutte le sostanze chimiche sono considerati rifiuti pericolosi. Lo smaltimento è regolato da leggi nazionali. Per ulteriori informazioni contattare l'autorità locale.

13. INFORMAZIONI PER GLI ORDINI

Numero del prodotto:	MUMG0340	Virus della Mumps IgG ELISA (96 determinazioni)

ESPAÑOL

1. INTRODUCCIÓN

Los Virus de la Parotiditis son los virus que causan la papera, son virus de A.R.N. pertenecientes a la familia de los Paramyxoviridae. Tienen una envoltura lípida, un diámetro de 150 a 200nm y una forma pleomorfa. La papera es de distribución mundial manifestandose en epidemias infantiles, sobre todo en temporadas frías. En aprox. el 30% de los casos la enfermedad pasa inadvertida. La transmisión aerógena o por gotas de fluido contaminado de hombre a hombre dejando una inmunidad para el resto de la vida.

El virus de la papera infecta al principio las epitelias del tracto respiratorio superior, del tracto gastrointestinal o los ojos. Después de una reproducción inicial el virus se disgrega en nódulos linfáticos regionales donde después de otras fases de reproducción se produce una viremia produciendo infecciones en otros órganos como las glándulas salivares, las glándulas mamarias, en los testículos, en los ovarios, en el sistema nervioso y en el páncreas. Los síntomas aparecen después de un período de incubación de 7 a 21 días con fiebre, dolores de cabeza y sensación general de enfermedad. El síntoma clave de la papera epidémica es la inflamación dolorosa de una de las glándulas salivares, inflamándose la otra uno o dos días más tarde.

Complicaciones:

- Orquitis, epididymitis en 20% de los casos (lleva a la esterilidad en el 80% de los casos si la infección esta en ambos lados)
- Meningitis en el 20% de los pacientes el día 18 después del comienzo de la enfermedad (por regla general remisión rápida y total)
- Meningoencefalitis (muy raras veces (<1%) en comparación con la meningitis pero con una letalidad más alta (5%); la remisión con residuos es posible (5%)).
- Miocarditis, pancreatitis, adnexitis en las mujeres (raras veces complicaciones)

Como profilaxis existe una vacuna viva (simple o en combinación con otras).

Especies	Enfermedad	Síntomas (p.ej.)	Vía de transmisión
Virus de la Parotiditis (En inglés Mumps Virus)	Parotiditis epidémica (paperas)	Fiebre, dolores de cabeza, sensación de enfermedad, inflamación de la parotis de un o ambos lados (Gl. Sublinguales y submandibulares están afectados amenudo también) Complicaciones: orquitis, meningoencefalitis, pancreatitis.	aerógena, por gotitas de fluido contaminado

Detección de infecciones o de agentes patógenos de:

- PCR
- Serología: p.ej. ELISA

2. USO PREVISTO

El ensayo inmunoensayo Mumps Virus IgG ELISA se utiliza para la determinación cualitativa de anticuerpos IgG específicos contra Mumps (**Virus de la Parotiditis**) en suero o plasma (citrato, heparina) humano.

3. PRINCIPIO DEL ENSAYO

La determinación inmunoenzimática cualitativa de anticuerpos específicos se basa en la técnica ELISA (Enzyme-linked Immunosorbent Assay).

Las microplacas están recubiertas con antígenos específicos unen a los anticuerpos de la muestra. Después de lavar los pocillos para eliminar todo el material de muestra no unido, el conjugado de peroxidasa de rábano (HRP) se añade. Este conjugado se une a los anticuerpos capturados. En una segunda etapa de lavado se retira el conjugado no unido. El complejo inmune formado por el conjugado unido se visualizó añadiendo sustrato tetrametilbencidina (TMB), que da un producto de reacción azul.

La intensidad de este producto es proporcional a la cantidad de anticuerpos específicos en la muestra. se añade ácido sulfúrico para detener la reacción. Esto produce un cambio de color de azul a amarillo. La extinción a 450/620 nm se mide con un lector de microplacas ELISA.

4. MATERIALES

4.1. Reactivos suministrados

- **Mumps Virus IgG microplaca recubierta:** 12 tiras de 8 pocillos rompibles, recubiertos con antígenos de Mumps Virus, en bolsa de aluminio.
- **Diluyente para IgG de la muestra:** 1 botella de 100 ml de solución de tampón de fosfato (10 mM) para diluir la muestra; pH $7,2 \pm 0,2$; color amarillo; listo para ser utilizado; tapa blanca.
- **Solución de parada:** 1 botella de 15 ml de ácido sulfúrico, 0,2 mol/l, listo para ser utilizado; tapa roja.
- **Tampón de lavado (20x conc.):** 1 botella de 50 ml de una solución de tampón de fosfato 20x concentrado (0,2 M) para lavar los pocillos; pH $7,2 \pm 0,2$; tapa blanca.
- **Conjugado Mumps Virus anti-IgG:** 1 botella de 20 ml de conjugado de anticuerpos IgG anti-humano con peroxidasa en tampón de fosfato (10 mM); color azul; tapa negra; listo para ser utilizado.
- **Solución de sustrato de TMB:** 1 botella de 15 ml 3,3',5,5'-tetrametilbenzindina (TMB), < 0,1 %; listo para ser utilizado; tapa amarilla; < 5% NMP.
- **Control positivo Mumps Virus IgG:** 1 botella de 2 ml control (suero o plasma humano); color amarillo; tapa roja; listo para ser utilizado.
- **Control cut-off Mumps Virus IgG:** 1 botella de 3 ml control (suero o plasma humano); color amarillo; tapa verde; listo para ser utilizado.
- **Control negativo Mumps Virus IgG:** 1 botella de 2 ml control (suero o plasma humano); color amarillo; tapa azul; listo para ser utilizado.

Para sustancias potencialmente peligrosas por favor revise la ficha de datos de seguridad.

4.2. Accesorios suministrados

- 1 lámina autoadhesiva
- 1 instrucciones de uso
- 1 esquema de la placa

4.3. Materiales e instrumentos necesarios

- Fotómetro de microplaca con filtros de 450/620 nm
- Incubadora 37 °C
- Dispositivo de lavado manual o automático
- Micropipetas para uso de (10-1000 µl)
- Mezcladora Vortex
- Agua destilada
- Tubos de plástico desechables

5. ESTABILIDAD Y ALMACENAJE

Almacene el kit a 2...8 °C. Los reactivos abiertos son estables hasta la fecha de caducidad indicada en la etiqueta cuando se almacena a 2...8 °C.

6. PREPARACIÓN DE LOS REACTIVOS

Es muy importante llevar Todos los reactivos y las muestras para a la temperatura ambiente (20...25 °C) y mezclarlos antes de ser utilizados!

6.1. Microplaca recubierta

As tiras rompibles están recubiertas con antígeno de Mumps Virus. Inmediatamente después de la eliminación de las tiras, las tiras restantes deben sellarse de nuevo en el papel de aluminio junto con la bolsita de dióxido de silicio y almacenar a 2...8 °C.

6.2. Tampón de lavado (20x conc.)

Diluir la Tampón de lavado 1+19; por ejemplo: 10 ml de la Tampón de lavado + 190 ml de agua destilada. La muestra de tampón diluido es estable durante 5 días a temperatura ambiente (20...25 °C). Caso aparecen cristales en el concentrado, calentar la solución a 37 °C, por ejemplo, en un baño María. Mezclar bien antes de la dilución.

6.3. Solución de sustrato de TMB

La solución está listo para su uso y debe almacenarse a 2...8 °C, protegida de la luz. La solución debe ser incolora o podría tener un color ligeramente azul claro. Si el sustrato se convierte en azul, es posible que haya sido contaminado y no puede ser utilizada en el ensayo.

7. TOMA Y PREPARACIÓN DE LAS MUESTRAS

Usar muestras de suero o plasma (citrato, heparina) humano. Las instrucciones de uso ABVL0001 deben ser usadas para LCR. Si el ensayo se realiza dentro de 5 días después de la toma de sangre, las muestras pueden ser almacenadas de 2...8 °C, en caso contrario deben ser alicotadas y almacenadas congeladas (-70...-20 °C). Agitar bien las muestras descongeladas antes de diluirlas. Evitar congelaciones y descongelaciones repetidas.

No se recomienda la inactivación por calor de las muestras.

7.1. Dilución de las muestras

Antes del ensayo, las muestras tienen que estar diluidas en relación 1 + 100 con el tampón de dilución para la muestra de IgG, p. e. 10 µl de la muestra con 1 ml de tampón IgG, mezclar bien con la mezcladora Vortex.

8. PROCEDIMIENTO

8.1. Preparación del ensayo

Por favor, leer cuidadosamente las instrucciones de uso del ensayo **antes** de realizarlo. Para el buen funcionamiento de la técnica es necesario seguir las instrucciones. El siguiente procedimiento es válido solamente para el método manual. Si se realiza el ensayo en los sistemas automáticos de ELISA es aconsejable elevar el número de lavado de de 3 a 5 veces y el volumen de Tampón de lavado de 300 µl a 350 µl para excluir efectos de lavado. Preste atención al capítulo 12. Antes de comenzar, especificar exactamente la repartición y posición de las muestras y de los estándares/controles (recomienda determinar en doble) en lo esquema de la placa suministrada. Usar la cantidad necesaria de tiras o pozos e insertarlos en el soporte.

Realizar el ensayo en el orden indicado y sin retraso.

Para cada paso de pipeteado en los estándares/controles y en las muestras, usar siempre puntas de pipeta de un solo uso.

Graduar la incubadora a $37 \pm 1^\circ\text{C}$.

1. Pipetear 100 µl de estándares/controles y muestras en los pocillos respectivos. Dejar el pocillo A1 para el blanco.
2. Recubrir las tiras con los autoadhesivos suministrados.
3. **Incubar 1 h $\pm$ 5 min a $37 \pm 1^\circ\text{C}$.**
4. Después de la incubación, retirar el autoadhesivo, aspirar el líquido de la tira y lavarla tres veces con 300µl de la Tampón de lavado. Evitar el rebosamiento de los pocillos. El intervalo entre lavado y aspiración debe ser > 5 segundos. Para sacar el líquido restante de las tiras, es conveniente sacudirlas sobre papel absorbente.
Nota: El lavado es muy importante! Un mal lavado insuficiente provoca una baja precisión y resultados falsamente elevados!
5. Pipetar 100 µl de conjugado en cada pocillo con excepción del blanco sustrato A1.
6. **Incubar 30 min a la temperatura ambiente ($20...25^\circ\text{C}$).** Evitar la luz solar directa.
7. Repetir el lavado como en el paso numero 4.
8. Pipetar 100 µl de la Solución de sustrato de TMB en todos los pocillos.
9. **Incubar exactamente 15 min en oscuridad a temperatura ambiente ($20...25^\circ\text{C}$).** Un color azul se produce en las muestras positivas debido a la reacción enzimática.
10. Pipetear en todos los pocillos 100 µl de la solución de parada en el mismo orden y mismo intervalo de tiempo como con la Solución de sustrato de TMB, por lo tanto un cambio de color de azul a amarillo se produce.
11. Medir la extinción con 450/620 nm en un periodo de 30 min después de añadir la solución de parada.

8.2. Medición

Ajustar el lector de microplaca (fotómetro) Elisa **al cero** utilizando **el Blanco**.

Si por razones técnicas el lector de ELISA no se puede ajustar a cero utilizando el Blanco, el valor de la absorbancia de esto debe ser sustraído de los demás valores de absorbancia medidos con el fin de obtener resultados fiables!

Medir la **extinción** de todos los pocillos con **450 nm** y anotar los resultados de los estándares/controles y de las muestras en la esquema de la placa.

Es aconsejable realizar la medición **bicromática** a una longitud de onda de referencia de 620 nm.

Si se efectuaron análisis en duplicado o múltiples, hay que calcular **el promedio de los valores de extinción** de los pocillos correspondientes.

9. CALCULO DE LOS RESULTADOS

9.1. Criterios de validez del ensayo

El ensayo es válido si se cumplen los siguientes criterios:

- **Blanco:** valor de la extinción **< 0,100**
- **Control negativo:** valor de la extinción **< 0,200 y < Cut-off**
- **Control cut-off:** valor de la extinción **0,150 – 1,300**
- **Control positivo:** valor de la extinción **> Cut-off**

Si estos criterios no se cumplen, la prueba no es válida y deberá repetirse.

9.2. Calculo del valor de la medición

El Cut-off se obtiene de los valores de la extinción de lo Control cut-off.

Ejemplo: $0,42 \text{ OD Control cut-off} + 0,44 \text{ OD Control cut-off} = 0,86 : 2 = 0,43$
Cut-off = 0,43

9.2.1. Resultados en unidades [NTU]

Promedio valor de la extinción de la muestra $\times 10$ = [NovaTec-unidades = NTU]
Cut-off

Ejemplo: $\frac{1,591 \times 10}{0,43} = 37$ NTU

9.3. Interpretación de los resultados

Cut-off	10 NTU	-
Positivo	> 11 NTU	Los anticuerpos contra el patógeno están presentes. Ha producido un contacto con el antígeno (patógeno resp. vacuna).
Zona intermedia	9 – 11 NTU	Los anticuerpos contra el patógeno no se pudieron detectar claramente. Se recomienda repetir la prueba con una muestra fresca en 2 a 4 semanas. Si el resultado es de nuevo en la zona intermedia, la muestra se considera como negativa .
Negativo	< 9 NTU	La muestra no contiene anticuerpos contra el patógeno. Un contacto previo con el antígeno (patógeno resp. vacuna) es poco probable.
El diagnóstico de una infección no solamente se debe basar en el resultado del ensayo. Es necesario considerar la anamnesis y la sintomatología del paciente junto al resultado serológico. Estos resultados sólo tienen valor restringido en pacientes inmunodeprimidos o en neonatos.		

9.3.1. Isotipos de anticuerpo y Estado de la Infección

Serología	Significado
IgM	Característica de la respuesta primaria del anticuerpo Alto título de IgM con bajo título de IgG → sugieren una infección muy reciente o aguda Raras: → persistente IgM
IgG	Característica de la respuesta secundaria del anticuerpo Pueden persistir por varios años El alto título de IgG con bajo título de IgM: → pueden indicar una infección pasada

10. CARACTERÍSTICAS DEL ENSAYO

Los resultados están basados en lo gruppó de pruebas investigado; no se trata de especificaciones garantizadas.

Para obtener más información sobre las características del ensayo, por favor, entre en contacto NovaTec Immundiagnostica GmbH.

10.1. Precisión

Intra ensayo	n	Promedio (E)	CV (%)
#1	24	0,411	4,14
#2	24	1,173	4,32
#3	24	1,338	1,41
Inter ensayo	n	Promedio (NTU)	CV (%)
#1	12	30,07	4,58
#2	12	31,66	5,52
#3	12	2,87	9,27

10.2. Especificad diagnóstica

La especificidad del ensayo se define como la probabilidad que tiene el ensayo de dar un resultado negativo en ausencia del analítico específico. Es 95,83% (95% Intervalo de confianza: 85,75% - 99,49%).

10.3. Sensibilidad de diagnóstico

La sensibilidad del ensayo se define como la probabilidad que tiene el ensayo de dar un resultado positivo en presencia del analítico específico. Es 93,55% (95% Intervalo de confianza: 89,41% - 96,43%).

10.4. Interferencias

Las muestras lipémicas, ictericas e hemolíticas no mostraron interferencias con este equipo ELISA hasta una concentración de 5 mg/ml para triglicéridos, de 0,5 mg/ml para bilirrubina y de 10 mg/ml hemoglobina.

10.5. Reactividad cruzada

Pruebas realizadas con un panel de muestras con distinta actividad de anticuerpos para estudiar parámetros de reactividad no dieron falsos positivos debidos a reactividad cruzada.

11. LIMITACIONES DEL ENSAYO

Una contaminación de las muestras con bacterias, o una congelación y descongelación repetida pueden producir cambios en los valores de la extinción.

12. PRECAUCIONES Y ADVERTENCIAS

- En cumplimiento con el artículo 1 párrafo 2b de la directiva europea 98/79/EC, la utilización de sistemas médicos para diagnóstico in vitro tiene la intención por parte del fabricante de asegurar la adecuación, realizaciones y seguridad del producto. Por lo tanto, el procedimiento, la información, las precauciones y los avisos de las instrucciones de uso han de ser seguidas estrictamente. La utilización de equipos con analizadores y equipamiento similar tiene que ser validada. No se autorizan cambios en el diseño, composición y procedimiento, así como cualquier utilización en combinación con otros productos no aprobados por el fabricante; el usuario debe hacerse responsable de estos cambios. El fabricante no responderá ante falsos resultados e incidentes debidos a estas razones. El fabricante no responderá ante cualquier resultado por análisis visual de las muestras de los pacientes.
- Solo para diagnóstico in vitro.
- Todos los materiales de origen humano o animal deberán ser considerados y tratados como potencialmente infecciosos.
- Todos los componentes de origen humano han sido examinados y resultaron no reactivos a anticuerpos contra el VIH, VHC y HbsAG.
- No intercambiar reactivos y placas de microtítulo de cargas diferentes.
- No usar reactivos de otro fabricante para este ensayo.
- No usar después de la fecha de caducidad.
- Sólo usar recambios de pipetas, dispensadores y materiales de laboratorio limpios.
- No intercambiar las tapas de los diferentes reactivos, para evitar la contaminación cruzada.
- Para evitar la evaporación y una contaminación microbiana, cierre inmediatamente las botellas después de usarlas.
- Después de abrirlas y posterior almacenaje, asegurarse de que no existe contaminación microbiana antes de seguir usándolas.
- Para evitar contaminaciones cruzadas y resultados erróneamente aumentados, pipetear cuidadosamente las muestras y los reactivos en los pocillos sin salpicar.
- El ELISA está pensado exclusivamente para su uso por personal especializado que domine perfectamente las técnicas de trabajo.

12.1. Indicaciones para la eliminación de residuos

Por regla general, los productos químicos y las preparaciones son residuos peligrosos. Su eliminación está sometida a las leyes y los decretos nacionales sobre la eliminación de residuos. Las autoridades informan sobre la eliminación de residuos peligroso.

13. INFORMACIONES PARA PEDIDOS

N° del producto:	MUMG0340	Mumps Virus IgG ELISA (96 determinaciones)

PORTUGUÊS

1. INTRODUÇÃO

Os vírus da parotidite são vírus ARN da família Paramyxoviridae. Os viriões são partículas esféricas com um diâmetro de 150-250 nm, compostas de ribonucleoproteína com simetria helicoidal e providas de um invólucro de proteína matrix e bicamada lipídica, que contém duas estruturas de espigões: Hemaglutinina viral (H) e neuraminidase viral (N). O vírus da parotidite afecta principalmente a parótida e glândulas salivares relacionadas; no entanto, a infecção pode dar origem a doenças do SNC e a uma acumulação do vírus no LCR. A papeira (parotidite epidémica) é uma doença viral aguda contagiosa, que ocorre sobretudo em crianças. Perto de 50% do total de infecções é subclínico. A incidência mais elevada de manifestações clínicas encontra-se no grupo etário entre os 4 e os 15 anos. As infecções secundárias são raras, devido à imunização duradoura. 10 a 35% dos casos de papeira dão origem a orquite, que ocorre quase sempre depois da puberdade. O processo é maioritariamente unilateral e o prognóstico é geralmente favorável. O vírus da parotidite foi uma das causas mais importantes de doenças virais do SNC (meningite e encefalite) nos EUA; a administração de vacinas reduziu significativamente esta incidência..

Espécies	Doença	Sintomas (p.ex.)	Via de transmissão
Vírus da Parotidite	Parotidite infecciosa (Papeira, Caxumba)	Febre e edema unilateral ou bilateral da glândula parótida; as glândulas sublinguais e submandibulares também podem ser afectadas. Complicações: Orquite, Meningoencefalite, Pancreatite	Por via aérea, a transmissão do vírus ocorre por infecção por gotículas;

A presença de agentes patogénicos ou de infecção pode ser identificada através

- PCR
- Serologia: p.ex. ELISA

2. UTILIZAÇÃO PRETENDIDA

O kit Mumps Virus IgG ELISA destina-se à determinação qualitativa de anticorpos da classe IgG contra Mumps Virus (Vírus da Parotidite) no soro ou plasma (citrato, heparina) humanos.

3. PRINCÍPIO DO ENSAIO

A determinação imunoenzimática qualitativa de anticorpos específicos é baseado na técnica de ELISA (do inglês Enzyme-linked Immunosorbent Assay).

As microplacas são revestidas com antígenos específicos que se ligam os anticorpos correspondentes da amostra. Após lavagem dos poços, para remover todo o material de amostra não ligado, um conjugado de peroxidase de rábano (HRP) é adicionado. Este conjugado se liga aos anticorpos capturados. Num segundo passo de lavagem o conjugado não ligado é removido. O complexo imune formado pelo conjugado ligado é visualizado por adição de substrato de tetrametilbenzidina (TMB), o que dá um produto de reacção azul.

A intensidade deste produto é proporcional à quantidade de anticorpos específicos da amostra. O ácido sulfúrico é adicionado para parar a reacção. Isso produz uma mudança de cor de azul para amarelo.

Absorvância a 450/620 nm é lida utilizando um leitor de microplacas ELISA.

4. MATERIAIS

4.1. Reagentes fornecidos

- **Mumps Virus IgG microplaca revestida:** 12 tiras de 8 poços, destacáveis e quebráveis, revestidas com antígeno do Vírus Mumps, em bolsas de folha de alumínio com fecho.
- **Diluinte de Amostra IgG:** 1 frasco contendo 100 ml de tampão fosfato (10 mM) para diluição da amostra, pH 7,2 ± 0,2; de cor amarela; pronto a usar; tampa branca.
- **Solução de Bloqueio:** 1 frasco contendo 15 ml ácido sulfúrico; 0,2 mol/l; pronto a usar; tampa vermelha.
- **Tampão de lavagem (conc. 20x):** 1 frasco contendo 50 ml de um tampão fosfato (0,2 M); concentrado 20 vezes (pH 7,2 ± 0,2) para a lavagem dos poços; tampa branca.
- **Conjugado Mumps Virus anti-IgG:** 1 frasco contendo 20 ml de anticorpo IgG anti-humana marcados com peroxidase no tampão fosfato (10 mM); de cor azul, pronto a usar; tampa preta.
- **Solução Substrato TMB:** 1 frasco contendo 15 ml de 3,3',5,5'-tetrametilbenzidina (TMB), < 0,1 %; pronto a usar; tampa amarela;; < 5% NMP.
- **Controle positivo Mumps Virus IgG:** 1 frasco contendo 2 ml controle (soro ou plasma humanos); de cor amarela; pronto a usar; tampa vermelha.
- **Controle cut-off Mumps Virus IgG:** 1 frasco contendo 3 ml controle (soro ou plasma humanos); de cor amarela; pronto a usar; tampa verde.
- **Controle negativo Mumps Virus IgG:** 1 frasco contendo 2 ml controle (soro ou plasma humanos); de cor amarela; pronto a usar; tampa azul.

Para substâncias potencialmente perigosas verifique a ficha de dados de segurança.

4.2. Materiais fornecidos

- 1 Película de cobertura
- 1 Instruções de uso
- 1 Layout da placa

4.3. Materiais e Equipamento necessários

- Leitor de microplacas ELISA, equipado para a medição da absorvância a 450/620 nm
- Incubadora 37 °C
- Equipamento manual ou automático para a lavagem dos poços
- Pipetas para dispensar volumes entre 10 e 1000 µl
- Agitador de tubos tipo Vortex
- Água destilada
- Tubos descartáveis

5. ESTABILIDADE E ARMAZENAMENTO

Armazene o kit a 2...8 °C. Os reagentes abertos são estáveis até o prazo de validade impresso no rótulo quando armazenado a 2...8 °C.

6. PREPARAÇÃO DOS REAGENTES

É muito importante deixar todos os reagentes e amostras estabilizar à temperatura ambiente (20...25 °C) misturá-los antes de iniciar o teste!

6.1. Microplaca revestidas

As tiras quebráveis são revestidas com antigénio Vírus Mumps. Imediatamente após a remoção das tiras necessárias, as tiras restantes devem ser lacradas de novo na folha de alumínio juntamente com o saquinho de silício fornecido e armazenadas a 2...8 °C.

6.2. Tampão de lavagem (conc. 20x)

Diluir o Tampão de lavagem 1+19; por exemplo, 10 ml do Tampão de lavagem + 190 ml de água destilada. A amostra do tampão diluída é estável durante 5 dias à temperatura ambiente (20...25 °C). Caso apareça cristais no concentrado, aquecer a solução a 37 °C por exemplo, em banho Maria. Misture bem antes da diluição.

6.3. Solução Substrato TMB

A solução está pronta para uso e tem de ser armazenada à 2...8 °C, protegida da luz. A solução deve ser incolor ou poderia ter uma ligeira coloração azul claro. Se o substrato se transforma em azul, pode ter sido contaminado e não pode ser usado no teste.

7. COLHEITA E PREPARAÇÃO DAS AMOSTRAS

Usar com este ensaio amostras de soro ou plasma (citrato, heparina) humanos. Para LCR, por favor utilize a instrução de uso ABVL0001. Se o ensaio for realizado dentro de 5 dias após colheita da amostra, o espécime deve ser mantido a 2...8 °C; caso contrário devem ser alicotadas e armazenadas congeladas (-70...-20 °C). Se as amostras forem armazenadas congeladas, misturar bem as amostras descongeladas antes de testar. Evitar congelar e descongelar repetidamente. Não é recomendada a inativação por calor das amostras.

7.1. Diluição das amostras

Antes de testar todas as amostras devem ser diluídas 1 + 100 com Diluente de Amostra IgG. Dispensar 10 µl de amostra e 1 ml de Diluente de Amostra IgG em tubos para obter uma diluição 1 + 100 e misturar meticulosamente com um vortex.

8. PROCEDIMENTO DO ENSAIO

8.1. Preparação do Teste

Por favor, ler atentamente as instruções de uso **antes** de realizar o teste. A fiabilidade dos resultados depende da adesão estrita às instruções de uso, conforme descritas. O procedimento de ensaio a seguir está validado apenas para o procedimento manual. Se o teste for realizado em sistemas automáticos para teste ELISA é recomendável aumentar os passos de lavagem de três para cinco e o volume do Tampão de lavagem de 300 µl para 350 µl para evitar efeitos de lavagem. Preste atenção ao capítulo 12. Antes de iniciar o teste, o plano de distribuição e identificação de todas as amostras e calibradores/controles (é recomendado determinar em duplicidade) deve ser cuidadosamente estabelecido no Layout da placa fornecida no kit. Selecionar o número necessário de tiras ou poços e inserir os mesmos no suporte.

Realizar todas as etapas do teste na ordem indicada e sem atrasos significativos.

Na pipetagem deve ser utilizada uma ponta limpa e descartável para dispensar cada controle e amostra.

Ajustar a incubadora para 37 ± 1 °C.

1. Dispensar 100 µl dos calibradores/controles e das amostras diluídas nos poços respectivos. Deixar o poço A1 vazio para o branco substrato.
2. Cobrir os poços com a película fornecida no kit.
3. **Incubar durante 1 hora $\pm$ 5 min a 37 ± 1 °C.**
4. Quando terminar a incubação, remover a película, aspirar o conteúdo dos poços e lavar cada poço três vezes com 300 µl de Tampão de lavagem. Evitar que os poços de reacção transbordem. O intervalo entre a lavagem e a aspiração deve ser > 5 seg. No final, retirar cuidadosamente o fluido restante batendo delicadamente as tiras sobre papel absorvente, antes da próxima etapa!
Nota: A lavagem é muito importante! Lavagem insuficiente resulta em baixa precisão e falsos resultados.
5. Dispensar 100 µl de Conjugado em todos os poços, excepto no poço do Branco substrato A1.
6. **Incubar durante 30 min à temperatura ambiente (20...25°C).** Não expor diretamente à luz solar.
7. Repetir a etapa 4.
8. Dispensar 100 µl de Solução Substrato TMB em todos os poços.
9. **Incubar durante exactamente 15 min à temperatura ambiente (20...25°C) e no escuro.** A cor azul devido a uma reacção enzimática.
10. Dispensar 100 µl de Solução de bloqueio em todos os poços, pela mesma ordem e com a mesma velocidade a que foi dispensada a Solução Substrato TMB, desse modo uma mudança de cor de azul para amarelo ocorre.
11. Medir a absorvância a 450/620 nm dentro de 30 min após a adição da solução de bloqueio.

8.2. Medição

Ajustar o Leitor de Microplacas ELISA a zero usando o **Branco substrato**.

Se - devido às razões técnicas – o leitor ELISA não puder ser ajustado a zero usando o Branco substrato, valor da absorvância deste deve ser subtraído de todos os outros valores de absorvância medidos de forma a obter resultados fiáveis!

Medir a absorvância de todos os poços a **450 nm** e registar os valores da absorvância para cada calibrador/controle e amostra no Layout da placa.

É recomendado fazer a medição **dicromática** usando como referência um comprimento de onda de 620 nm.

Se determinações duplas foram realizadas, calcular **os valores médios de absorvância**.

9. RESULTADOS

9.1. Critérios de validação do ensaio

Para que um ensaio seja considerado válido, devem ser cumpridos os seguintes critérios:

- **Branco substrato:** Valor de Absorvância < **0,100**
- **Controle negativo:** Valor de Absorvância < **0,200** e < **Cut-off**
- **Controle cut-off:** Valor de Absorvância **0,150 – 1,300**
- **Controle positivo:** Valor de Absorvância > **Cut-off**

Se estes critérios não forem cumpridos, o teste não é válido e deve ser repetido.

9.2. Cálculo dos Resultados

O Cut-off é o valor médio da absorvância das determinações do Controle cut-off.

Exemplo: Valor da absorvância do Controle cut-off 0,42 + valor da absorvância do Controle cut-off 0,44 = 0,86: 2 = 0,43
Cut-off = 0,43

9.2.1. Resultados em Unidades [NTU]

$$\frac{\text{Valor da absorvância (média) da amostra} \times 10}{\text{Cut-off}} = [\text{Unidades NovaTec} = \text{NTU}]$$

Exemplo:
$$\frac{1,591 \times 10}{0,43} = 37 \text{ NTU}$$

9.3. Interpretação dos Resultados

Cut-off	10 NTU	-
Positivo	> 11 NTU	Os anticorpos contra o agente patogênico estão presente. Houve um contacto com o antígeno (patógeno resp vacina).
Zona cinzenta	9 – 11 NTU	Os anticorpos contra o agente patogênico não puderam ser claramente detectados. Recomenda-se a repetir o teste com uma amostra fresca em 2 a 4 semanas. Se o resultado estiver novamente dentro da zona cinzenta, a amostra é julgada como negativa .
Negativo	< 9 NTU	A amostra não contém os anticorpos contra o agente patogênico. Um contato prévio com o antígeno (patógeno resp. vacina) é improvável.
O diagnóstico de uma doença infecciosa não deve ser estabelecido com base num único resultado do teste. Um diagnóstico preciso deve ter em consideração a história clínica, a sintomatologia bem como dados serológicos. Em paciente imunossuprimidos e recém-nascidos os dados serológicos têm apenas valor restrito.		

9.3.1. Isotipos de anticorpos e Estado da Infecção

Sorologia	Significado
IgM	Característica da resposta primária do anticorpo Alto título de IgM com baixo título de IgG: → sugere uma infecção muito recente ou aguda Raros: → persistente IgM
IgG	Característica da resposta secundária do anticorpo Podem persistir por vários anos Alto título de IgG com baixo título de IgM: → pode indicar uma infecção passada

10. CARACTERÍSTICAS DE DESEMPENHO ESPECÍFICAS

Os resultados referem-se aos grupos de amostras investigados; estas não são especificações garantidas.

Para mais informações sobre as características de desempenho específicas, por favor, entre em contato NovaTec Immundiagnostica GmbH.

10.1. Precisão

Intra ensaio	n	Média (E)	CV (%)
#1	24	0,411	4,14
#2	24	1,173	4,32
#3	24	1,338	1,41
Inter ensaio	n	Média (NTU)	CV (%)
#1	12	30,07	4,58
#2	12	31,66	5,52
#3	12	2,87	9,27

10.2. Especificidade Diagnóstica

A especificidade diagnóstica é definida como a probabilidade do ensaio ser negativo na ausência do analito específico.
É de 95,83% (95% Intervalo de confiança: 85,75% - 99,49%).

10.3. Sensibilidade Diagnóstica

A sensibilidade diagnóstica é definida como a probabilidade do ensaio ser positivo na presença do analito específico.
É de 93,55% (95% Intervalo de confiança: 89,41% - 96,43%).

10.4. Interferências

Não são observadas interferências com amostras hemolisadas, lipêmicas ou ictericas até uma concentração de hemoglobina de 10 mg/ml, de triglicerídeos de 5 mg/ml e de bilirrubina de 0,5 mg/ml.

10.5. Reacção cruzada

A investigação do painel de amostras com atividades de anticorpos em parâmetros com potencial de reacção cruzada não revelou nenhuma evidência de resultados falso-positivos devido a reacções cruzadas.

11. LIMITAÇÕES DO PROCEDIMENTO

Contaminação bacteriana ou a repetição de ciclos de congelação-descongelação do espécime podem afectar os valores da absorvância.

12. PRECAUÇÕES E AVISOS

- Em cumprimento com o artigo 1 parágrafo 2b da directiva Europeia 98/79/EC o uso de dispositivos médicos para diagnóstico in vitro destina-se segundo o fabricante a assegurar adequabilidade, desempenhos e segurança do produto. Portanto o procedimento do teste, as informações, as precauções e avisos nas instruções para utilização têm de ser rigorosamente seguidas. O uso de kits de teste com analisadores e equipamento similar tem de ser validado. Qualquer alteração no desenho, composição e procedimento do teste bem como qualquer utilização em combinação com outros produtos não aprovados pelo fabricante não estão autorizados; o próprio utilizador é responsável por tais alterações. O fabricante não é legalmente responsável por resultados falsos e incidentes originados por estes motivos. O fabricante não é legalmente responsável por quaisquer resultados obtidos por análise visual das amostras dos pacientes.
- Apenas para uso no diagnóstico in-vitro.
- Todos os materiais de origem humana ou animal devem ser considerados e tratados como potencialmente infectantes.
- Todos os componentes de origem humana usados para a produção destes reagentes foram testados para anticorpos anti-HIV, anticorpos anti-HCV e HBsAg e foram considerados não-reactivos.
- Não trocar e juntar reagentes ou tiras de lotes de produção diferentes.
- nenhuns reagentes de outros fabricantes devem ser usados juntamente com reagentes deste kit de teste.
- Não usar reagentes após a data de validade indicada no rótulo.
- Usar apenas pontas de pipeta, dispensadores e material de laboratório limpos.
- Não trocar as tampas dos frascos dos reagentes para evitar contaminação cruzada.
- Fechar firmemente os frascos dos reagentes imediatamente após a utilização para evitar evaporação e contaminação microbiana.
- Após a primeira abertura e armazenamento subsequente verificar se existe contaminação microbiana dos frascos do conjugado e dos calibradores/controles antes de utiliza-los novamente.
- Para evitar contaminação-cruzada e resultados falsamente elevados, pipetar as amostras dos pacientes e dispensar os reagentes precisamente nos poços sem salpicar.
- O ELISA foi desenhado apenas para pessoal qualificado e que esteja familiarizado com boas práticas laboratoriais.

12.1. Considerações de Eliminação

Resíduos de químicos e preparações são geralmente considerados como resíduos perigosos. A eliminação deste tipo de resíduos está regulada por leis e normativas nacionais e regionais. Contactar as autoridades locais ou empresas de gestão de resíduos as quais podem aconselhar sobre como eliminar resíduos perigosos.

13. INFORMAÇÃO DE PEDIDO

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

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ABBREVIATIONS / ABKÜRZUNGEN / ABRÉVIATIONS / ABBREVIAZIONI / ABREVIACIONES / ABREVIATURAS

NMP	N-Methyl-2-pyrrolidone

SYMBOLS KEY / SYMBOLSCHLÜSSEL / EXPLICATION DES SYMBOLES / LEGENDA / SIMBOLOS / TABELA DE SIMBOLOS

	Manufactured by / Hergestellt von / Fabriqué par / Prodotto da / Fabricado por / Fabricado por
IVD	In Vitro Diagnostic Medical Device / In Vitro Diagnosticum / Dispositif médical de diagnostic in vitro / Diagnostico in vitro / Producto para diagnóstico In vitro / Dispositivo Médico para Diagnóstico In Vitro
LOT	Lot Number / Chargenbezeichnung / Numéro de lot / Lotto / Número de lote / Número de lote
	Expiration Date / Verfallsdatum / Date de péremption / Scadenza / Fecha de caducidad / Data de Validade
	Storage Temperature / Lagertemperatur / Température de conservation / Temperatura di conservazione / Temperatura de almacenamiento / Temperatura de Armazenamento
CE	CE Mark / CE-Zeichen / Marquage CE / Marchio CE / Marca CE / Marca CE
REF	Catalogue Number / Katalog Nummer / Référence du catalogue / Numero di codice / Número de Catálogo / Número de Catálogo
	Consult Instructions for Use / Arbeitsanleitung beachten / Consulter la notice d'utilisation / Consultare le istruzioni per l'uso / Consulte las Instrucciones de Uso / Consultar as Instruções de Utilização
MTP	Microplate / Mikrotiterplatte / Microplaque / Micropiastra / Microplaca / Microplaca
CONJ	Conjugate / Konjugat / Conjugué / Coniugato / Conjugado / Conjugado
CONTROL -	Negative Control / Negativkontrolle / Contrôle négatif / Controllo negativo / Control negativo / Controle negativo
CONTROL +	Positive Control / Positivkontrolle / Contrôle positif / Controllo positivo / Control positivo / Controle positivo
CUT OFF	Cut-off Control / Cut-off Kontrolle / Contrôle cut-off / Controllo cut-off / Control cut-off / Controle cut-off
DIL G	IgG Sample Diluent / IgG-Probenverdünnungspuffer / Diluant pour échantillon IgG / Tampone diluente IgG / Diluyente para IgG de la muestra / Diluente de Amostra IgG
SOLN STOP	Stop Solution / Stopplösung / Solution d'arrêt / Soluzione bloccante / Solución de Parada/ Solução de bloqueio
SUB TMB	TMB Substrate solution / TMB-Substratlösung / Solution de substrat TMB / Soluzione substrato TMB / Solución de sustrato de TMB / Solução substrato TMB
WASH BUF 20x	Washing Buffer 20x concentrated / Waschpuffer 20x konzentriert / Tampon de lavage concentré 20 x / Tampone di lavaggio concentrazione x20 / Tampón de lavado concentrado x20 / Tampão de lavagem concentrada 20x
	Contains sufficient for "n" tests / Ausreichend für "n" Tests / Contenu suffisant pour "n" tests / Contenido suficiente per "n" saggi / Contenido suficiente para "n" tests / Conteúdo suficiente para "n" testes

SCHEME OF THE ASSAY

Mumps Virus IgG ELISA

Test Preparation

Prepare reagents and samples as described. Establish the distribution and identification plan for all samples and standards/controls on the plate layout supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder.

Assay Procedure

	Substrate Blank (A1)	Negative Control	Cut-off Control	Positive Control	Sample (diluted 1+100)
Negative Control	-	100 µl	-	-	-
Cut-off Control	-	-	100 µl	-	-
Positive Control	-	-	-	100 µl	-
Sample (diluted 1+100)	-	-	-	-	100 µl
Cover wells with foil supplied in the kit Incubate for 1 h at 37 °C Wash each well three times with 300 µl of Washing Buffer					
Conjugate	-	100 µl	100 µl	100 µl	100 µl
Incubate for 30 min at room temperature (20...25 °C) Do not expose to direct sunlight Wash each well three times with 300 µl of Washing Buffer					
TMB Substrate solution	100 µl	100 µl	100 µl	100 µl	100 µl
Incubate for exactly 15 min at room temperature (20...25 °C) in the dark					
Stop Solution	100 µl	100 µl	100 µl	100 µl	100 µl
Photometric measurement at 450 nm (reference wavelength: 620 nm)					

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Mumps Virus IgM

ELISA

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Only for in-vitro diagnostic use

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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 occurring 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

The presence of pathogen or infection 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.

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

- **Mumps Virus Coated Microplate (IgM):** 12 break-apart 8-well snap-off strips coated with Mumps Virus 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.
- **Mumps Virus 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.
- **Mumps Virus IgM Positive Control:** 1 vial containing 2 ml control (human serum or plasma); coloured yellow; ready to use; red cap.
- **Mumps Virus IgM Cut-off Control:** 1 vial containing 3 ml control (human serum or plasma); coloured yellow; ready to use; green cap.
- **Mumps Virus 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 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. 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 $\pm$ 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]

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

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

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.

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.: MUMM0340 Mumps Virus IgM ELISA (96 Determinations)

DEUTSCH

1. EINLEITUNG

Mumpsviren sind Einzelstrang-RNA-Viren der Familie der Paramyxoviridae. Sie haben eine lipidhaltige Hülle, einen Durchmesser von etwa 150-250nm und eine pleomorphe Gestalt. Das Mumpsvirus ist der Erreger der Parotitis epidemica (Mumps).

Mumps ist weltweit verbreitet und tritt meist epidemisch im Kindesalter auf, bevorzugt in der kalten Jahreszeit. Bei etwa 30% der Infizierten verläuft die Erkrankung inapparent. Die Übertragung erfolgt aerogen durch Tröpfchen, selten durch Schmierinfektion, direkt von Mensch zu Mensch und hinterlässt lebenslange Immunität.

Das Mumpsvirus infiziert primär die Epithelien des oberen Respirationstraktes, des Gastrointestinaltraktes oder der Augen. Nach initialer Replikation kommt es zur Aussaat in regionale Lymphknoten, von wo aus nach weiteren Replikationsschritten eine erste Virämie zur Infektion weiterer Organe wie Speicheldrüsen, Brustdrüsen, Testes, Ovarien, ZNS und Pankreas führt. Beim symptomatischen Verlauf treten nach der Inkubationszeit (ca. 7-21 Tage) Fieber, Kopfschmerzen und ein allgemeines Krankheitsgefühl auf. Leitsymptom der Parotitis epidemica ist die schmerzhafte Schwellung erst der einen, nach ein bis zwei Tagen auch der anderen Ohrspeicheldrüse. Komplikationen der Infektion sind:

Orchitis und Epididymitis bei etwa 20% der Infizierten (beim doppelseitigen Befall führt sie in 80% zur Sterilität)

Meningitis bei etwa 20% der Patienten etwa um den 18. Tag nach Krankheitsbeginn (in der Regel rasche, folgenlose Rückbildung)

Meningoenzephalitis (sehr viel seltener (<1%) als die reine Meningitis, aber mit höherer Letalität (5%); Ausheilung mit Residuen möglich (5%))

Myokarditis, Pankreatitis, Adnexitis bei Frauen (seltene Komplikationen)

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

Spezies	Erkrankung	Symptome (z.B.)	Infektionsweg
Mumps Virus	Parotitis epidemica (Mumps, Ziegenpeter)	Fieber, Kopfschmerzen, Krankheitsgefühl, ein- oder beidseitige Parotisschwellung; Glandulae sublinguales und submandibulares häufig mitbetroffen Komplikationen: Orchitis, Epididymitis, Meningitis, Meningoenzephalitis, Myokarditis, Pankreatitis, Adnexitis	Aerogen durch Tröpfchen selten durch Schmierinfektion

Nachweis des Erregers bzw. der Infektion durch:

- PCR
- Serologie: z.B. ELISA

2. VERWENDUNGSZWECK

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

3. TESTPRINZIP

Die qualitative immunoenzymatische 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 Waschschriff 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

- **Mumps Virus beschichtete Mikrotiterplatte (IgM):** 12 teilbare 8er-Streifen, beschichtet mit Mumps Virus Antigenen; in wieder verschließbarem Aluminiumbeutel.
- **IgM-Probenverdünnungspuffer:** 1 Flasche mit 100 ml Phosphatpuffer (10 mM) zur Probenverdünnung; pH $7,2 \pm 0,2$; anti-human IgG (RF- Absorbens); grün gefärbt; gebrauchsfertig; weiße Verschlusskappe.
- **Stopplösung:** 1 Flasche mit 15 ml Schwefelsäure, 0,2 mol/l; gebrauchsfertig; rote Verschlusskappe.
- **Waschpuffer (20x konz.):** 1 Flasche mit 50 ml eines 20-fach konzentrierten Phosphatpuffers (0,2 M), zum Waschen der Kavitäten; pH $7,2 \pm 0,2$; weiße Verschlusskappe.
- **Mumps Virus anti-IgM Konjugat:** 1 Flasche mit 20 ml Peroxidase-konjugierten Antikörpern gegen humanes IgM in Phosphatpuffer (10 mM); rot gefärbt; gebrauchsfertig; schwarze Verschlusskappe.
- **TMB-Substratlösung:** 1 Flasche mit 15 ml 3,3',5,5'-Tetramethylbenzidin (TMB), < 0,1 %; gebrauchsfertig; gelbe Verschlusskappe; < 5% NMP.
- **Mumps Virus IgM Positivkontrolle:** 1 Fläschchen mit 2 ml Kontrolle (humanes Serum oder Plasma); gelb gefärbt; rote Verschlusskappe; gebrauchsfertig.
- **Mumps Virus IgM Cut-off Kontrolle:** 1 Fläschchen mit 3 ml Kontrolle (humanes Serum oder Plasma); gelb gefärbt; grüne Verschlusskappe; gebrauchsfertig.
- **Mumps Virus IgM Negativkontrolle:** 1 Fläschchen mit 2 ml Kontrolle (humanes Serum oder Plasma); gelb gefärbt; blaue Verschlusskappe; gebrauchsfertig.

Für potenzielle Gefahrstoffe überprüfen Sie bitte das Sicherheitsdatenblatt.

4.2. Mitgeliefertes Zubehör

- 1 selbstklebende Abdeckfolie
- 1 Arbeitsanleitung
- 1 Plattenlayout

4.3. Erforderliche Materialien und Geräte

- Mikrotiterplatten-Photometer mit Filtern 450/620 nm
- Inkubator 37°C
- Manuelle oder automatische Waschvorrichtung
- Mikropipetten (10 - 1000 µl)
- Vortex-Mischer
- Destilliertes Wasser
- Plastikröhrchen für den einmaligen Gebrauch

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. Beschichtete Mikrotiterplatte

Die abbrechbaren Streifen sind mit Mumps Virus Antigenen beschichtet. Nicht verbrauchte Vertiefungen im Aluminiumbeutel zusammen mit dem Trockenmittel sofort wieder verschließen und bei 2...8 °C lagern.

6.2. Waschpuffer (20x konz.)

Der Waschpuffer ist im Verhältnis 1 + 19 zu verdünnen; z.B. 10 ml Waschpuffer + 190 ml destilliertes Wasser.

Der verdünnte Puffer ist bei Raumtemperatur (20...25 °C) 5 Tage haltbar. Sollten Kristalle im Konzentrat auftreten, die Lösung z.B. in einem Wasserbad auf 37 °C erwärmen und vor dem Verdünnen gut mischen.

6.3. TMB-Substratlösung

Die gebrauchsfertige Lösung ist bei 2...8 °C vor Licht geschützt aufzubewahren. Die Lösung ist farblos, kann aber auch leicht hellblau sein. Sollte die TMB-Substratlösung blau sein, ist sie kontaminiert und kann nicht im Test verwendet werden.

7. ENTNAHME UND VORBEREITUNG DER PROBEN

Es sollten humane Serum- oder Plasmaproben (Citrat, Heparin) verwendet werden. Für Liquor ist die Arbeitsanleitung ABVL0001 zu verwenden. Werden die Bestimmungen innerhalb von 5 Tagen nach Blutentnahme durchgeführt, können die Proben bei 2...8 °C aufbewahrt werden, sonst aliquotieren und tiefgefrieren (-70...-20 °C). Wieder aufgetaute Proben vor dem Verdünnen gut schütteln. Wiederholtes Tiefgefrieren und Auftauen vermeiden! Hitzeeinaktivierung der Proben wird nicht empfohlen.

7.1. Probenverdünnung

Proben vor Testbeginn im Verhältnis 1 + 100 mit IgM-Probenverdünnungspuffer verdünnen, z. B. 10 µl Probe und 1 ml IgM-Probenverdünnungspuffer in die entsprechenden Röhrchen pipettieren, um eine Verdünnung von 1 + 100 zu erhalten; gut mischen (Vortex).

8. TESTDURCHFÜHRUNG

8.1. Testvorbereitung

Arbeitsanleitung **vor** Durchführung des Tests sorgfältig lesen. Für die Zuverlässigkeit der Ergebnisse ist es notwendig, die Arbeitsanleitung genau zu befolgen. Die folgende Testdurchführung ist für die manuelle Methode validiert. Beim Arbeiten mit ELISA Automaten empfehlen wir, um Wascheffekte auszuschließen, die Zahl der Waschschrte von drei auf fünf und das Volumen des Waschpuffers von 300 µl auf 350 µl zu erhöhen. Kapitel 12 beachten. Vor Testbeginn auf dem mitgelieferten Plattenlayout die Verteilung bzw. Position der Proben und der Standards/Kontrollen (Doppelbestimmung empfohlen) genau festlegen. Die benötigte Anzahl von Mikrotiterstreifen (Kavitäten) in den Streifenhalter einsetzen.

Den Test in der angegebenen Reihenfolge und ohne Verzögerung durchführen.

Für jeden Pipettierschritt der Standards/Kontrollen und Proben saubere Einmalspitzen verwenden.

Den Inkubator auf 37 ± 1 °C einstellen.

1. Je 100 µl Standards/Kontrollen und vorverdünnte Proben in die entsprechenden Vertiefungen pipettieren. Vertiefung A1 ist für den Substratleerwert vorgesehen.
2. Die Streifen mit der mitgelieferten Abdeckfolie bedecken.
3. **1 h ± 5 min bei 37 °C inkubieren.**
4. Am Ende der Inkubationszeit Abdeckfolie entfernen und die Inkubationsflüssigkeit aus den Teststreifen absaugen. Anschließend dreimal mit 300 µl Waschpuffer waschen. Überfließen von Flüssigkeit aus den Vertiefungen vermeiden. Das Intervall zwischen Waschen und Absaugen sollte > 5 sec betragen. Nach dem Waschen die Teststreifen auf Fließpapier ausklopfen, um die restliche Flüssigkeit zu entfernen.
Beachte: Der Waschvorgang ist wichtig, da unzureichendes Waschen zu schlechter Präzision und falschen Messergebnissen führt!
5. 100 µl Konjugat in alle Vertiefungen, mit Ausnahme der für die Berechnung des Leerwertes A1 vorgesehenen, pipettieren.
6. **30 min bei Raumtemperatur (20...25 °C) inkubieren.** Nicht dem direkten Sonnenlicht aussetzen.
7. Waschvorgang gemäß Punkt 4 wiederholen.
8. 100 µl TMB-Substratlösung in alle Vertiefungen pipettieren.
9. **Genau 15 min im Dunkeln bei Raumtemperatur (20...25 °C) inkubieren.** Bei enzymatischer Reaktion findet eine Blaufärbung statt.
10. In alle Vertiefungen 100 µl Stopplösung in der gleichen Reihenfolge und mit den gleichen Zeitintervallen wie bei Zugabe der TMB-Substratlösung pipettieren, dadurch erfolgt ein Farbwechsel von blau nach gelb.
11. Die Extinktion der Lösung in jeder Vertiefung bei 450/620 nm innerhalb von 30 min nach Zugabe der Stopplösung messen.

8.2. Messung

Mit Hilfe des Substratleerwertes den **Nullabgleich** des Mikrotiterplatten-Photometers vornehmen.

Falls diese Eichung aus technischen Gründen nicht möglich ist, muss nach der Messung der Extinktionswert des Substratleerwertes von allen anderen Extinktionswerten subtrahiert werden, um einwandfreie Ergebnisse zu erzielen!

Extinktion aller Kavitäten bei **450 nm** messen und die Messwerte der Standards/Kontrollen und Proben in das Plattenlayout eintragen.

Eine **bichromatische** Messung mit der Referenzwellenlänge 620 nm wird empfohlen.

Falls Doppel- oder Mehrfachbestimmungen durchgeführt wurden, den **Mittelwert der Extinktionswerte** berechnen.

9. BERECHNUNG DER ERGEBNISSE

9.1. Testgültigkeitskriterien

Der Test wurde richtig durchgeführt, wenn er folgende Kriterien erfüllt:

- **Substrat-Leerwert:** Extinktionswert < **0,100**
- **Negativkontrolle:** Extinktionswert < **0,200** und < **Cut-off**
- **Cut-off Kontrolle:** Extinktionswert **0,150 – 1,300**
- **Positivkontrolle:** Extinktionswert > **Cut-off**

Sind diese Kriterien nicht erfüllt, ist der Testlauf ungültig und muss wiederholt werden.

9.2. Messwertberechnung

Der Cut-off ergibt sich aus dem Mittelwert der gemessenen Extinktionen der Cut-off Kontrolle.

Beispiel: $0,44 \text{ OD Cut-off Kontrolle} + 0,42 \text{ OD Cut-off Kontrolle} = 0,86: 2 = 0,43$

Cut-off = 0,43

9.2.1. Ergebnisse in Einheiten [NTU]

$\frac{\text{Mittlere Extinktion der Probe} \times 10}{\text{Cut-off}} = [\text{NovaTec Einheiten} = \text{NTU}]$

Beispiel: $\frac{1,591 \times 10}{0,43} = 37 \text{ NTU}$

9.3. Interpretation der Ergebnisse

Cut-off	10 NTU	-
Positiv	> 11 NTU	Es liegen Antikörper gegen den Erreger vor. Ein Kontakt mit dem Antigen (Erreger bzw. Impfstoff) hat stattgefunden.
Grenzwertig	9 – 11 NTU	Antikörper gegen den Erreger können nicht eindeutig nachgewiesen werden. Es wird empfohlen den Test nach 2 bis 4 Wochen mit einer frischen Patientenprobe zu wiederholen. Finden sich die Ergebnisse erneut im grenzwertigen Bereich, gilt die Probe als negativ .
Negativ	< 9 NTU	Es liegen keine Antikörper gegen den Erreger vor. Ein vorausgegangener Kontakt mit dem Antigen (Erreger bzw. Impfstoff) ist unwahrscheinlich.
Die Diagnose einer Infektionskrankheit darf nicht allein auf der Basis des Ergebnisses einer Bestimmung gestellt werden. Die anamnestischen Daten sowie die Symptomatologie des Patienten müssen zusätzlich zu den serologischen Ergebnissen in Betracht gezogen werden. Bei Immunsupprimierten und Neugeborenen besitzen die Ergebnisse serologischer Tests nur einen begrenzten Wert.		

9.3.1. Antikörper-Isotypen und Infektionsstatus

Serologie	Bedeutung
IgM	Typisch für Primärantwort Hoher IgM-Titer bei gleichzeitig niedrigem IgG-Titer: → Hinweis auf relativ frische Infektion Selten: → persistierendes IgM
IgG	Typisch für Sekundärantwort Können auch noch nach Jahren nachweisbar sein Hoher IgG-Titer bei gleichzeitig niedrigem IgM-Titer: → wahrscheinlich länger zurückliegende Infektion

10. TESTMERKMALE

Die Ergebnisse beziehen sich auf die untersuchten Probenkollektive; es handelt sich nicht um garantierte Spezifikationen.

Für weitere Informationen zu den Testmerkmalen kontaktieren Sie bitte NovaTec Immundiagnostica GmbH.

10.1. Präzision

Intraassay	n	Mittelwert (E)	Vk (%)
#1	24	0,600	6,05
#2	24	1,039	4,81
#3	24	0,639	3,71
Interassay	n	Mittelwert (NTU)	Vk (%)
#1	12	21,38	7,50
#2	12	13,88	8,95
#3	12	1,39	10,35

10.2. Diagnostische Spezifität

Die diagnostische Spezifität ist definiert als die Wahrscheinlichkeit des Tests, ein negatives Ergebnis bei Fehlen des spezifischen Analyten zu liefern. Sie beträgt 97,68% (95% Konfidenzintervall: 95,03% - 99,15%).

10.3. Diagnostische Sensitivität

Die diagnostische Sensitivität ist definiert als die Wahrscheinlichkeit des Tests, ein positives Ergebnis bei Vorhandensein des spezifischen Analyten zu liefern. Sie ist 94,44% (95% Konfidenzintervall: 72,71% - 99,86%).

10.4. Interferenzen

Hämolytische, lipämische und ikterische Proben ergaben bis zu einer Konzentration von 10 mg/ml für Hämoglobin, von 5 mg/ml Triglyceride und von 0,5 mg/ml für Bilirubin keine Interferenzen im vorliegenden ELISA.

10.5. Kreuzreaktivität

Die Untersuchung eines Probenpanels mit Antikörperaktivitäten gegen potenziell kreuzreagierende Parameter ließ keine Anzeichen von falsch-positiven Ergebnissen aufgrund von Kreuzreaktivitäten erkennen.

11. GRENZEN DES VERFAHRENS

Kontamination der Proben durch Bakterien oder wiederholtes Einfrieren und Auftauen können zu einer Veränderung der Messwerte führen.

12. SICHERHEITSMASSNAHMEN UND WARNHINWEISE

- Gemäß Art. 1 Abs. 2b der EU-Richtlinie 98/79/EG legt der Hersteller die Zweckbestimmung von In-vitro-Diagnostika fest, um deren Eignung, Leistung und Sicherheit sicherzustellen. Daher sind die Testdurchführung, die Information, die Sicherheitsmaßnahmen und Warnhinweise in der Arbeitsanleitung strikt zu befolgen. Bei Anwendung des Testkits auf Diagnostika-Geräten ist die Testmethode zu validieren. Jede Änderung am Aussehen, der Zusammensetzung und der Testdurchführung sowie jede Verwendung in Kombination mit anderen Produkten, die der Hersteller nicht autorisiert hat, ist nicht zulässig; der Anwender ist für solche Änderungen selbst verantwortlich. Der Hersteller haftet für falsche Ergebnisse und Vorkommnisse aus solchen Gründen nicht. Auch für falsche Ergebnisse aufgrund von visueller Auswertung wird keine Haftung übernommen.
- Nur für in-vitro-Diagnostik.
- Alle Materialien menschlichen oder tierischen Ursprungs sind als potentiell infektiös anzusehen und entsprechend zu behandeln.
- Alle verwendeten Bestandteile menschlichen Ursprungs sind auf Anti-HIV-AK, Anti-HCV-AK und HBsAg nicht-reaktiv getestet.
- Reagenzien und Mikrotiterplatten unterschiedlicher Chargen nicht untereinander austauschen.
- Keine Reagenzien anderer Hersteller zusammen mit den Reagenzien dieses Testkits verwenden.
- Nicht nach Ablauf des Verfallsdatums verwenden.
- Nur saubere Pipettenspitzen, Dispenser und Labormaterialien verwenden.
- Verschlusskappen der einzelnen Reagenzien nicht untereinander vertauschen, um Kreuzkontaminationen zu vermeiden.
- Flaschen sofort nach Gebrauch fest verschließen, um Verdunstung und mikrobielle Kontamination zu vermeiden.
- Nach dem ersten Öffnen Konjugat und Standards/Kontrollen vor weiterem Gebrauch auf mikrobielle Kontamination prüfen.
- Zur Vermeidung von Kreuzkontamination und falsch erhöhten Resultaten, Reagenzien sorgfältig in die Kavitäten pipettieren.
- Der ELISA ist nur für die Anwendung durch Fachpersonal vorgesehen, welches die Arbeitstechniken einwandfrei beherrscht.

12.1. Entsorgungshinweise

Chemikalien und Zubereitungen sind in der Regel Sonderabfälle. Deren Beseitigung unterliegt den nationalen abfallrechtlichen Gesetzen und Verordnungen. Die zuständige Behörde informiert über die Entsorgung von Sonderabfällen.

13. BESTELLINFORMATIONEN

Produktnummer: MUMM0340 Mumps Virus IgM ELISA (96 Bestimmungen)

FRANÇAIS

1. INTRODUCTION

Les virus des oreillons sont des virus ARN de la famille Paramyxoviridae. Les virions sont des particules sphériques de 150-250 nm de diamètre qui se composent d'une ribonucléoprotéine avec une symétrie hélicoïdale et qui sont enveloppées par une protéine matrice et une double couche lipidique qui contient deux autres protéines: hemagglutinin viral (H) et neuraminidase virale (N). Le virus des oreillons atteint surtout la parotide et les glandes salivaires reliées; cependant l'infection peut mener à une maladie du SNC et à l'accumulation du virus dans le liquide céphalo-rachidien. Les oreillons (parotidite épidémique) sont une maladie virale contagieuse aiguë qui se produit surtout chez les enfants. Presque 50% de toutes les infections sont subcliniques. Le plus haute incidence des manifestations cliniques est trouvée dans la catégorie d'âge de 4 à 15 ans. Les infections secondaires sont rares à cause de l'immunité durable. 10 à 35 % des cas d'oreillons développent l'orchite qui se produit presque toujours après la puberté. Pour la plupart du temps, le processus est unilatéral et d'habitude le pronostic est bon. Le virus des oreillons a été l'une des causes les plus importantes de la maladie virale du SNC (méningite et encéphalite) aux Etats-Unis; l'administration des vaccinations a considérablement réduit cette maladie.

Espèce	La maladie	Symptômes (p.ex.)	Modes de transmission
Virus des oreillons (en Anglais Mumps Virus)	Oreillons	Fièvre et gonflement unilatéral ou bilatéral de la glande parotide; les glandes sublingual peuvent également être impliquées Complications: Orchite, Méningo-encéphalite, Pancréatites	La transmission du virus se produit par l'infection de gouttelettes; La transmission du virus se produit par une infection gouttelette;

L'identification de l'agent pathogène ou d'une infection par:

- PCR
- Sérologie: p.ex. ELISA

2. INDICATION D'UTILISATION

La trousse Mumps Virus IgM ELISA est prévue pour la détection qualitative des anticorps IgM anti-Mumps Virus dans le sérum humain ou plasma (citrate, héparine).

3. PRINCIPE DU TEST

La détermination immunoenzymatique qualitative des anticorps spécifiques est basée sur la technique ELISA (du anglais, Enzyme-Linked Immunosorbent Assay).

Microplaques sont recouvertes d'antigènes spécifiques pour lier les anticorps correspondants de l'échantillon. Après le lavage des puits pour éliminer l'échantillon détaché, le conjugué peroxydase de raifort (HRP) est ajouté. Ce conjugué se lie aux anticorps capturés. Dans une deuxième étape de lavage, le conjugué non lié est éliminé. Le complexe immun formé par le conjugué lié est visualisé par l'addition tétraméthylbenzidine (TMB) qui donne un produit de réaction bleu.

L'intensité de ce produit est proportionnelle à la quantité d'anticorps spécifiques dans l'échantillon. L'acide sulfurique est ajouté pour arrêter la réaction. Cela produit un changement du bleu au jaune. L'absorbance à 450/620 nm est lue en utilisant un lecteur de microplaques ELISA.

4. MATERIEL

4.1. Réactifs fournis

- **Mumps Virus (IgM) microplaque revêtus:** 12 barrettes de 8 puits sécables revêtus d'antigène d'Mumps Virus; en sachets d'aluminium refermables.
- **Diluant pour échantillon IgM:** 1 flacon contenant 100 ml de tampon phosphaté (10 mM) pour la dilution de l'échantillon; pH $7,2 \pm 0,2$; anti-humaine IgG (RF-Absorbant); prêt à l'emploi; couleur vert; bouchon blanc.
- **Solution d'arrêt:** 1 flacon contenant 15 ml d'acide sulfurique, 0,2 mol/l; prêt à l'emploi; bouchon rouge.
- **Tampon de lavage (concentré x 20):** 1 flacon contenant 50 ml d'un tampon phosphaté (0,2 M) concentré 20 fois (pH $7,2 \pm 0,2$) pour laver les puits; bouchon blanc.
- **Conjugué Mumps Virus anti-IgM:** 1 flacon contenant 20 ml d'anticorps IgM anti-humaines conjuguées à de la peroxydase du raifort dans le tampon phosphaté (10 mM); prêt à l'emploi; couleur rouge, bouchon noir.
- **Solution de substrat TMB:** 1 flacon contenant 15 ml de 3,3',5,5'-tétraméthylbenzidine (TMB), < 0,1 %; prêt à l'emploi; bouchon jaune; < 5% NMP.
- **Contrôle positif Mumps Virus IgM:** 1 flacon contenant 2 ml contrôle (sérum humain ou plasma); prêt à l'emploi; couleur jaune; bouchon rouge.
- **Contrôle cut-off Mumps Virus IgM:** 1 flacon contenant 3 ml contrôle (sérum humain ou plasma); prêt à l'emploi; couleur jaune; bouchon vert.
- **Contrôle négatif Mumps Virus IgM:** 1 flacon contenant 2 ml contrôle (sérum humain ou plasma); prêt à l'emploi; couleur jaune; bouchon bleu.

Pour les substances potentiellement dangereuses s'il vous plaît vérifiez la fiche de données de sécurité.

4.2. Matériel fourni

- 1 couvercle autocollante
- 1 instructions d'utilisation
- 1 présentation de la plaque

4.3. Matériel et équipement requis

- Lecteur de microplaques ELISA, pour mesurer l'absorbance à 450/620 nm
- Incubateur 37 °C
- Laveur manuel ou automatique pour le lavage des puits
- Pipettes pour utilisation entre 10 et 1000 µl
- Mélangeur Vortex
- Eau distillée
- Tubes jetables

5. STABILITE ET CONSERVATION

Conserver le kit à 2...8 °C. Les réactifs ouverts sont stables jusqu'à la date de péremption indiquée sur l'étiquette lorsqu'il est conservé à 2...8°C.

6. PREPARATION DES REACTIFS

Il est très important porter tous les réactifs et échantillons à température ambiante (20 ... 25 °C) et les mélanger avant de commencer le test!

6.1. Microplaque revêtues

Les barrettes sécables sont revêtues d'antigène d'Mumps Virus. Immédiatement après avoir prélevé les barrettes nécessaires, les barrette restantes doivent être scellés le vide dans de feuille d'aluminium avec le sac de silicium (le déshydratant) fourni et emmagasiner à 2...8 °C.

6.2. Tampon de lavage (conc. x 20)

Diluer le Tampon de lavage 1+19; par exemple 10 ml du Tampon de lavage + 190 ml d'eau distillée. L'échantillon de tampon diluée est stable pendant 5 jours à la température ambiante (20...25 °C). Cas apparaissent des cristaux dans le concentré, chauffer la solution à 37 °C par exemple dans un bain-marie mélangez bien avant dilution.

6.3. Solution de substrat TMB

La solution est prête à utiliser et doit être emmagasiné à 2...8 °C, à l'abri de la lumière. La solution doit être incolore ou pourrait avoir une légère couleur bleu clair. Si le substrat devient bleu, il peut avoir été contaminé et ne peut pas être utilisé dans le test.

7. PRELEVEMENT ET PREPARATION DES ECHANTILLONS

Utiliser des échantillons humain de sérum ou plasma (citrate, héparine) pour ce test. Les instructions d'utilisation ABVL0001 doit être utilisé pour le LCS. Si le test est réalisé dans les 5 jours après le prélèvement, les échantillons doivent être conservés à 2...8 °C; autrement ils doivent être aliquotés et conservés surgelés (-70...-20 °C). Si les échantillons sont conservés congelés, bien mélanger les échantillons décongelés avant le test. Éviter les cycles répétés de congélation et décongélation. L'inactivation par la chaleur des échantillons n'est pas recommandée.

7.1. Dilution de l'échantillon

Avant du test, tous les échantillons doivent être dilués 1 + 100 avec diluant de l'échantillon IgM. Diluer 10 µl d'échantillon avec 1 ml I diluant de l'échantillon IgM dans des tubes pour obtenir une dilution 1 + 100 et mélanger soigneusement sur un Vortex.

8. PROCEDE DE TEST

8.1. Préparation du test

Lire attentivement les instructions d'utilisation **avant de** réaliser le test. La fiabilité des résultats dépend du suivi strict d'utilisation comme décrit. La technique de test suivante a été validée uniquement pour une procédure manuelle. Si le test doit être effectué sur un systèmes automatiques pour ELISA, nous conseillons d'augmenter le nombre d'étapes de lavage de trois à cinq et le volume du Tampon de lavage de 300 à 350 µl. Faites attention au chapitre 12. Avant de commencer le test, le plan de distribution et d'identification de tous les échantillons et les étalons/contrôles (il est recommandé déterminer en double) doivent être soigneusement établi sur la feuille présentation de la plaque prévue dans le conseil de kit. Sélectionner le nombre de barrettes ou de puits nécessaires et les placer sur le support.

Réaliser toutes les étapes du test dans l'ordre donné et sans délai.

Un embout de pipette propre et jetable doit être utilisé pour distribuer chaque étalon/contrôle et échantillon.

Régler l'incubateur à 37 ± 1 °C.

1. Pipeter 100 µl de étalons/contrôles et d'échantillons dilués dans leurs puits respectifs. Garder le puits A1 pour le blanc substrat.
2. Couvrir les puits avec le couvercle, fourni dans le kit.
3. **Incuber pendant 1 heure $\pm$ 5 minutes à 37 ± 1 °C.**
4. A la fin de l'incubation, enlever le couvercle, aspirer le contenu des puits et laver chaque puits trois fois avec 300 µl du Tampon de lavage. Éviter les débordements des puits de réaction. L'intervalle entre le cycle de lavage et l'aspiration doit être > 5 sec. À la fin, enlever soigneusement le liquide restant en tapotant les barrettes sur du papier absorbant avant la prochaine étape.
Note: L'étape de lavage est très importante! Un lavage insuffisant peut conduire à une précision faible et de faux résultats !
5. Pipeter 100 µl du conjugué dans tous les puits sauf le puits Blanc A1.
6. **Incuber pendant 30 minutes à température ambiante (20...25°C).** N'exposer pas à la lumière directe du soleil.
7. Répéter l'étape numéro 4.
8. Pipeter 100 µl de la solution de substrat TMB dans tous les puits.
9. **Incuber pendant exactement 15 minutes à température ambiante (20...25°C) dans l'obscurité.** Une couleur bleue se produit en raison d'une réaction enzymatique.
10. Pipeter 100 µl de la solution d'arrêt dans tous les puits dans le même ordre et à la même vitesse que pour la solution de substrat TMB, ainsi, il y a un changement du bleu au jaune.
11. Mesurer l'absorbance à 450/620 nm dans les 30 minutes après l'addition de la solution d'arrêt.

8.2. Mesure

Réglez le lecteur de microplaques ELISA à **zéro** en utilisant le **Blanc substrat**.

Si - pour des raisons techniques - le lecteur de microplaques ELISA ne peut pas être ajusté à zéro en utilisant le Blanc substrat, la valeur d'absorbance de cette doit être soustraire la valeur d'absorbance de toutes les autres valeurs d'absorbance mesurées afin d'obtenir des résultats fiables!

Mesurer l'absorbance de tous les puits à **450 nm** et enregistrer les valeurs d'absorbance pour chaque étalon/contrôle et échantillon dans la présentation de la plaque.

Il est recommandé d'effectuer la mesure **dichromatique** utilisant 620 nm comme longueur d'onde de référence.

Si doubles déterminations ont été effectuées, calculer **les valeurs moyennes d'absorbance**.

9. RESULTATS

9.1. Critères de validation

Afin de valider le test, les critères suivants doivent être respectés:

- **Blanc Substrat:** Valeur d'absorbance < **0,100**
- **Contrôle négatif:** Valeur d'absorbance < **0,200** et < **Cut-off**
- **Contrôle cut-off:** Valeur d'absorbance **0,150 – 1,300**
- **Contrôle positif:** Valeur d'absorbance > **Contrôle cut-off**

Lorsque ces critères ne sont pas remplis, le test n'est pas valide et doit être recommencé.

9.2. Calcul des résultats

La valeur seuil correspond à la moyenne des valeurs d'absorbance du Contrôle cut-off.

Exemple: $0,44 \text{ DO Contrôle cut-off} + 0,42 \text{ DO Contrôle cut-off} = 0,86 : 2 = 0,43$
Cut-off = 0,43

9.2.1. Résultats en unités [NTU]

Valeur (moyenne) d'absorbance de l'échantillon x 10 = [unités NovaTec = NTU]
Cut-off

Exemple: $\frac{1,591 \times 10}{0,43} = 37 \text{ NTU}$

9.3. Interprétation des résultats

Cut-off	10 NTU	-
Positif	> 11 NTU	Les anticorps dirigés contre l'agent pathogène sont présents. Il ya eu un contact avec l'antigène (pathogène resp. vaccin).
Zone grise	9 – 11 NTU	Les anticorps dirigés contre l'agent pathogène ne pouvaient pas être détectés clairement. Il est recommandé de répéter le test avec un échantillon frais dans 2 à 4 semaines. Si le résultat est encore dans la zone grise l'échantillon est jugé négatif .
Négatif	< 9 NTU	L'échantillon ne contient pas d'anticorps contre l'agent pathogène. Un contact préalable avec l'antigène (pathogène resp. vaccin) est peu probable.

Le diagnostic d'une maladie infectieuse ne devrait pas être établi sur la base du résultat d'une seule analyse. Un diagnostic précis devrait prendre en considération l'histoire clinique, la symptomatologie ainsi que les données sérologiques. Les données sérologiques sont de valeur limitée dans le cas des patients immunodéprimés et des nouveau-nés.

9.3.1. Isotypes d'anticorps et l'Etat de l'infection

Sérologie	Signification
IgM	Caractéristique de la réponse primaire du anticorps Titre élevé d'IgM avec une faible titre d'IgG: → suggère une infection très récente ou aiguë Rare: → persistante IgM
IgG	Caractéristique de la réponse secondaire du anticorps Peut persister pendant plusieurs années Des titres élevés d'IgG à faible titre d'IgM: → peuvent indiquer une infection ancienne

10. PERFORMANCES DU TEST

Ces résultats s'appuient sur les groupes d'échantillons étudiés; il n'agit pas de caractéristiques techniques garanties.
Pour plus d'informations sur les performances du test s'il vous plaît contactez NovaTec Immundiagnostica GmbH.

10.1. Précision

Intra-essai	n	moyenne (E)	CV (%)
#1	24	0,600	6,05
#2	24	1,039	4,81
#3	24	0,639	3,71
Inter-essai	n	moyenne (NTU)	CV (%)
#1	12	21,38	7,50
#2	12	13,88	8,95
#3	12	1,39	10,35

10.2. Spécificité diagnostique

La spécificité diagnostique est définie comme la probabilité d'obtenir un résultat négatif en l'absence d'un analyte spécifique.
Elle est 97,68% (95% Intervalle de confiance: 95,03% - 99,15%).

10.3. Sensibilité diagnostique

La sensibilité diagnostique est définie comme la probabilité d'obtenir un résultat positif en présence d'un analyte spécifique.
Elle est 94,44% (95% Intervalle de confiance: 72,71% - 99,86%).

10.4. Interférences

Des échantillons hémolytiques ou lipémiques ou ictériques n'ont pas montré d'interférences, avec des concentrations jusqu'à 10 mg/ml de hémoglobine, 5 mg/ml de triglycérides et 0,5 mg/ml de bilirubine.

10.5. Réaction croisée

L'étude d'un panel d'échantillons avec des anticorps dirigés contre différents paramètres interférents n'a pas révélé de preuves de résultats faussement positifs dus à des réactions croisées.

11. LIMITES DE LA TECHNIQUE

Une contamination bactérienne ou des cycles de congélation/décongélation répétés de l'échantillon peuvent affecter les valeurs d'absorption.

12. PRECAUTIONS ET AVERTISSEMENTS

- En accord avec l'article 1 paragraphe 2b de la directive européenne 98/79/EC, l'utilisation des dispositifs médicaux de diagnostic in vitro est destinée par le fabricant à garantir le bien-fondé, les performances et la sécurité du produit. Par conséquent, la procédure de test, l'information, les précautions et mises en garde de la notice d'emploi, doivent être suivies de façon stricte. L'utilisation de ces trousse avec des automates ou dispositifs similaires doit être validée. Aucun changement de la conception, composition et procédure de test, ainsi que l'utilisation avec d'autres produits non approuvés par le fabricant, ne sont pas autorisés; seul l'utilisateur est responsable de tels changements. Le fabricant n'est pas responsable des faux résultats et des incidents dus à ces motifs. Le fabricant n'est pas responsable des résultats fournis par analyse visuelle des échantillons des patients.
- Uniquement pour diagnostic in vitro.
- Tous les matériaux d'origine humaine ou animale doivent être considérés et traités comme étant potentiellement infectieux.
- Tous les composants d'origine humaine utilisés pour la fabrication de ces réactifs ont été analysés et ont été trouvés non réactifs en Ag HBs, en anticorps anti-VHI 1 et 2 et en anticorps anti-VHC.
- Ne pas échanger les réactifs ou les barrettes provenant de différents lots de production.
- Ne pas utiliser de réactifs provenant d'autres fabricants avec les réactifs de cette trousse.
- Ne pas utiliser les réactifs après la date de péremption indiquée sur l'étiquette.
- Utiliser seulement des embouts de pipette, des distributeurs et du matériel de laboratoire propres.
- Ne pas échanger les bouchons des flacons, pour éviter la contamination croisée.
- Fermer soigneusement les flacons après utilisation pour éviter l'évaporation et la contamination microbienne.
- Avant une nouvelle utilisation, vérifier les flacons de conjugué et de étalon/contrôle, déjà utilisés, pour exclure une contamination microbienne.
- Pour éviter la contamination croisée et des résultats faussement élevés, introduire les échantillons de patients et les réactifs exactement au fond des puits sans éclabousser.
- Le ELISA est uniquement destinée à l'utilisation par un personnel compétent, maîtrisant parfaitement les techniques de travail.

12.1. Elimination des déchets

Les résidus des produits chimiques et des préparations sont considérés en général comme des déchets dangereux. L'élimination de ce type de déchet est réglementée par des lois et réglementations nationales et régionales. Contacter les autorités compétentes ou les sociétés de gestion des déchets pour obtenir des renseignements sur l'élimination des déchets dangereux.

13. INFORMATION POUR LES COMMANDES

Référence:	MUMM0340	Mumps Virus IgM ELISA (96 déterminations)

ITALIANO

1. INTRODUZIONE

Il Virus della parotite è a RNA a singola elica e appartiene alla famiglia dei paramyxoviridae. Presenta un involucro contenente lipidi e ha un diametro di ca 150-250 nm. Il virus causa la parotite epidemica (orecchioni), una malattia presente in tutto il mondo, contratta soprattutto da i bambini nel periodo freddo dell'anno. Nel 30 % circa dei casi la malattia non manifesta sintomi clinici. Viene trasmessa attraverso goccioline e risulta con immunità durevole a vita. Il virus infetta soprattutto gli epitelii delle vie aeree superiori ma anche il tratto gastrointestinale e gli occhi. Dopo la replicazione iniziale si introduce nei linfonodi per poi infettare anche altri organi come le ghiandole salivari, le ghiandole mammarie, i testicoli, le ovaie, l'SNC e il pancreas. Dopo un periodo di incubazione di 7-21 giorni si manifesta con febbre, mal di testa e malessere in generale. Il sintomo classico della parotite è l'ingrossamento doloroso di una o delle due ghiandole parotidi. Le complicazioni dell'infezione sono: l'orchite e l'epididimite (20%) (nel 80% dei casi risulta con sterilità), meningite (20%), dopo il 18esimo giorno della infezione meningoencefalite (raramente <1% con una letalità del 5%), miocardite e pancreatite. Esiste un vaccino specifico.

Specie	Malattia	Sintomi (p.es.)	Via di trasmissione
Virus della parotite, Paramyxovirus (In inglese Mumps Virus)	Parotite epidemica (orecchioni)	La febbre e gonfiore unilaterale o bilaterale della parotite; le ghiandole sublinguali e sottomascellari possono anche essere coinvolti. Complicanze: Orchite, meningoencefalite, pancreatite	Trasmissione: aerea; contatto diretto con una persona infetta

La presenza di agenti patogeni o infezione può essere identificato mediante:

- PCR
- Sierologia: p.es. ELISA

2. USO PREVISTO

Il Mumps Virus IgM ELISA è un kit per la determinazione qualitativa degli anticorpi specifici della classe IgM per Mumps Virus nel siero o plasma (citrato, eparina) umano.

3. PRINCIPIO DEL TEST

La determinazione immunoenzimatica qualitativa degli anticorpi specifici si basa sulla tecnica ELISA (d'inglese Enzyme-linked immunosorbent assay).

Micropiastre sono rivestite con antigeni specifici che si legano agli anticorpi presenti nel campione. Dopo aver lavato i pozzetti per rimuovere tutto il materiale campione non legato, il coniugato di perossidasi di rafano (HRP) è aggiunto. Questo coniugato si lega agli anticorpi catturati. In una seconda fase di lavaggio coniugato, non legato è rimosso. Il complesso immunitario formato dal coniugato legato sarà evidenziato aggiungendo tetrametilbenzidina (TMB) substrato che dà una colorazione blu. L'intensità di questa colorazione è direttamente proporzionale alla quantità di anticorpi specifici presenti nel campione. Acido solforico è aggiunto per bloccare la reazione. Questo produce un cambiamento di colore dal blu al giallo. Assorbanza a 450/620 nm viene letto utilizzando un lettore di micropiastre ELISA.

4. MATERIALI

4.1. Reagenti forniti

- **Mumps Virus (IgM) micropiastre rivestite:** 12 strisce divisibili in 8 pozzetti, con adesivi antigeni della Mumps Virus; dentro una busta d'alluminio richiudibile.
- **Tampone diluente IgM:** 1 flacone contenente 100 ml di tampone fosfato (10 mM) per diluire i campioni; pH 7,2 ± 0,2; anti-umane IgG (mezzo di assorbimento RF); colore verde; pronto all'uso; tappo bianco.
- **Soluzione bloccante:** 1 flacone contenente 15 ml di acido solforico, 0,2 mol/l, pronto all'uso; tappo rosso.
- **Tampone di lavaggio (20x conc.):** 1 flacone contenente 50 ml di un tampone fosfato concentrato 20 volte (0,2 M) per il lavaggio dei pozzetti; pH 7,2 ± 0,2; tappo bianco.
- **Coniugato Mumps Virus anti-IgM:** 1 flacone contenente 20 ml di anticorpi anti-IgM umani, coniugati a perossidasi in tampone fosfato (10 mM); colore rosso; pronto all'uso; tappo nero.
- **Soluzione Substrato TMB:** 1 flacone contenente 15 ml di 3,3',5,5'-Tetrametilbenzidina (TMB), < 0,1 %; pronto all'uso; tappo giallo; < 5% NMP.
- **Controllo positivo Mumps Virus IgM:** 1 flacone da 2 ml controllo (siero o plasma umano); colore giallo; tappo rosso; pronto all'uso.
- **Controllo cut-off Mumps Virus IgM:** 1 flacone da 3 ml controllo (siero o plasma umano); colore giallo; tappo verde; pronto all'uso.
- **Controllo negativo Mumps Virus IgM:** 1 flacone da 2 ml controllo (siero o plasma umano); colore giallo; tappo blu; pronto all'uso.

Per le sostanze potenzialmente pericolose si prega di leggere la scheda di dati di sicurezza.

4.2. Accessori forniti

- 1 supporto per micropiastre
- 1 istruzione per l'uso
- 1 schema della piastra

4.3. Materiali e attrezzature necessari

- Fotometro per micropiastre con filtri da 450/620 nm
- Incubatrice 37°C
- Lavatore, manuale o automatico, di micropiastre
- Micropipette per l'uso tra 10-1000 µl
- Vortex-Mixer
- Acqua distillata
- Provette monouso

5. MODALITÀ DI CONSERVAZIONE

Conservare il kit a 2...8 °C. I reagenti aperti sono stabili fino alla data di scadenza indicata sull'etichetta quando sono conservati a 2...8 °C.

6. PREPARAZIONE DEI REAGENTI

È molto importante, portare tutti i reagenti e campioni a temperatura ambiente (20...25 °C) e mescolare prima di iniziare il test.

6.1. Micropiastre rivestita

Le strisce divisibili sono rivestite con l'antigeni della Mumps Virus. Immediatamente dopo la rimozione degli strisce necessari, le strisce rimanenti devono essere sigillare nuovamente in un foglio di alluminio insieme con il sacchetto di gel di silice conservati a 2...8 °C.

6.2. Tampone di lavaggio (20x conc.)

Diluire il Tampone di lavaggio 1+19; per esempio: 10 ml del Tampone di lavaggio + 190 ml di acqua distillata. Il campione di tampone diluito è stabile per 5 giorni a temperatura ambiente (20...25 °C). Se cristalli appaiono nel concentrato, riscaldare la soluzione a 37 °C per esempio in un bagnomaria. Mescolare bene prima della diluizione.

6.3. Soluzione Substrato TMB

La soluzione sta pronta all'uso e deve essere conservata a 2...8 °C, al riparo dalla luce. La soluzione deve essere incolore o potrebbe avere un leggero colore blu chiaro. Se il substrato diventa blu, potrebbe essere stato contaminato e non può essere utilizzato nel test.

7. PRELIEVO E PREPARAZIONE DEI CAMPIONI

Per questo test si prega di usare campioni di siero o plasma (citrato, eparina) umano. Per il CSF, si prega di utilizzare le istruzioni per l'uso ABVL0001. Se il test è fatto entro 5 giorni dal prelievo i campioni possono essere conservati tra 2...8 °C. Altrimenti devono essere aliquotati e congelati tra (-70...-20 °C). Se i campioni sono conservati congelati, mescolare bene i campioni scongelati prima del test. Evitare cicli ripetuti di congelamento/scongelamento. L'inattivazione dei campioni per mezzo del calore non è raccomandata.

7.1. Diluizione dei campioni

Prima del test, diluire i campioni 1+100 con tampone diluente IgM. Per esempio, pipettare nelle provette 10 µl di campione + 1 ml di tampone IgM e mescolare bene (Vortex).

8. PROCEDIMENTO

8.1. Preparazione del test

Leggere bene le istruzioni per l'uso **prima** di iniziare il test. L'affidabilità dei risultati dipende dalla stretta aderenza alle istruzioni per l'uso di prova come descritto. La seguente procedura è stata validata per l'esecuzione manuale. Per un'esecuzione su strumentazione automatica si consiglia di incrementare il numero di lavaggi da 3 a 5 volte e il volume della Tampone di lavaggio da 300 a 350 µl per evitare effetti di lavaggio. Prestare attenzione al capitolo 12. Stabilire innanzitutto il piano di distribuzione e identificazione dei campioni e standards/controlli (è raccomandato determinare in duplicato) sullo schema della piastra fornito con il kit. Inserire i pozzetti necessari nel supporto.

Eseguire il test nell'ordine stabilito dalle istruzioni, senza ritardi.

Sul pipettaggio utilizzare puntali nuovi e puliti per ogni campione e standard/controllo.

Regolare l'incubatore a $37 \pm 1^\circ\text{C}$.

1. Pipettare 100 µl di standard/controllo e di campione diluito nei relativi pozzetti. Usare il pozzetto A1 per il Bianco-substrato.
2. Coprire i pozzetti con la pellicola adesiva, fornita nel kit.
3. **Incubare 1 ora $\pm$ 5 min a $37 \pm 1^\circ\text{C}$.**
4. Al termine dell'incubazione, togliere la pellicola e aspirare il liquido dai pozzetti. Successivamente lavare i pozzetti tre volte con 300 µl di tampone di lavaggio. Evitare che la soluzione trabocchi dai pozzetti. L'intervallo tra il lavaggio e l'aspirazione deve essere > 5 sec. Dopo il lavaggio picchiare delicatamente i pozzetti su una carta assorbente per togliere completamente il liquido, prima del passo successivo.
Attenzione: Il lavaggio è una fase molto importante. Da lavaggio insufficiente risulta una bassa precisione e risultati falsi!
5. Pipettare 100 µl di Coniugato in tutti i pozzetti, escludendo quello con il Bianco-substrato (Blank) A1.
6. **Incubare per 30 min a temperatura ambiente ($20...25^\circ\text{C}$).** Non esporre a fonti di luce diretta.
7. Ripetere il lavaggio secondo punto 4.
8. Pipettare 100 µl di Soluzione Substrato TMB in tutti i pozzetti.
9. **Incubare precisamente per 15 min a temperatura ambiente ($20...25^\circ\text{C}$) al buio.** Un colore blu verifica a causa della reazione enzimatica.
10. Pipettare 100 µl di Soluzione bloccante in tutti i pozzetti, nello stesso ordine della Soluzione Substrato TMB, in tal modo un cambiamento di colore dal blu al giallo si verifica.
11. Misurare l'assorbanza a 450/620 nm entro 30 min dopo l'aggiunta della Soluzione bloccante.

8.2. Misurazione

Regolare il fotometro per le micropiastre ELISA **a zero** usando il substrato-Bianco (Blank).

Se, per motivi tecnici, non è possibile regolare il fotometro a zero usando il Bianco-substrato, il valore di assorbanza di questo deve essere sottratto dai valori dell'assorbanza da tutti i valori delle altre assorbanze per ottenere risultati affidabili!

Misurare l'assorbanza di tutti i pozzetti a **450 nm** e inserire tutti i valori misurati nello schema della piastra.

È raccomandato fare le misurazioni delle onde **bichrome** (due colori). Utilizzando la lunghezza d'onda di 620 nm come misura di riferimento.

Dove sono state misurate in doppio, calcolare **la media delle assorbanze**.

9. RISULTATI

9.1. Validazione del test

Il test è valido se risponde ai prossimi criteri:

- **Substrato Bianco (Blank):** Valore di assorbanza $< 0,100$
- **Controllo negativo:** Valore di assorbanza $< 0,200$ e $< \text{Cut-off}$
- **Controllo cut-off:** Valore di assorbanza $0,150 - 1,300$
- **Controllo positivo:** Valore di assorbanza $> \text{Cut-off}$

Se non sono soddisfatti questi criteri, il test non è valido e deve essere ripetuto.

9.2. Calcolo dei risultati

Il Cut-off è la media dei valori di assorbanza dei Controlli cut-off.

Esempio: Valore di assorbanza del Controllo cut-off 0,44 + valore di assorbanza del Controllo cut-off 0,42 = $0,86/2 = 0,43$
Cut-off = 0,43

9.2.1. Risultati in unità [NTU]

$$\frac{\text{Assorbanza media del campione} \times 10}{\text{Cut-off}} = [\text{unità NovaTec} = \text{NTU}]$$

Esempio:
$$\frac{1,591 \times 10}{0,43} = 37 \text{ NTU}$$

9.3. Interpretazione dei risultati

Cut-off	10 NTU	-
Positivo	> 11 NTU	Anticorpi contro il patogeno sono presenti. C'è stato un contatto con l'antigene (patogeno resp. vaccino).
zona grigia	9 – 11 NTU	Anticorpi contro il patogeno non è stato possibile rilevare chiaramente. Si consiglia di ripetere il test con un nuovo campione in 2-4 settimane. Se il risultato è nuovamente nella zona grigia, il campione viene giudicato come negativo .
Negativo	< 9 NTU	Il campione non contiene anticorpi contro il patogeno. Un precedente contatto con l'antigene (patogeno resp. vaccino) è improbabile.
La diagnosi di una malattia infettiva non deve essere fatta soltanto sulla risultanza di un unico test. È importante considerare anche l'anamnesi ed i sintomi del paziente. I risultati del test da pazienti immunosoppressi e neonati hanno un valore limitato.		

9.3.1. Isotipi degli anticorpi e Stato dell'infezione

Sierologia	Significato
IgM	Caratteristica della risposta primaria dell'anticorpo Alto titolo IgM con basso titolo IgG: → suggerisce una infezione molto recente o acuta Raro: → IgM persistente
IgG	Caratteristica della risposta secondaria dell'anticorpo Può persistere per diversi anni Alto titolo IgG con basso titolo IgM: → può indicare un'infezione passata

10. CARATTERISTICHE DEL TEST

I risultati si riferiscono al gruppo di campioni investigato; questi non sono specifiche garantite.

Per ulteriori informazioni su caratteristiche del test, si prega, di contattare NovaTec Immundiagnostica GmbH.

10.1. Precisione

Intradosaggio	n	Media (E)	CV (%)
#1	24	0,600	6,05
#2	24	1,039	4,81
#3	24	0,639	3,71
Interdosaggio	n	Media (NTU)	CV (%)
#1	12	21,38	7,50
#2	12	13,88	8,95
#3	12	1,39	10,35

10.2. Specificità diagnostica

La specificità diagnostica è la probabilità del test di fornire un risultato negativo in assenza di analiti specifici.

La specificità diagnostica è 97,68% (95% intervallo di confidenza: 95,03% - 99,15%).

10.3. Sensibilità diagnostica

La sensibilità diagnostica è la probabilità del test di fornire un risultato positivo alla presenza di analiti specifici.

La sensibilità diagnostica è 94,44% (95% intervallo di confidenza: 72,71% - 99,86%).

10.4. Possibili interferenze

Campioni emolitici, lipidici ed itterici contenenti fino a 10 mg/ml di emoglobina, 5 mg/ml di trigliceridi e 0,5 mg/ml di bilirubina non hanno presentato fenomeni d'interferenza nel presente test.

10.5. Reattività crociata

L'investigazione di un gruppo di campioni con attività di anticorpi contro parametri potenzialmente interferenti non ha rivelato alcuna evidenza di risultati falsamente positivi dovuto a reattività crociata.

11. LIMITAZIONI

Una contaminazione da microorganismi o ripetuti cicli di congelamento-scongelo possono alterare i valori delle assorbanze.

12. PRECAUZIONI E AVVERTENZE

- In ottemperanza all'articolo 1, paragrafo 2 della direttiva Europea 98/79/EC, l'uso dei diagnostici medici in vitro è inteso da parte del produttore ad assicurare la congruenza, le prestazioni e la sicurezza del prodotto. Di conseguenza la procedura analitica, le informazioni, le precauzioni e le avvertenze contenute nelle istruzioni per l'uso devono essere seguite scrupolosamente. L'uso dei kit con analizzatori e attrezzature simili deve essere previamente convalidato. Qualunque cambiamento nello scopo, nel progetto, nella composizione o struttura e nella procedura analitica, così come qualunque uso dei kit in associazione ad altri prodotti non approvati dal produttore non è autorizzato; l'utilizzatore stesso è responsabile di questi eventuali cambiamenti. Il produttore non è responsabile per falsi risultati e incidenti che possano essere causati da queste ragioni. Il produttore non è responsabile per qualunque risultato ottenuto attraverso esame visivo dei campioni dei pazienti.
- Solo per uso diagnostico in-vitro.
- Tutti i materiali di origine umana o animale devono essere considerati potenzialmente contagiosi e infettivi.
- Tutti i componenti di origine umana sono stati trovati non reattivi con Anti-HIV-Ab, Anti-HCV-Ab e HBsAg.
- Non scambiare reagenti e micropiastre di lotti diversi.
- Non utilizzare reagenti di altri produttori insieme con i reagenti di questo kit.
- Non usare dopo la data di scadenza.
- Utilizzare soltanto punte per pipette, distributori, e articoli da laboratorio puliti.
- Non scambiare i tappi dei flaconi, per evitare contaminazione incrociata.
- Richiudere i flaconi immediatamente dopo l'uso per evitare la vaporizzazione e contaminazione.
- Una volta aperti e dopo relativo stoccaggio verificare i reagenti per una loro eventuale contaminazione prima dell'uso.
- Per evitare contaminazioni crociate e risultati erroneamente alti pipettare i campioni e reagenti con molta precisione nei pozzetti senza spruzzi.
- Il ELISA è previsto soltanto per essere impiegato da parte di personale specializzato che conosce perfettamente le tecniche di lavoro.

12.1. Smaltimento

In genere tutte le sostanze chimiche sono considerati rifiuti pericolosi. Lo smaltimento è regolato da leggi nazionali. Per ulteriori informazioni contattare l'autorità locale.

13. INFORMAZIONI PER GLI ORDINI

Numero del prodotto:	MUMM0340	Mumps Virus IgM ELISA (96 determinazioni)

ESPAÑOL

1. INTRODUCCIÓN

Los Virus de la Parotiditis son los virus que causan la papera, son virus de A.R.N. pertenecientes a la familia de los Paramyxoviridae. Tienen una envoltura lípida, un diámetro de 150 a 200nm y una forma pleomorfa. La papera es de distribución mundial manifestándose en epidemias infantiles, sobre todo en temporadas frías. En aprox. el 30% de los casos la enfermedad pasa inadvertida. La transmisión aerógena o por gotas de fluido contaminado de hombre a hombre dejando una inmunidad para el resto de la vida.

El virus de la papera infecta al principio las epitelias del tracto respiratorio superior, del tracto gastrointestinal o los ojos. Después de una reproducción inicial el virus se disgrega en nódulos linfáticos regionales donde después de otras fases de reproducción se produce una viremia produciendo infecciones en otros órganos como las glándulas salivares, las glándulas mamarias, en los testículos, en los ovarios, en el sistema nervioso y en el páncreas. Los síntomas aparecen después de un período de incubación de 7 a 21 días con fiebre, dolores de cabeza y sensación general de enfermedad. El síntoma clave de la papera epidémica es la inflamación dolorosa de una de las glándulas salivares, inflamándose la otra uno o dos días más tarde.

Complicaciones:

- Orquitis, epididymitis en 20% de los casos (lleva a la esterilidad en el 80% de los casos si la infección esta en ambos lados)
- Meningitis en el 20% de los pacientes el día 18 después del comienzo de la enfermedad (por regla general remisión rápida y total)
- Meningoencefalitis (muy raras veces (<1%) en comparación con la meningitis pero con una letalidad más alta (5%); la remisión con residuos es posible (5%)).
- Miocarditis, pancreatitis, adnexitis en las mujeres (raras veces complicaciones)

Como profilaxis existe una vacuna viva (simple o en combinación con otras).

Especies	Enfermedad	Síntomas (p.ej.)	Vía de transmisión
Virus de la Parotiditis (En inglés Mumps Virus)	Parotiditis epidémica (papera)	Fiebre, dolores de cabeza, sensación de enfermedad, inflamación de la parotis de un o ambos lados (Gl. Sublinguales y submandibulares están afectados amenudo también) Complicaciones: orquitis, meningoencefalitis, pancreatitis.	aerógena, por gotitas de fluido contaminado

Detección de infecciones o de agentes patógenos de:

- PCR
- Serología: p.ej. ELISA

2. USO PREVISTO

El ensayo de inmunoenzima Mumps Virus IgM ELISA se utiliza para la determinación cualitativa de anticuerpos IgM específicos contra Mumps Virus en suero o plasma (citratado, heparinado) humano.

3. PRINCIPIO DEL ENSAYO

La determinación inmunoenzimática cualitativa de anticuerpos específicos se basa en la técnica ELISA (Enzyme-linked Immunosorbent Assay).

Las microplacas están recubiertas con antígenos específicos unen a los anticuerpos de la muestra. Después de lavar los pocillos para eliminar todo el material de muestra no unido, el conjugado de peroxidasa de rábano (HRP) se añade. Este conjugado se une a los anticuerpos capturados. En una segunda etapa de lavado se retira el conjugado no unido. El complejo inmune formado por el conjugado unido se visualizó añadiendo sustrato tetrametilbencidina (TMB), que da un producto de reacción azul.

La intensidad de este producto es proporcional a la cantidad de anticuerpos específicos en la muestra. se añade ácido sulfúrico para detener la reacción. Esto produce un cambio de color de azul a amarillo. La extinción a 450/620 nm se mide con un lector de microplacas ELISA.

4. MATERIALES

4.1. Reactivos suministrados

- **Mumps Virus IgM microplaca recubierta:** 12 tiras de 8 pocillos rompibles, recubiertos con antígenos de Mumps Virus, en bolsa de aluminio.
- **Diluyente para IgM de la muestra:** 1 botella de 100 ml de solución de tampón de fosfato (10 mM) para diluir la muestra; pH $7,2 \pm 0,2$; anti-humana IgG (RF Absorbente); color verde; listo para ser utilizado; tapa blanca.
- **Solución de parada:** 1 botella de 15 ml de ácido sulfúrico, 0,2 mol/l, listo para ser utilizado; tapa roja.
- **Tampón de lavado (20x conc.):** 1 botella de 50 ml de una solución de tampón de fosfato 20x concentrado (0,2 M) para lavar los pocillos; pH $7,2 \pm 0,2$; tapa blanca.
- **Conjugado Mumps Virus anti-IgM:** 1 botella de 20 ml de conjugado de anticuerpos IgM anti-humano con peroxidasa en tampón de fosfato (10 mM); color rojo; tapa negra; listo para ser utilizado.
- **Solución de sustrato de TMB:** 1 botella de 15 ml 3,3',5,5'-tetrametilbenzindina (TMB), < 0,1 %; listo para ser utilizado; tapa amarilla; < 5% NMP.
- **Control positivo Mumps Virus IgM:** 1 botella de 2 ml control (suero o plasma humano); color amarillo; tapa roja; listo para ser utilizado.
- **Control cut-off Mumps Virus IgM:** 1 botella de 3 ml control (suero o plasma humano); color amarillo; tapa verde; listo para ser utilizado.
- **Control negativo Mumps Virus IgM:** 1 botella de 2 ml control (suero o plasma humano); color amarillo; tapa azul; listo para ser utilizado.

Para sustancias potencialmente peligrosas por favor revise la ficha de datos de seguridad.

4.2. Accesorios suministrados

- 1 lámina autoadhesiva
- 1 instrucciones de uso
- 1 esquema de la placa

4.3. Materiales e instrumentos necesarios

- Fotómetro de microplaca con filtros de 450/620 nm
- Incubadora 37°C
- Dispositivo de lavado manual o automático
- Micropipetas para uso de (10-1000 µl)
- Mezcladora Vortex
- Agua destilada
- Tubos de plástico desechables

5. ESTABILIDAD Y ALMACENAJE

Almacene el kit a 2...8 °C. Los reactivos abiertos son estables hasta la fecha de caducidad indicada en la etiqueta cuando se almacena a 2...8 °C.

6. PREPARACIÓN DE LOS REACTIVOS

Es muy importante llevar Todos los reactivos y las muestras para a la temperatura ambiente (20...25 °C) y mezclarlos antes de ser utilizados!

6.1. Microplaca recubierta

As tiras rompibles están recubiertas con antígeno de Mumps Virus. Inmediatamente después de la eliminación de las tiras, las tiras restantes deben sellarse de nuevo en el papel de aluminio junto con la bolsita de dióxido de silicio y almacenar a 2...8 °C.

6.2. Tampón de lavado (20x conc.)

Diluir la Tampón de lavado 1+19; por ejemplo. 10 ml de la Tampón de lavado + 190 ml de agua destilada. La muestra de tampón diluido es estable durante 5 días a temperatura ambiente (20...25 °C). Caso aparecen cristales en el concentrado, calentar la solución a 37 °C, por ejemplo, en un baño María. Mezclar bien antes de la dilución.

6.3. Solución de sustrato de TMB

La solución está listo para su uso y debe almacenarse a 2...8 °C, protegida de la luz. La solución debe ser incolora o podría tener un color ligeramente azul claro. Si el sustrato se convierte en azul, es posible que haya sido contaminado y no puede ser utilizada en el ensayo.

7. TOMA Y PREPARACIÓN DE LAS MUESTRAS

Usar muestras de suero o plasma (citrato, heparina) humano. Las instrucciones de uso ABVL0001 deben ser usadas para LCR. Si el ensayo se realiza dentro de 5 días después de la toma de sangre, las muestras pueden ser almacenadas de 2...8 °C, en caso contrario deben ser alicotadas y almacenadas congeladas (-70...-20 °C). Agitar bien las muestras descongelaadas antes de diluirlas. Evitar congelaciones y descongelaciones repetidas.

No se recomienda la inactivación por calor de las muestras.

7.1. Dilución de las muestras

Antes del ensayo, las muestras tienen que estar diluidas en relación 1 + 100 con el tampón de dilución para la muestra de IgM, p. e. 10 µl de la muestra con 1 ml de tampón IgM, mezclar bien con la mezcladora Vortex.

8. PROCEDIMIENTO

8.1. Preparación del ensayo

Por favor, leer cuidadosamente las instrucciones de uso del ensayo **antes** de realizarlo. Para el buen funcionamiento de la técnica es necesario seguir las instrucciones. El siguiente procedimiento es válido solamente para el método manual. Si se realiza el ensayo en los sistemas automáticos de ELISA es aconsejable elevar el número de lavado de de 3 a 5 veces y el volumen de Tampón de lavado de 300 µl a 350 µl para excluir efectos de lavado. Preste atención al capítulo 12. Antes de comenzar, especificar exactamente la repartición y posición de las muestras y de los estándares/controles (recomienda determinar en doble) en lo esquema de la placa suministrada. Usar la cantidad necesaria de tiras o pozos e insertarlos en el soporte.

Realizar el ensayo en el orden indicado y sin retraso.

Para cada paso de pipeteado en los estándares/controles y en las muestras, usar siempre puntas de pipeta de un solo uso.

Graduar la incubadora a $37 \pm 1^\circ\text{C}$.

1. Pipetear 100 µl de estándares/controles y muestras en los pocillos respectivos. Dejar el pocillo A1 para el blanco.
2. Recubrir las tiras con los autoadhesivos suministrados.
3. **Incubar 1 h $\pm$ 5 min a $37 \pm 1^\circ\text{C}$.**
4. Después de la incubación, retirar el autoadhesivo, aspirar el líquido de la tira y lavarla tres veces con 300µl de la Tampón de lavado. Evitar el rebosamiento de los pocillos. El intervalo entre lavado y aspiración debe ser > 5 segundos. Para sacar el líquido restante de las tiras, es conveniente sacudirlas sobre papel absorbente.
Nota: El lavado es muy importante! Un mal lavado insuficiente provoca una baja precisión y resultados falsamente elevados!
5. Pipetar 100 µl de conjugado en cada pocillo con excepción del blanco substrato A1.
6. **Incubar 30 min a la temperatura ambiente (20...25 °C).** Evitar la luz solar directa.
7. Repetir el lavado como en el paso numero 4.
8. Pipetar 100 µl de la Solución de sustrato de TMB en todos los pocillos.
9. **Incubar exactamente 15 min en oscuridad a temperatura ambiente (20...25 °C).** Un color azul se produce en las muestras positivas debido a la reacción enzimática.
10. Pipetear en todos los pocillos 100 µl de la solución de parada en el mismo orden y mismo intervalo de tiempo como con la Solución de sustrato de TMB, por lo tanto un cambio de color de azul a amarillo se produce.
11. Medir la extinción con 450/620 nm en un periodo de 30 min después de añadir la solución de parada.

8.2. Medición

Ajustar el lector de microplaca (fotómetro) Elisa **al cero** utilizando **el Blanco**.

Si por razones técnicas el lector de ELISA no se pueren ajustar a cero utilizando el Blanco, el valor de la absorbancia de esto debe ser sustraído de los demás valores de absorbancia medidos con el fin de obtener resultados fiables!

Medir la **extinción** de todos los pocillos con **450 nm** y anotar los resultados de los estándares/controles y de las muestras en la esquema de la placa.

Es aconsejable realizar la medición **bicromática** a una longitud de onda de referencia de 620 nm.

Si se efectuaron análisis en duplicado o múltiples, hay que calcular **el promedio de los valores de extinción** de los pocillos correspondientes.

9. CALCULO DE LOS RESULTADOS

9.1. Criterios de validez del ensayo

El ensayo es válido si se cumplen los siguientes criterios:

- **Blanco:** valor de la extinción < **0,100**
- **Control negativo:** valor de la extinción < **0,200 y < Cut-off**
- **Control cut-off:** valor de la extinción **0,150 – 1,300**
- **Control positivo:** valor de la extinción > **Cut-off**

Si estos criterios no se cumplen, la prueba no es válida y deberá repetirse.

9.2. Calculo del valor de la medición

El Cut-off se obtiene de los valores de la extinción de lo Control cut-off.

Ejemplo: $0,42 \text{ OD Control cut-off} + 0,44 \text{ OD Control cut-off} = 0,86:2 = 0,43$

Cut-off = 0,43

9.2.1. Resultados en unidades [NTU]

$\frac{\text{Promedio valor de la extinción de la muestra} \times 10}{\text{Cut-off}} = [\text{NovaTec-unidades} = \text{NTU}]$

Ejemplo: $\frac{1,591 \times 10}{0,43} = 37 \text{ NTU}$

9.3. Interpretación de los resultados

Cut-off	10 NTU	-
Positivo	> 11 NTU	Los anticuerpos contra el patógeno están presentes. Ha producido un contacto con el antígeno (patógeno resp. vacuna).
Zona intermedia	9 – 11 NTU	Los anticuerpos contra el patógeno no se pudieron detectar claramente. Se recomienda repetir la prueba con una muestra fresca en 2 a 4 semanas. Si el resultado es de nuevo en la zona intermedia, la muestra se considera como negativa .
Negativo	< 9 NTU	La muestra no contiene anticuerpos contra el patógeno. Un contacto previo con el antígeno (patógeno resp. vacuna) es poco probable.
El diagnóstico de una infección no solamente se debe basar en el resultado del ensayo. Es necesario considerar la anamnesis y la sintomatología del paciente junto al resultado serológico. Estos resultados sólo tienen valor restringido en pacientes inmunodeprimidos o en neonatos.		

9.3.1. Isotipos de anticuerpo y Estado de la Infección

Serología	Significado
IgM	Característica de la respuesta primaria del anticuerpo Alto título de IgM con bajo título de IgG → sugieren una infección muy reciente o aguda Raras: → persistente IgM
IgG	Característica de la respuesta secundario del anticuerpo Pueden persistir por varios años El alto título de IgG con bajo título de IgM: → pueden indicar una infección pasada

10. CARACTERÍSTICAS DEL ENSAYO

Los resultados están basados en lo grupo de pruebas investigado; no se trata de especificaciones garantizadas.

Para obtener más información sobre las características del ensayo, por favor, entre en contacto NovaTec Immundiagnostica GmbH.

10.1. Precisión

Intra ensayo	n	Promedio (E)	CV (%)
#1	24	0,600	6,05
#2	24	1,039	4,81
#3	24	0,639	3,71
Inter ensayo	n	Promedio (NTU)	CV (%)
#1	12	21,38	7,50
#2	12	13,88	8,95
#3	12	1,39	10,35

10.2. Especificidad diagnóstica

La especificidad del ensayo se define como la probabilidad que tiene el ensayo de dar un resultado negativo en ausencia del analítico específico. Es 97,68% (95% Intervalo de confianza: 95,03% - 99,15%).

10.3. Sensibilidad de diagnóstico

La sensibilidad del ensayo se define como la probabilidad que tiene el ensayo de dar un resultado positivo en presencia del analítico específico. Es 94,44% (95% Intervalo de confianza: 72,71% - 99,86%).

10.4. Interferencias

Las muestras lipémicas, ictericas e hemolíticas no mostraron interferencias con este equipo ELISA hasta una concentración de 5 mg/ml para triglicéridos, de 0,5 mg/ml para bilirrubina y de 10 mg/ml hemoglobina.

10.5. Reactividad cruzada

Pruebas realizadas con un panel de muestras con distinta actividad de anticuerpos para estudiar parámetros de reactividad no dieron falsos positivos debidos a reactividad cruzada.

11. LIMITACIONES DEL ENSAYO

Una contaminación de las muestras con bacterias, o una congelación y descongelación repetida pueden producir cambios en los valores de la extinción.

12. PRECAUCIONES Y ADVERTENCIAS

- En cumplimiento con el artículo 1 párrafo 2b de la directiva europea 98/79/EC, la utilización de sistemas médicos para diagnóstico in vitro tiene la intención por parte del fabricante de asegurar la adecuación, realizaciones y seguridad del producto. Por lo tanto, el procedimiento, la información, las precauciones y los avisos de las instrucciones de uso han de ser seguidas estrictamente. La utilización de equipos con analizadores y equipamiento similar tiene que ser validada. No se autorizan cambios en el diseño, composición y procedimiento, así como cualquier utilización en combinación con otros productos no aprobados por el fabricante; el usuario debe hacerse responsable de estos cambios. El fabricante no responderá ante falsos resultados e incidentes debidos a estas razones. El fabricante no responderá ante cualquier resultado por análisis visual de las muestras de los pacientes.
- Solo para diagnóstico in vitro.
- Todos los materiales de origen humano o animal deberán ser considerados y tratados como potencialmente infecciosos.
- Todos los componentes de origen humano han sido examinados y resultaron no reactivos a anticuerpos contra el VIH, VHC y HbsAG.
- No intercambiar reactivos y placas de microtítulo de cargas diferentes.
- No usar reactivos de otro fabricante para este ensayo.
- No usar después de la fecha de caducidad.
- Sólo usar recambios de pipetas, dispensadores y materiales de laboratorio limpios.
- No intercambiar las tapas de los diferentes reactivos, para evitar la contaminación cruzada.
- Para evitar la evaporación y una contaminación microbiana, cierre inmediatamente las botellas después de usarlas.
- Después de abrirlas y posterior almacenaje, asegurarse de que no existe contaminación microbiana antes de seguir usándolas.
- Para evitar contaminaciones cruzadas y resultados erróneamente aumentados, pipetear cuidadosamente las muestras y los reactivos en los pocillos sin salpicar.
- El ELISA está pensado exclusivamente para su uso por personal especializado que domine perfectamente las técnicas de trabajo.

12.1. Indicaciones para la eliminación de residuos

Por regla general, los productos químicos y las preparaciones son residuos peligrosos. Su eliminación esta sometida a las leyes y los decretos nacionales sobre la eliminación de residuos. Las autoridades informan sobre la eliminación de residuos peligroso.

13. INFORMACIONES PARA PEDIDOS

Nº del producto: MUMM0340 Mumps Virus IgM ELISA (96 determinaciones)

PORTUGUÊS

1. INTRODUÇÃO

Os vírus da parotidite são vírus ARN da família Paramyxoviridae. Os viriões são partículas esféricas com um diâmetro de 150-250 nm, compostas de ribonucleoproteína com simetria helicoidal e providas de um invólucro de proteína matrix e bicamada lipídica, que contém duas estruturas de espigões: Hemaglutinina viral (H) e neuraminidase viral (N). O vírus da parotidite afecta principalmente a parótida e glândulas salivares relacionadas; no entanto, a infecção pode dar origem a doenças do SNC e a uma acumulação do vírus no LCR. A papeira (parotidite epidémica) é uma doença viral aguda contagiosa, que ocorre sobretudo em crianças. Perto de 50% do total de infecções é subclínico. A incidência mais elevada de manifestações clínicas encontra-se no grupo etário entre os 4 e os 15 anos. As infecções secundárias são raras, devido à imunização duradoura. 10 a 35% dos casos de papeira dão origem a orquite, que ocorre quase sempre depois da puberdade. O processo é maioritariamente unilateral e o prognóstico é geralmente favorável. O vírus da parotidite foi uma das causas mais importantes de doenças virais do SNC (meningite e encefalite) nos EUA; a administração de vacinas reduziu significativamente esta incidência.

Espécies	Doença	Sintomas (p.ex.)	Via de transmissão
Vírus da Parotidite (em inglês, Mumps Virus)	Parotidite infecciosa (Papeira, Caxumba)	Febre e edema unilateral ou bilateral da glândula parótida; as glândulas sublinguais e submandibulares também podem ser afectadas. Complicações: Orquite, Meningoencefalite, Pancreatite	Por via aérea, a transmissão do vírus ocorre por infecção por gotículas.

A presença de agentes patogénicos ou de infecção pode ser identificada através

- PCR
- Serologia: p.ex. ELISA

2. UTILIZAÇÃO PRETENDIDA

O kit Mumps Virus IgM ELISA destina-se à determinação qualitativa de anticorpos da classe IgM contra Mumps Virus no soro ou plasma (citrato, heparina) humanos.

3. PRINCÍPIO DO ENSAIO

A determinação imunoenzimática qualitativa de anticorpos específicos é baseado na técnica de ELISA (do inglês Enzyme-linked Immunosorbent Assay).

As microplacas são revestidas com antígenos específicos que se ligam os anticorpos correspondentes da amostra. Após lavagem dos poços, para remover todo o material de amostra não ligado, um conjugado de peroxidase de rábano (HRP) é adicionado. Este conjugado se liga aos anticorpos capturados. Num segundo passo de lavagem o conjugado não ligado é removido. O complexo imune formado pelo conjugado ligado é visualizado por adição de substrato de tetrametilbenzidina (TMB), o que dá um produto de reacção azul.

A intensidade deste produto é proporcional à quantidade de anticorpos específicos da amostra. O ácido sulfúrico é adicionado para parar a reacção. Isso produz uma mudança de cor de azul para amarelo.

Absorvância a 450/620 nm é lida utilizando um leitor de microplacas ELISA.

4. MATERIAIS

4.1. Reagentes fornecidos

- **Mumps Virus IgM microplaca revestida:** 12 tiras de 8 poços, destacáveis e quebráveis, revestidas com antígeno de Mumps Virus, em bolsas de folha de alumínio com fecho.
- **Diluyente de Amostra IgM:** 1 frasco contendo 100 ml de tampão fosfato (10 mM) para diluição da amostra, pH 7,2 ± 0,2; anti-humana IgG (RF Absorbent); de cor verde; pronto a usar; tampa branca.
- **Solução de bloqueio:** 1 frasco contendo 15 ml ácido sulfúrico; 0,2 mol/l; pronto a usar; tampa vermelha.
- **Tampão de lavagem (conc. 20x):** 1 frasco contendo 50 ml de um tampão fosfato (0,2 M); concentrado 20 vezes (pH 7,2 ± 0,2) para a lavagem dos poços; tampa branca.
- **Conjugado Mumps Virus:** 1 frasco contendo 20 ml de anticorpo para IgM humana marcados com peroxidase no tampão fosfato (10 mM); de cor vermelho, pronto a usar; tampa preta.
- **Solução Substrato TMB:** 1 frasco contendo 15 ml de 3,3',5,5'-tetrametilbenzidina (TMB), < 0,1 %; pronto a usar; tampa amarela; < 5% NMP.
- **Controle positivo Mumps Virus IgM:** 1 frasco contendo 2 ml controle (soro ou plasma humanos); de cor amarela; pronto a usar; tampa vermelha.
- **Controle cut-off Mumps Virus IgM:** 1 frasco contendo 3 ml controle (soro ou plasma humanos); de cor amarela; pronto a usar; tampa verde.
- **Controle negativo Mumps Virus IgM:** 1 frasco contendo 2 ml controle (soro ou plasma humanos); de cor amarela; pronto a usar; tampa azul.

Para substâncias potencialmente perigosas verifique a ficha de dados de segurança.

4.2. Materiais fornecidos

- 1 Película de cobertura
- 1 Instruções de uso
- 1 Layout da placa

4.3. Materiais e Equipamento necessários

- Leitor de microplacas ELISA, equipado para a medição da absorvância a 450/620 nm
- Incubadora 37 °C
- Equipamento manual ou automático para a lavagem dos poços
- Pipetas para dispensar volumes entre 10 e 1000 µl
- Agitador de tubos tipo Vortex
- Água destilada
- Tubos descartáveis

5. ESTABILIDADE E ARMAZENAMENTO

Armazene o kit a 2...8 °C. Os reagentes abertos são estáveis até o prazo de validade impresso no rótulo quando armazenado a 2...8 °C.

6. PREPARAÇÃO DOS REAGENTES

É muito importante deixar todos os reagentes e amostras estabilizar à temperatura ambiente (20...25 °C) misturá-los antes de iniciar o teste!

6.1. Microplaca revestidas

As tiras quebráveis são revestidas com antígeno Mumps Virus. Imediatamente após a remoção das tiras necessárias, as tiras restantes devem ser lacradas de novo na folha de alumínio juntamente com o saquinho de silício fornecido e armazenadas a 2...8 °C.

6.2. Tampão de lavagem (conc. 20x)

Diluir o Tampão de lavagem 1+19; por exemplo. 10 ml do Tampão de lavagem + 190 ml de água destilada. A amostra do tampão diluída é estável durante 5 dias à temperatura ambiente (20...25 °C). Caso apareça cristais no concentrado, aquecer a solução a 37 °C por exemplo, em banho Maria. Misture bem antes da diluição.

6.3. Solução Substrato TMB

A solução está pronta para uso e tem de ser armazenada à 2...8 °C, protegida da luz. A solução deve ser incolor ou poderia ter uma ligeira coloração azul claro. Se o substrato se transforma em azul, pode ter sido contaminado e não pode ser usado no teste.

7. COLHEITA E PREPARAÇÃO DAS AMOSTRAS

Usar com este ensaio amostras de soro ou plasma (citrato, heparina) humanos. Para LCR, por favor utilize a instrução de uso ABVL0001. Se o ensaio for realizado dentro de 5 dias após colheita da amostra, o espécime deve ser mantido a 2...8 °C; caso contrário devem ser alicotadas e armazenadas congeladas (-70...-20 °C). Se as amostras forem armazenadas congeladas, misturar bem as amostras descongeladas antes de testar. Evitar congelar e descongelar repetidamente. Não é recomendada a inativação por calor das amostras.

7.1. Diluição das amostras

Antes de testar todas as amostras devem ser diluídas 1 + 100 com Diluente de Amostra IgM. Dispensar 10 µl de amostra e 1 ml de Diluente de Amostra IgM em tubos para obter uma diluição 1 + 100 e misturar meticulosamente com um vortex.

8. PROCEDIMENTO DO ENSAIO

8.1. Preparação do Teste

Por favor, ler atentamente as instruções de uso **antes** de realizar o teste. A fiabilidade dos resultados depende da adesão estrita ao as instruções de uso, conforme descritas. O procedimento de ensaio a seguir está validado apenas para o procedimento manual. Se o teste for realizado em sistemas automáticos para teste ELISA é recomendável aumentar os passos de lavagem de três para cinco e o volume do Tampão de lavagem de 300 µl para 350 µl para evitar efeitos de lavagem. Preste atenção ao capítulo 12. Antes de iniciar o teste, o plano de distribuição e identificação de todas as amostras e calibradores/controles (é recomendado determinar em duplicidade) deve ser cuidadosamente estabelecido no Layout da placa fornecida no kit. Seleccionar o número necessário de tiras ou poços e inserir os mesmos no suporte.

Realizar todas as etapas do teste na ordem indicada e sem atrasos significativos.

Na pipetagem deve ser utilizada uma ponta limpa e descartável para dispensar cada controle e amostra.

Ajustar a incubadora para 37 ± 1 °C.

1. Dispensar 100 µl dos calibradores/controles e das amostras diluídas nos poços respectivos. Deixar o poço A1 vazio para o branco substrato.
2. Cobrir os poços com a película fornecida no kit.
3. **Incubar durante 1 hora $\pm$ 5 min a 37 ± 1 °C.**
4. Quando terminar a incubação, remover a película, aspirar o conteúdo dos poços e lavar cada poço três vezes com 300 µl de Tampão de lavagem. Evitar que os poços de reacção transbordem. O intervalo entre a lavagem e a aspiração deve ser > 5 seg. No final, retirar cuidadosamente o fluido restante batendo delicadamente as tiras sobre papel absorvente, antes da próxima etapa!
Nota: A lavagem é muito importante! Lavagem insuficiente resulta em baixa precisão e falsos resultados.
5. Dispensar 100 µl de Conjugado em todos os poços, excepto no poço do Branco substrato A1.
6. **Incubar durante 30 min à temperatura ambiente (20...25°C).** Não expor diretamente à luz solar.
7. Repetir a etapa 4.
8. Dispensar 100 µl de Solução Substrato TMB em todos os poços.
9. **Incubar durante exactamente 15 min à temperatura ambiente (20...25°C) e no escuro.** A cor azul devido a uma reacção enzimática.
10. Dispensar 100 µl de Solução de bloqueio em todos os poços, pela mesma ordem e com a mesma velocidade a que foi dispensada a Solução Substrato TMB,desse modo uma mudança de cor de azul para amarelo ocorre.
11. Medir a absorvância a 450/620 nm dentro de 30 min após a adição da solução de bloqueio.

8.2. Medição

Ajustar o Leitor de Microplacas ELISA a zero usando o **Branco substrato** .

Se - devido à razões técnicas – o leitor ELISA não puder ser ajustado a zero usando o Branco substrato, valor da absorvância deste deve ser subtraído de todos os outros valores de absorvância medidos de forma a obter resultados fiáveis!

Medir a absorvância de todos os poços a **450 nm** e registar os valores da absorvância para cada calibrador/controle e amostra no Layout da placa.

É recomendado fazer a medição **dicromática** usando como referência um comprimento de onda de 620 nm.

Se determinações duplas foram realizadas, calcular **os valores médios de absorvância**.

9. RESULTADOS

9.1. Critérios de validação do ensaio

Para que um ensaio seja considerado válido, devem ser cumpridos os seguintes critérios:

- **Branco substrato:** Valor de Absorvância < **0,100**
- **Controle negativo:** Valor de Absorvância < **0,200** e < **Cut-off**
- **Controle cut-off:** Valor de Absorvância **0,150 – 1,300**
- **Controle positivo:** Valor de Absorvância > **Cut-off**

Se estes critérios não forem cumpridos, o teste não é válido e deve ser repetido.

9.2. Cálculo dos Resultados

O Cut-off é o valor médio da absorvância das determinações do Controle cut-off.

Exemplo: Valor da absorvância do Controle cut-off 0,42 + valor da absorvância do Controle cut-off 0,44 = 0,86: 2 = 0,43
Cut-off = 0,43

9.2.1. Resultados em Unidades [NTU]

$$\frac{\text{Valor da absorvância (média) da amostra} \times 10}{\text{Cut-off}} = [\text{Unidades NovaTec} = \text{NTU}]$$

Exemplo:
$$\frac{1,591 \times 10}{0,43} = 37 \text{ NTU}$$

9.3. Interpretação dos Resultados

Cut-off	10 NTU	-
Positivo	> 11 NTU	Os anticorpos contra o agente patogênico estão presente. Houve um contacto com o antígeno (patógeno resp vacina).
Zona cinzenta	9 – 11 NTU	Os anticorpos contra o agente patogênico não puderam ser claramente detectados. Recomenda-se a repetir o teste com uma amostra fresca em 2 a 4 semanas. Se o resultado estiver novamente dentro da zona cinzenta, a amostra é julgada como negativa .
Negativo	< 9 NTU	A amostra não contém os anticorpos contra o agente patogênico. Um contato prévio com o antígeno (patógeno resp. vacina) é improvável.
O diagnóstico de uma doença infecciosa não deve ser estabelecido com base num único resultado do teste. Um diagnóstico preciso deve ter em consideração a história clínica, a sintomatologia bem como dados serológicos. Em pacientes imunossuprimidos e recém-nascidos os dados serológicos têm apenas valor restrito.		

9.3.1. Isotipos de anticorpos e Estado da Infecção

Sorologia	Significado
IgM	Característica da resposta primária do anticorpo Alto título de IgM com baixo título de IgG: → sugere uma infecção muito recente ou aguda Raros: → persistente IgM
IgG	Característica da resposta secundária do anticorpo Podem persistir por vários anos Alto título de IgG com baixo título de IgM: → pode indicar uma infecção passada

10. CARACTERÍSTICAS DE DESEMPENHO ESPECÍFICAS

Os resultados referem-se aos grupos de amostras investigados; estas não são especificações garantidas.

Para mais informações sobre as características de desempenho específicas, por favor, entre em contato NovaTec Immundiagnostica GmbH.

10.1. Precisão

Intra ensaio	n	Média (E)	CV (%)
#1	24	0,600	6,05
#2	24	1,039	4,81
#3	24	0,639	3,71
Inter ensaio	n	Média (NTU)	CV (%)
#1	12	21,38	7,50
#2	12	13,88	8,95
#3	12	1,39	10,35

10.2. Especificidade Diagnóstica

A especificidade diagnóstica é definida como a probabilidade do ensaio ser negativo na ausência do analito específico.
É de 97,68% (95% Intervalo de confiança: 95,03% - 99,15%).

10.3. Sensibilidade Diagnóstica

A sensibilidade diagnóstica é definida como a probabilidade do ensaio ser positivo na presença do analito específico.
É de 94,44% (95% Intervalo de confiança: 72,71% - 99,86%).

10.4. Interferências

Não são observadas interferências com amostras hemolisadas, lipêmicas ou ictericas até uma concentração de hemoglobina de 10 mg/ml, de triglicerídeos de 5 mg/ml e de bilirrubina de 0,5 mg/ml.

10.5. Reacção cruzada

A investigação do painel de amostras com atividades de anticorpos em parâmetros com potencial de reacção cruzada não revelou nenhuma evidência de resultados falso-positivos devido a reacções cruzadas.

11. LIMITAÇÕES DO PROCEDIMENTO

Contaminação bacteriana ou a repetição de ciclos de congelação-descongelação do espécime podem afectar os valores da absorvância.

12. PRECAUÇÕES E AVISOS

- Em cumprimento com o artigo 1 parágrafo 2b da directiva Europeia 98/79/EC o uso de dispositivos médicos para diagnóstico in vitro destina-se segundo o fabricante a assegurar adequabilidade, desempenhos e segurança do produto. Portanto o procedimento do teste, as informações, as precauções e avisos nas instruções para utilização têm de ser rigorosamente seguidas. O uso de kits de teste com analisadores e equipamento similar tem de ser validado. Qualquer alteração no desenho, composição e procedimento do teste bem como qualquer utilização em combinação com outros produtos não aprovados pelo fabricante não estão autorizados; o próprio utilizador é responsável por tais alterações. O fabricante não é legalmente responsável por resultados falsos e incidentes originados por estes motivos. O fabricante não é legalmente responsável por quaisquer resultados obtidos por análise visual das amostras dos pacientes.
- Apenas para uso no diagnóstico in-vitro.
- Todos os materiais de origem humana ou animal devem ser considerados e tratados como potencialmente infectantes.
- Todos os componentes de origem humana usados para a produção destes reagentes foram testados para anticorpos anti-HIV, anticorpos anti-HCV e HBsAg e foram considerados não-reactivos.
- Não trocar e juntar reagentes ou tiras de lotes de produção diferentes.
- nenhuns reagentes de outros fabricantes devem ser usados juntamente com reagentes deste kit de teste.
- Não usar reagentes após a data de validade indicada no rótulo.
- Usar apenas pontas de pipeta, dispensadores e material de laboratório limpos.
- Não trocar as tampas dos frascos dos reagentes para evitar contaminação cruzada.
- Fechar firmemente os frascos dos reagentes imediatamente após a utilização para evitar evaporação e contaminação microbiana.
- Após a primeira abertura e armazenamento subsequente verificar se existe contaminação microbiana dos frascos do conjugado e dos calibradores/controles antes de utiliza-los novamente.
- Para evitar contaminação-cruzada e resultados falsamente elevados, pipetar as amostras dos pacientes e dispensar os reagentes precisamente nos poços sem salpicar.
- O ELISA foi desenhado apenas para pessoal qualificado e que esteja familiarizado com boas práticas laboratoriais.

12.1. Considerações de Eliminação

Resíduos de químicos e preparações são geralmente considerados como resíduos perigosos. A eliminação deste tipo de resíduos está regulada por leis e normativas nacionais e regionais. Contactar as autoridades locais ou empresas de gestão de resíduos as quais podem aconselhar sobre como eliminar resíduos perigosos.

13. INFORMAÇÃO DE PEDIDO

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

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ABBREVIATIONS / ABKÜRZUNGEN / ABRÉVIATIONS / ABBREVIAZIONI / ABREVIACIONES / ABREVIATURAS

NMP	N-Methyl-2-pyrrolidone

SYMBOLS KEY / SYMBOLSCHLÜSSEL / EXPLICATION DES SYMBOLES / LEGENDA / SIMBOLOS / TABELA DE SIMBOLOS

	Manufactured by / Hergestellt von / Fabriqué par / Prodotto da / Fabricado por / Fabricado por
IVD	In Vitro Diagnostic Medical Device / In Vitro Diagnosticum / Dispositif médical de diagnostic in vitro / Diagnostico in vitro / Producto para diagnóstico In vitro / Dispositivo Médico para Diagnóstico In Vitro
LOT	Lot Number / Chargenbezeichnung / Numéro de lot / Lotto / Número de lote / Número de lote
	Expiration Date / Verfallsdatum / Date de péremption / Scadenza / Fecha de caducidad / Data de Validade
	Storage Temperature / Lagertemperatur / Température de conservation / Temperatura di conservazione / Temperatura de almacenamiento / Temperatura de Armazenamento
CE	CE Mark / CE-Zeichen / Marquage CE / Marchio CE / Marca CE / Marca CE
REF	Catalogue Number / Katalog Nummer / Référence du catalogue / Numero di codice / Número de Catálogo / Número de Catálogo
	Consult Instructions for Use / Arbeitsanleitung beachten / Consulter la notice d'utilisation / Consultare le istruzioni per l'uso/ Consulte las Instrucciones de Uso / Consultar as Instruções de Utilização
MTP	Microplate / Mikrotiterplatte / Microplaque / Micropiastra / Microplaca / Microplaca
CONJ	Conjugate / Konjugat / Conjugué / Coniugato / Conjugado / Conjugado
CONTROL -	Negative Control / Negativkontrolle / Contrôle négatif / Controllo negativo / Control negativo / Controle negativo
CONTROL +	Positive Control / Positivkontrolle / Contrôle positif / Controllo positivo / Control positivo / Controle positivo
CUT OFF	Cut-off Control / Cut-off Kontrolle / Contrôle cut-off / Controllo cut-off / Control cut-off / Controle cut-off
DIL M	IgM Sample Diluent / IgM-Probenverdünnungspuffer / Diluant pour échantillon IgM / Tampone diluente IgM / Diluyente para IgM de la muestra / Diluente de Amostra IgM
SOLN STOP	Stop Solution / Stopplösung / Solution d'arrêt / Soluzione bloccante / Solución de parada/ Solução de bloqueio
SUB TMB	TMB Substrate solution / TMB-Substratlösung / Solution de substrat TMB / Soluzione substrato TMB / Solución de sustrato de TMB / Solução substrato TMB
WASH BUF 20x	Washing Buffer 20x concentrated / Waschpuffer 20x konzentriert / Tampon de lavage concentré 20 x / Tampone di lavaggio concentrazione x20 / Tampón de lavado concentrado x20 / Tampão de lavagem concentrada 20x
	Contains sufficient for "n" tests / Ausreichend für "n" Tests / Contenu suffisant pour "n" tests / Contenuto sufficiente per "n" saggi / Contenido suficiente para "n" tests / Conteúdo suficiente para "n" testes

SCHEME OF THE ASSAY

Mumps Virus IgM ELISA

Test Preparation

Prepare reagents and samples as described. Establish the distribution and identification plan for all samples and standards/controls on the plate layout supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder.

Assay Procedure

	Substrate Blank (A1)	Negative Control	Cut-off Control	Positive Control	Sample (diluted 1+100)
Negative Control	-	100 µl	-	-	-
Cut-off Control	-	-	100 µl	-	-
Positive Control	-	-	-	100 µl	-
Sample (diluted 1+100)	-	-	-	-	100 µl
Cover wells with foil supplied in the kit Incubate for 1 h at 37 °C Wash each well three times with 300 µl of Washing Buffer					
Conjugate	-	100 µl	100 µl	100 µl	100 µl
Incubate for 30 min at room temperature (20...25 °C) Do not expose to direct sunlight Wash each well three times with 300 µl of Washing Buffer					
TMB Substrate solution	100 µl	100 µl	100 µl	100 µl	100 µl
Incubate for exactly 15 min at room temperature (20...25 °C) in the dark					
Stop Solution	100 µl	100 µl	100 µl	100 µl	100 µl
Photometric measurement at 450 nm (reference wavelength: 620 nm)					

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Certificate

**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

TÜVRheinland

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

A. Fechner



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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



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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 pseudomembranous 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 Lyster 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 / $\geq$ 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 <i>C. difficile</i> 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 <i>C. difficile</i> 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 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 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 / $\geq$ 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

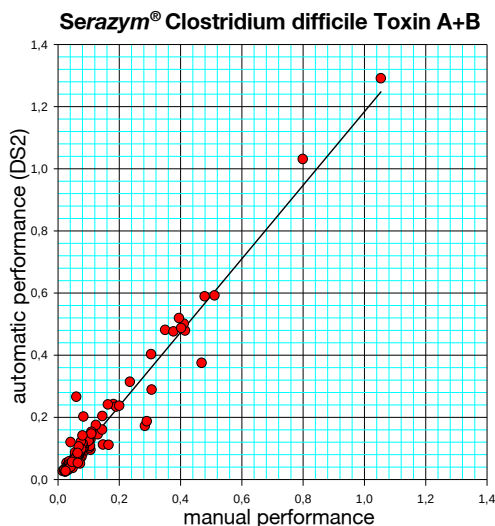
There is no correlation between measured absorbance and seriousness of the infection. It is also not allowed to correlate absorbances of the samples with that of the positive control. Cross contamination of reagents and samples can produce false positive results. Incorrect dilutions, not sufficiently 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
<i>Serazym®</i> ELISA positive	103	4
<i>Serazym®</i> 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 $\geq 10^8$ colony forming units of the following microorganisms and tested negative with the *Serazym®* ELISA (OD 450 / 620 nm < Cut-Off):

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

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



Manufacturer



Date of manufacture



Use by



Batch code



Catalog number



Keep away from sunlight



Temperature limits



Biological risks



Do not reuse



Consult instructions for use



Caution



In-vitro-diagnostic medical device



Contains sufficient for <n> tests

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 



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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 Lyster 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
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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 / ≥ 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- <i>C.difficile</i> 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 <i>C. difficile</i> 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 <i>C. difficile</i> 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- <i>C.difficile</i> 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 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. 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 1 x 96 or 2 x 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

<i>Serazym</i>® Clostridium difficile GDH	
Positive	$\geq$ 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
<i>Serazym</i> [®] ELISA positive	101	3**
<i>Serazym</i> [®] 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
<i>Serazym</i> [®] ELISA positive	69	1*
<i>Serazym</i> [®] 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):

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

<i>Klebsiella pneumoniae</i>	(ATCC 13883)
<i>Peptostreptococcus anaerobius</i>	(ATCC 27337)
<i>Proteus vulgaris</i>	(ATCC 8427)
<i>Pseudomonas aeruginosa</i>	(ATCC 10145)
<i>Salmonella enterica</i> Sero var <i>enteritidis</i>	(ATCC 13076)
<i>Salmonella enterica</i> Sero var <i>typhimurium</i>	(ATCC 14028)
<i>Shigella flexneri</i>	(ATCC 12022)
<i>Shigella sonnei</i>	(ATCC 25931)
<i>Staphylococcus aureus</i>	(ATCC 25923)
<i>Staphylococcus epidermidis</i>	(ATCC 12228)
<i>Vibrio parahaemolyticus</i>	(ATCC 17802)
<i>Vibrio cholerae</i>	Clinical isolate
<i>Yersinia enterocolitica</i> Serotyp <i>O3, O9</i>	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) **CONJ HRP** (6)
 +
 pipette
 100 µl **CONTROL +** (4)
 100 µl **CONTROL -** (5)
 100 µl **stool sample**, mix gently
 60 min incubation (room temperature)
alternatively 30 min while shaking
2.  5x wash with wash solution
3.  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

 Manufacturer	 Date of manufacture	 Use by	LOT Batch code	REF Catalog number
 Keep away from sunlight	 Temperature limits	 Biological risks	 Do not reuse	
 Consult instructions for use	 Caution	IVD In-vitro-diagnostic medical device	 Contains sufficient for <n> tests	

NovaLisa®

Clostridium tetani toxin IgG

ELISA



Only for in-vitro diagnostic use

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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 (trismus), 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
<i>Clostridium tetani</i>	Tetanus	Trismus, dysphagia, severe, painful spasms of whole muscle groups, hypersalivation, aspiration, asphyxia	Injury (Infection of the wound with <i>Clostridium tetani</i>)

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.

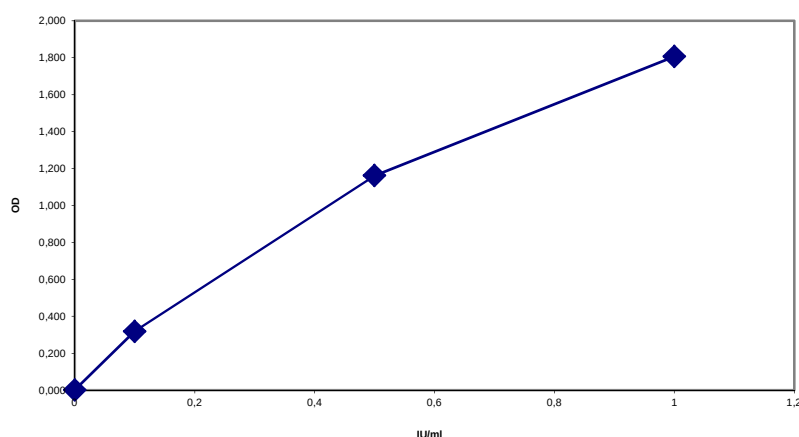
9.2. Calculation of Results

In order to obtain **quantitative results in IU/ml** plot 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)

NovaLisa®

Toxocara canis IgG

ELISA

CE

Only for in-vitro diagnostic use

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Product Number: TOCG0450 (96 Determinations)

ENGLISH

1. INTRODUCTION

Toxocara canis (an ascarid) is a parasitic nematode (roundworm) commonly found in the intestine of dogs. Humans are paratenic hosts who become infected by ingesting infective eggs in contaminated soil. After ingestion, the eggs yield larvae that penetrate the intestinal wall and are carried by the circulation to a wide variety of tissues (liver, heart, lungs, brain, muscle, eyes). While the larvae do not undergo any further development in these sites, they can cause several local reactions that are the basis of toxocariasis.

In most cases, *Toxocara* infections are not serious, and many people, especially adults infected by a small number of larvae (immature worms), may not notice any symptoms. The most severe cases are rare, but are more likely to occur in young children, who often play in dirt, or eat dirt contaminated by dog stool. The two main clinical presentations of toxocariasis are Ocular Larva Migrants (OLM), an eye disease that can cause blindness (each year more than 700 people infected with *Toxocara* experience permanent partial loss of vision), and Visceral Larva Migrants (VLM), a disease that causes swelling of ancillary body's organs or central nervous system

Species	Disease	Symptoms (e.g.)	Transmission route
<i>Toxocara canis</i>	Toxocariasis	Abdominal pain, gastrointestinal symptoms, weakness, pruritis	Infection after ingesting infective <i>Toxocara</i> eggs from larvae in soil or other contaminated surfaces.
	Ocular larva migrants (OLM)	Visual disturbance, endophthalmitis, retinal granuloma	
	Visceral larva migrants (VLM)	Fever, hepatomegaly, coughing, asthma or pneumonia	

Infection or presence of pathogen may be identified by:

- Microscopy
- Serology: Detection of antibodies by ELISA

2. INTENDED USE

The *Toxocara canis* IgG ELISA is intended for the qualitative determination of IgG class antibodies against *Toxocara canis* 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 *Toxocara canis* 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 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 *Toxocara canis* 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 $\pm$ 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 $< \text{Cut-off}$**
- **Cut-off Control:** Absorbance value **$0.150 - 1.300$**
- **Positive Control:** Absorbance value **$> \text{Cut-off}$**

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]

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

Example: $\frac{1.591 \times 10}{0.43} = 37 \text{ NTU}$

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	0.516	3.61
#2	24	0.904	2.90
#3	24	1.061	2.20
Interassay	n	Mean (NTU)	CV (%)
#1	12	18.81	4.83
#2	12	21.65	7.01
#3	12	2.23	12.17

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 98.63% (95% confidence interval: 96.52% - 99.62%).

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 96.92% (95% confidence interval: 89.32% - 99.63%).

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 *Ascaris lumbricoides* and *Schistosoma* 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

- 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 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.: TOCG0450 Toxocara canis IgG ELISA (96 Determinations)

DEUTSCH

1. EINLEITUNG

Toxocara canis ist ein Nematode (Rundwurm, Fadenwurm) der Familie der Ascarididae. Ascariden oder Spulwürmer sind große Rundwürmer. Die männlichen Individuen können bis zu 25 cm, die weiblichen bis zu 40 cm lang werden. *Toxocara canis* findet sich gewöhnlich bei Hunden. Befallen seine Larven den Menschen entwickelt sich das Larva-migrans-visceralis-Syndrom.

Die Infektion wird durch orale Aufnahme von Wurmeiern mit Erde, Sand, Nahrungsmitteln oder Wasser initiiert. Nach Ingestion schlüpfen die Larven, die die Darmwand durchbrechen und alle Organe des Menschen besiedeln können. Die meisten Hundespulwurminfektionen verlaufen unbemerkt, einige Infizierte haben jedoch bis zu mehrere Monate andauernde Symptome. Dazu zählen Eosinophilie und Hepatomegalie oder Fieberintervalle, gastro-intestinale Störungen, Asthma, cardiale Symptomatik, Lymph-adenopathie und urtikarielle Hautveränderungen, verbunden mit Leukozytose. Befall der Augen führt zu Endophthalmitis oder Chorioretinitis, die in eine Erblindung münden können. Zentralnervöse Störungen bedingen Lähmungen oder epileptiforme Anfälle. Die Infektion kann auch latent verlaufen. Etwa die Hälfte aller klinisch manifesten Fälle betrifft das Auge (Visusverlust).

Toxocara canis ist weltweit verbreitet. Neben Hundehaltern sind vor allem Kleinkinder betroffen

Spezies	Erkrankung	Symptome (z.B.)	Infektionsweg
<i>Toxocara canis</i>	Toxocariasis	Bauchschmerzen, gastrointestinale Symptome, Schwäche, Pruritus	Infektion nach Einnahme von infektiösen <i>Toxocara</i> -Eiern aus Larven im Boden oder anderen kontaminierten Oberflächen.
	Larva Migrans ocularis (auf english OLM)	Sehstörungen, Endophthalmitis, Netzhaut-Granulom	
	Larva-migrans-visceralis-Syndrom	Fieber, Hepatomegalie, Husten, Asthma oder Lungenentzündung	

Nachweis des Erregers bzw. der Infektion durch:

- Mikroskopie:
- Serologie: Nachweis der Antikörper mittels ELISA

2. VERWENDUNGSZWECK

Der *Toxocara canis* IgG ELISA ist für den qualitativen Nachweis spezifischer IgG-Antikörper gegen *Toxocara canis* in humanem Serum oder Plasma (Citrat, Heparin) bestimmt.

3. TESTPRINZIP

Die qualitative immunoenzymatische 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 Waschschrift 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.

Instructions For Use



Serazym[®] Adenovirus

Enzyme immunoassay for detection of *Adenovirus* in faecal samples

REF E-017 96 REF E-017-A2 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 <i>Adenovirus</i> 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 <i>Adenovirus</i> 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	$\geq$ 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

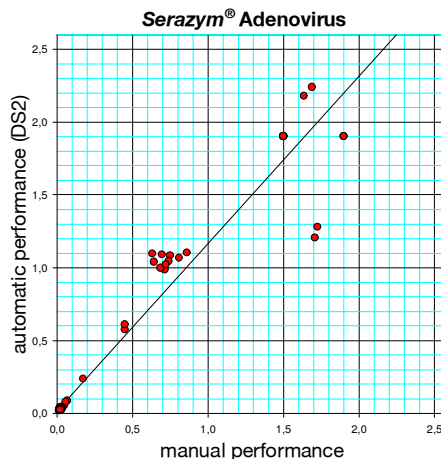
There is no correlation between measured absorbance and seriousness of the infection. It is also not allowed to correlate absorbances of the samples with that of the positive control. Cross contamination of reagents and samples can produce false positive results. Incorrect dilutions, not sufficiently 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
<i>Serazym</i> ® ELISA positive	55	1
<i>Serazym</i> ® ELISA negative	2	272

Specificity: 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):

<i>Aeromonas hydrophila</i>	(ATCC 7966)	<i>Klebsiella pneumoniae</i>	(ATCC 13883)
<i>Bacillus cereus</i>	(ATCC 11778)	<i>Peptostreptococcus anaerobius</i>	(ATCC 27337)
<i>Bacillus subtilis</i>	(ATCC 6633)	<i>Proteus vulgaris</i>	(ATCC 8427)
<i>Bacteroides fragilis</i>	(ATCC 25285)	<i>Pseudomonas aeruginosa</i>	(ATCC 10145)
<i>Candida albicans</i>	(ATCC 10231)	<i>Salmonella enterica</i> Serovar <i>enteritidis</i>	(ATCC 13076)
<i>Campylobacter coli</i>	(ATCC 33559)	<i>Salmonella enterica</i> Serovar <i>typhimurium</i>	(ATCC 14028)
<i>Campylobacter jejuni</i>	(ATCC 33291)	<i>Shigella flexneri</i>	(ATCC 12022)
<i>Citrobacter freundii</i>	(ATCC 8090)	<i>Shigella sonnei</i>	(ATCC 25931)
<i>Clostridium sordellii</i>	(ATCC 9714)	<i>Staphylococcus aureus</i>	(ATCC 25923)
<i>Enterobacter aerogenes</i>	(ATCC 13048)	<i>Staphylococcus epidermidis</i>	(ATCC 12228)
<i>Enterobacter cloacae</i>	(ATCC 13047)	<i>Vibrio parahaemolyticus</i>	(ATCC 17802)
<i>Enterococcus faecalis</i>	(ATCC 29212)	<i>Vibrio cholerae</i>	clinical isolate
<i>Escherichia coli</i>	(ATCC 25922)	<i>Yersinia enterocolitica</i> Serotyp <i>O3, O9</i>	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

 Manufacturer	 Date of manufacture	 Use by	LOT Batch code	REF Catalog number
 Keep away from sunlight	 Temperature limits	 Biological risks	 Do not reuse	
 Consult instructions for use	 Caution	IVD <i>In-vitro</i> -diagnostic medical device	 Contains sufficient for <n> tests	

Instructions For Use



Serazym® Astrovirus

Enzyme immunoassay for detection of *Astrovirus* in faecal samples

REF E-045 96 REF E-045-A2 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", Mikrobiologie, 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 / $\geq$ 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 <i>Astrovirus</i> 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 <i>Astrovirus</i> 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

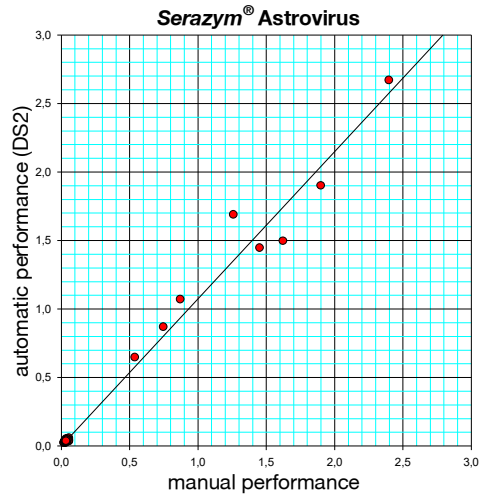
Performing the *Serazym*[®] Astrovirus on fully automated microplate processors (e.g. DS2, DSX) may cause elevated absorbances in comparison to the manual procedure due to individual differences concerning wash procedures and general technical specifications of the equipment. In these cases a maximum value of 0.3 absorbance units is permissible for the negative control. It is recommended to

<i>Serazym</i>[®] Astrovirus	
Positive	$\geq$ Cut-off
Negative	$<$ Cut-off

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 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
<i>Serazym®</i> ELISA positive	49	0
<i>Serazym®</i> 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):

<i>Aeromonas hydrophila</i>	(ATCC 7966)	<i>Klebsiella pneumoniae</i>	(ATCC 13883)
<i>Bacillus cereus</i>	(ATCC 11778)	<i>Peptostreptococcus anaerobius</i>	(ATCC 27337)
<i>Bacillus subtilis</i>	(ATCC 6633)	<i>Proteus vulgaris</i>	(ATCC 8427)
<i>Bacteroides fragilis</i>	(ATCC 25285)	<i>Pseudomonas aeruginosa</i>	(ATCC 10145)
<i>Candida albicans</i>	(ATCC 10231)	<i>Salmonella enterica</i> Serovar <i>enteritidis</i>	(ATCC 13076)
<i>Campylobacter coli</i>	(ATCC 33559)	<i>Salmonella enterica</i> Serovar <i>typhimurium</i>	(ATCC 14028)
<i>Campylobacter jejuni</i>	(ATCC 33291)	<i>Shigella flexneri</i>	(ATCC 12022)
<i>Citrobacter freundii</i>	(ATCC 8090)	<i>Shigella sonnei</i>	(ATCC 25931)
<i>Clostridium sordellii</i>	(ATCC 9714)	<i>Staphylococcus aureus</i>	(ATCC 25923)
<i>Enterobacter aerogenes</i>	(ATCC 13048)	<i>Staphylococcus epidermidis</i>	(ATCC 12228)
<i>Enterobacter cloacae</i>	(ATCC 13047)	<i>Vibrio parahaemolyticus</i>	(ATCC 17802)
<i>Enterococcus faecalis</i>	(ATCC 29212)	<i>Vibrio cholerae</i>	Clinical isolate
<i>Escherichia coli</i>	(ATCC 25922)	<i>Yersinia enterocolitica</i> Serotyp <i>O3, O9</i>	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)

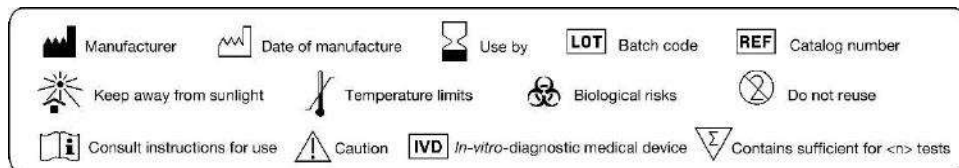
1.  2 drops (or 75 µl) **CONJ HRP** (6)
 +
 pipette
 75 µl **CONTROL +** (4)
 75 µl **CONTROL -** (5)
 50 µl **stool sample**, mix gently

 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 ▽ 96 REF E-061-A2 ▽ 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 / H₂O₂) 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 *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.

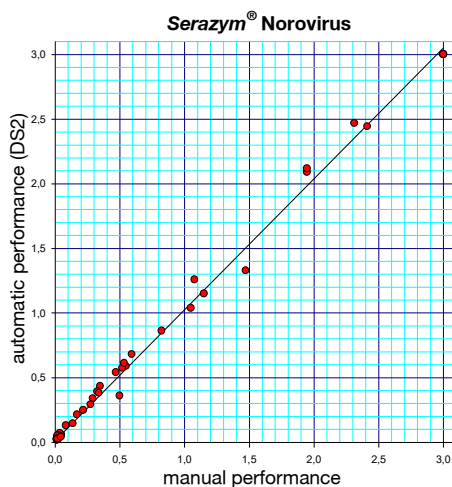
Automatic Processing

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
<i>Serazym®</i> ELISA positive	111	3
<i>Serazym®</i> 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 $\geq 10^8$ 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):

<i>Adenovirus</i>	typ e41	<i>Peptostreptococcus anaerobius</i>	ATCC 27337
<i>Aeromonas hydrophila</i>	ATCC 7966	<i>Proteus vulgaris</i>	ATCC 8427
<i>Astrovirus</i>	serotype 4	<i>Pseudomonas aeruginosa</i>	ATCC 10145
<i>Bacillus cereus</i>	ATCC 117788	<i>Rotavirus</i>	strain SA11
<i>Bacillus subtilis</i>	ATCC 6633	<i>Salmonella enterica</i> Serovar <i>thyphimurium</i>	ATCC 14028
<i>Bacteroides fragilis</i>	ATCC 25285	<i>Salmonella enterica</i> ssp. <i>galolyticus</i>	ATCC 13076
<i>Campylobacter jejuni</i>	ATCC 33291	<i>Shigella flexneri</i>	ATCC 12022
<i>Candida albicans</i>	clinical isolate	<i>Shigella sonnei</i>	ATCC 25931
<i>Citrobacter freundii</i>	ATCC 8090	<i>Staphylococcus aureus</i>	ATCC 25923
<i>Clostridium sordelli</i>	ATCC 9714	<i>Staphylococcus epidermidis</i>	ATCC 12228
<i>Enterobacter aerogenes</i>	ATCC 13048	<i>Vibrio cholerae</i>	RV 2011/ST5
<i>Enterobacter cloacae</i>	ATCC 13047	<i>Vibrio parahaemolyticus</i>	ATCC 17802
<i>Enterococcus faecalis</i>	ATCC 29212	<i>Yersinia enterocolitica</i> 0:9	clinical isolate
<i>Escherichia coli</i>	ATCC 25922	<i>Yersinia enterocolitica</i> 0:3	clinical isolate
<i>Klebsiella pneumonia</i>	ATCC 13883		

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 / $\geq$ 620 nm

 Manufacturer	 Date of manufacture	 Use by	LOT Batch code	REF Catalog number
 Keep away from sunlight	 Temperature limits	 Biological risks	 Do not reuse	
 Consult instructions for use	 Caution	IVD <i>In-vitro</i> -diagnostic medical device	 Contains sufficient for <n> tests	

NovaLisa®

Brucella IgG

ELISA

CE

Only for in-vitro diagnostic use

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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 contagiousity. 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 $\pm$ 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 $< \text{Cut-off}$
- **Cut-off Control:** Absorbance value **0.150 – 1.300**
- **Positive Control:** Absorbance value $> \text{Cut-off}$

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]

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

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

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 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 Durchmesser 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
<i>B. abortus</i> (Rind) <i>B. melitensis</i> (Ziegen, Schafe) <i>B. suis</i> (Schweine) <i>B. canis</i> (Hunde)	Brucella	Fieber, Schüttelfrost (Febris undulans), Unwohlsein, Arthritis, Hepatitis, Hepatomegalie, Endokarditis, Osteomyelitis	<u>oral</u> (unpasteurisierte Milch und Milchprodukte) <u>perkutan</u> (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 Waschschriff 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.

NovaLisa®

Brucella IgM

ELISA

CE

Only for in-vitro diagnostic use

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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 contagiousity. 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 $\pm$ 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 $< \text{Cut-off}$
- **Cut-off Control:** Absorbance value **0.150 – 1.300**
- **Positive Control:** Absorbance value $> \text{Cut-off}$

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]

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

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

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 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)

NovaLisa®

Hantavirus IgG

ELISA

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Only for in-vitro diagnostic use

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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 vision. 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.
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.	Bites by infected rodents. Human to human transmission can not be excluded (for New World strains).

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 $\pm$ 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]

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

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

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

<u>Intraassay</u>	<u>n</u>	<u>Mean (E)</u>	<u>Cv (%)</u>
#1	24	0.450	3.61
#2	24	1.333	6.41
#3	24	1.264	4.78
<u>Interassay</u>	<u>n</u>	<u>Mean (NTU)</u>	<u>Cv (%)</u>
#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, abdominale 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.
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.	Durch Nagerbisse. Eine Übertragung von Mensch zu Mensch kann nicht ausgeschlossen werden (v.a. bei den Neue Welt Stämmen).

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.

NovaLisa®

Hantavirus IgM

ELISA

CE

Only for in-vitro diagnostic use

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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 vision. 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.
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.	Bites by infected rodents. Human to human transmission can not be excluded (for New World strains).

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]

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

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

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)

Serazym® Rotavirus

Enzyme immunoassay for detection of *Rotavirus* in faecal samples

REF E-020  96 REF E-020-A2  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	$\geq$ 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

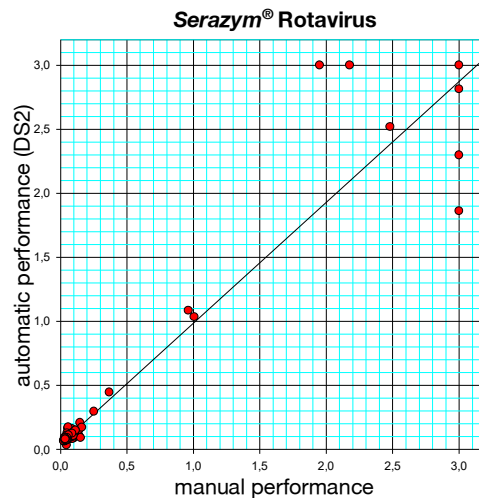
There is no correlation between measured absorbance and seriousness of the infection. It is also not allowed to correlate absorbances of the samples with that of the positive control. Cross contamination of reagents and samples can produce false positive results. Incorrect dilutions, not sufficiently 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 10⁶ 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
<i>Serazym</i> ® ELISA positive	246	0
<i>Serazym</i> ® ELISA negative	4	238

Specificity: 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 ≥ 10⁸ colony forming units of the following microorganisms and tested negative with the *Serazym*® ELISA (OD 450 / 620 nm < Cut-Off):

<i>Aeromonas hydrophila</i>	(ATCC 7966)	<i>Klebsiella pneumoniae</i>	(ATCC 13883)
<i>Bacillus cereus</i>	(ATCC 11778)	<i>Peptostreptococcus anaerobius</i>	(ATCC 27337)
<i>Bacillus subtilis</i>	(ATCC 6633)	<i>Proteus vulgaris</i>	(ATCC 8427)
<i>Bacteroides fragilis</i>	(ATCC 25285)	<i>Pseudomonas aeruginosa</i>	(ATCC 10145)
<i>Candida albicans</i>	(ATCC 10231)	<i>Salmonella enterica</i> Serovar <i>enteritidis</i>	(ATCC 13076)
<i>Campylobacter coli</i>	(ATCC 33559)	<i>Salmonella enterica</i> Serovar <i>typhimurium</i>	(ATCC 14028)
<i>Campylobacter jejuni</i>	(ATCC 33291)	<i>Shigella flexneri</i>	(ATCC 12022)
<i>Citrobacter freundii</i>	(ATCC 8090)	<i>Shigella sonnei</i>	(ATCC 25931)
<i>Clostridium sordellii</i>	(ATCC 9714)	<i>Staphylococcus aureus</i>	(ATCC 25923)
<i>Enterobacter aerogenes</i>	(ATCC 13048)	<i>Staphylococcus epidermidis</i>	(ATCC 12228)
<i>Enterobacter cloacae</i>	(ATCC 13047)	<i>Vibrio parahaemolyticus</i>	(ATCC 17802)
<i>Enterococcus faecalis</i>	(ATCC 29212)	<i>Vibrio cholerae</i>	clinical isolate
<i>Escherichia coli</i>	(ATCC 25922)	<i>Yersinia enterocolitica</i> Serotyp <i>O3, O9</i>	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), Simigel® (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 / $\geq$ 620 nm

 Manufacturer	 Date of manufacture	 Use by	LOT Batch code	REF Catalog number
 Keep away from sunlight	 Temperature limits	 Biological risks	 Do not reuse	
 Consult instructions for use	 Caution	IVD <i>In-vitro</i> -diagnostic medical device	 Contains sufficient for <n> tests	

Certificate

**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

TÜVRheinland

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

A. Fechner



Dipl.-Ing. A. Fechner

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NovaLisa®

Legionella pneumophila IgM

ELISA

CE

Only for in-vitro diagnostic use

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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
<i>Legionella pneumophila</i>	<i>Legionella</i> 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 <i>Legionella</i> bacteria or micro-aspiration of ingested water contaminated with <i>Legionella</i>
	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; $\leq 0.0015\%$ (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; $\leq 0.02\%$ (v/v) MIT.
- **Cut-off Control:** 1 vial containing 3 mL control; coloured yellow; ready to use; green cap; $\leq 0.02\%$ (v/v) MIT.
- **Negative Control:** 1 vial containing 2 mL control; coloured yellow; ready to use; blue cap; $\leq 0.0015\%$ (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 μ L
- 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]

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

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

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)

NovaLisa®

Legionella pneumophila IgG

ELISA

CE

Only for in-vitro diagnostic use

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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
<i>Legionella pneumophila</i>	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 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 $\pm$ 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 $< \text{Cut-off}$
- **Cut-off Control:** Absorbance value **0.150 – 1.300**
- **Positive Control:** Absorbance value $> \text{Cut-off}$

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]

Sample (mean) absorbance value x 10 = [NovaTec Units = NTU]
Cut-off

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)	Husten, Fieber und unspezifische Symptome (Unwohlsein, Muskel- und Kopfschmerzen); teilweise Schüttelfrost, Brustschmerzen,	mit Legionellen belastetes Wasser (Inhalation von Aerosolen oder Mikroaspiration)
	Pontiac-Fieber	Durchfall, neurologische Symptome; Influenza-ähnlich ohne Pneumonie: Kopf- und Muskelschmerzen, Frösteln, trockener Husten und Fieber	

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 Waschschriff 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.

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Leptospira IgM

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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; $\leq 0.0015\%$ (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; $\leq 0.02\%$ (v/v) MIT.
- **Cut-off Control:** 1 vial containing 3 mL control; coloured yellow; ready to use; green cap; $\leq 0.02\%$ (v/v) MIT.
- **Negative Control:** 1 vial containing 2 mL control; coloured yellow; ready to use; blue cap; $\leq 0.0015\%$ (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 μ L
- 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 μ L 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 $\pm$ 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 $< \text{Cut-off}$
- **Cut-off Control:** Absorbance value **0.150 – 1.300**
- **Positive Control:** Absorbance value $> \text{Cut-off}$

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]

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

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

<u>Intraassay</u>	<u>n</u>	<u>Mean (E)</u>	<u>CV (%)</u>
#1	24	0.531	5.34
#2	24	1.070	3.32
#3	24	1.900	2.80
<u>Interassay</u>	<u>n</u>	<u>Mean (NTU)</u>	<u>CV (%)</u>
#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 gemäßigttem 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 Reservoir der pathogenen Spezies *Leptospira interrogans* 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 Waschschriff 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.

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Leptospira IgG

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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; $\leq 0.0015\%$ (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; $\leq 0.02\%$ (v/v) MIT.
- **Cut-off Control:** 1 vial containing 3 mL control; coloured yellow; ready to use; green cap; $\leq 0.02\%$ (v/v) MIT.
- **Negative Control:** 1 vial containing 2 mL control; coloured yellow; ready to use; blue cap; $\leq 0.0015\%$ (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 $\pm$ 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 ($20\ldots 25$ °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 ($20\ldots 25$ °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 $< \text{Cut-off}$
- **Cut-off Control:** Absorbance value **$0.150 - 1.300$**
- **Positive Control:** Absorbance value $> \text{Cut-off}$

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]

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

Example: $\frac{1.591 \times 10}{0.43} = 37 \text{ NTU}$

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

<u>Intraassay</u>	<u>n</u>	<u>Mean (E)</u>	<u>CV (%)</u>
#1	24	0.470	2.95
#2	24	0.782	6.50
#3	24	0.460	3.49
<u>Interassay</u>	<u>n</u>	<u>Mean (NTU)</u>	<u>CV (%)</u>
#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)

NovaLisa®

Yersinia enterocolitica IgG

ELISA

CE

Only for in-vitro diagnostic use

Instructions for use

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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]

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

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

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
<i>Borrelia burgdorferi</i>	15	4	4
<i>Brucella</i>	10	9	9
<i>Campylobacter jejuni</i>	8	4	5
<i>Chlamydia pneumoniae</i>	12	4	2
Enterovirus	15	7	5
Epstein-Barr virus (EBV)	15	3	3
<i>Helicobacter pylori</i>	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



Набор реагентов
для иммуноферментного выявления
иммуноглобулинов класса М
к возбудителям иерсиниозов

ИНСТРУКЦИЯ ПО ПРИМЕНЕНИЮ

Утверждена 28.08.2013

Приказом Росздравнадзора № 4415-Пр/13

Иерсиния – IgM – ИФА – БЕСТ

НАБОР РЕАГЕНТОВ

D-3206

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 тетраметилбензида 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 (K^+), инаktivированный – 1 фл., 0,5 мл;
- отрицательный контрольный образец (K^-), инаktivированный – 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 $(37 \pm 1)^\circ\text{C}$;
- термошейкер, поддерживающий температуру $(37 \pm 1)^\circ\text{C}$, интенсивность перемешивания 400–800 об/мин;
- холодильник бытовой, поддерживающий температуру $(2-8)^\circ\text{C}$;
- 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 $(2-8)^{\circ}\text{C}$ не более 5 суток, либо при температуре минус $(20\pm 4)^{\circ}\text{C}$, 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) °C 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) °C.
- 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 объема 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 K^+** , в 2 другие лунки – по **10 мкл K^-** . 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 $(37 \pm 1)^\circ\text{C}$ **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 K^- не более 0,2 ($ОП_{cp} K^- \leq 0,2$).
- значение ОП в лунке с K^+ не менее 0,6 ($ОП K^+ \geq 0,6$);

Вычислить критическое значение ОП (ОП_{крит}) по формуле:

$$\text{ОП}_{\text{крит}} = \text{ОП}_{\text{ср}}(\text{K}^-) + 0,3,$$

где ОП_{ср}(K⁻) – среднее арифметическое значение ОП в лунках с отрицательным контрольным образцом.

Если ОП_{ср} (K⁻) имеет отрицательное значение, считать её равной нулю.

Для интерпретации результатов исследования сывороток использовать **коэффициент позитивности (КП)**:

$$\text{КП} = \text{ОП}_{\text{иссл. сыв.}} / \text{ОП}_{\text{крит.}}$$

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 приготовления.

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:

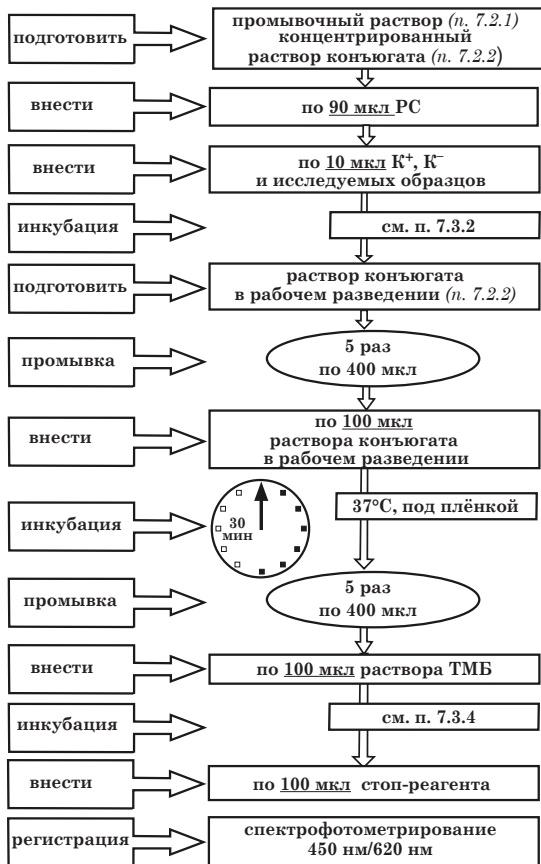
IgG	IgM	IgA	Интерпретация
+	—	—	перенесённая в прошлом инфекция
+	+, но КП снижается в течении 3–6 мес.	+, но КП снижается в течении 3–6 мес.	недавно перенесённая инфекция с исходом в выздоровление
+ или —	— или +, но КП снижается в динамике	+ более 6 мес.	персистенция возбудителя. Артриты, узловая эритема, 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 лабораторных исследований.

Схема анализа D-3206



ГРАФИЧЕСКИЕ СИМВОЛЫ

	Номер по каталогу		Медицинское изделие для диагностики <i>in vitro</i>
	Содержимого достаточно для проведения n количества тестов		Не стерильно
	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,
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E-mail: vbmarket@vector-best.ru

Internet: www.vector-best.ru

NovaLisa®

Yersinia enterocolitica IgA

ELISA

CE

Only for in-vitro diagnostic use

Instructions for use

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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]

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

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

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)

NovaLisa®

Taenia solium IgG

ELISA

CE

Only for in-vitro diagnostic use

English	2
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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
<i>Taenia solium</i>	Taeniasis Cysticercosis (Neurocysticercosis)	Abdominal pain; Nausea; Weakness and fatigue; Weight loss; Flatulence (gases); Diarrhea or constipation; Appetite changes (too much hunger or loss of appetite) Cysticerci in the brain may cause increased cranial pressure, convulsions and altered mental states	Ingestion of undercooked pork meat containing cysticerci or ingestion of <i>Taenia solium</i> eggs via fecally contaminated food or water

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; $\leq 0.0015\%$ (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; $\leq 0.02\%$ (v/v) MIT.
- **Cut-off Control:** 1 vial containing 3 mL control; coloured yellow; ready to use; green cap; $\leq 0.02\%$ (v/v) MIT.
- **Negative Control:** 1 vial containing 2 mL control; coloured yellow; ready to use; blue cap; $\leq 0.0015\%$ (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 $\pm$ 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 $< \text{Cut-off}$
- **Cut-off Control:** Absorbance value **0.150 – 1.300**
- **Positive Control:** Absorbance value $> \text{Cut-off}$

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]

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

Example:
$$\frac{1.591 \times 10}{0.43} = 37 \text{ NTU}$$

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)

Certificate



**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

TÜVRheinland

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



D. Wiedemuth

Dipl.-Ing. (FH) D. Wiedemuth
TÜV Rheinland LGA Products GmbH
Tillystraße 2 · 90431 Nürnberg · Germany

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Certificate



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

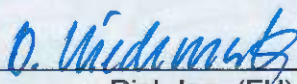
Report No.: 3313978-90

Effective date: 2020-05-19

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Dipl.-Ing. (FH) D. Wiedemuth
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Certificate



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




Dipl.-Ing. (FH) D. Wiedemuth
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Certificate



**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




Dipl.-Ing. (FH) D. Wiedemuth
TÜV Rheinland LGA Products GmbH
Tillystraße 2 · 90431 Nürnberg · Germany



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

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

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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	
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	
4. Universal buffer 10x concentrate	1 x 100 ml	8 x 100 ml	
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	
6. Adhesive foil	---	8 sheets	---
7. Test instruction	1 booklet	1 booklet	---
 Lot description			 Storage temperature
 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.

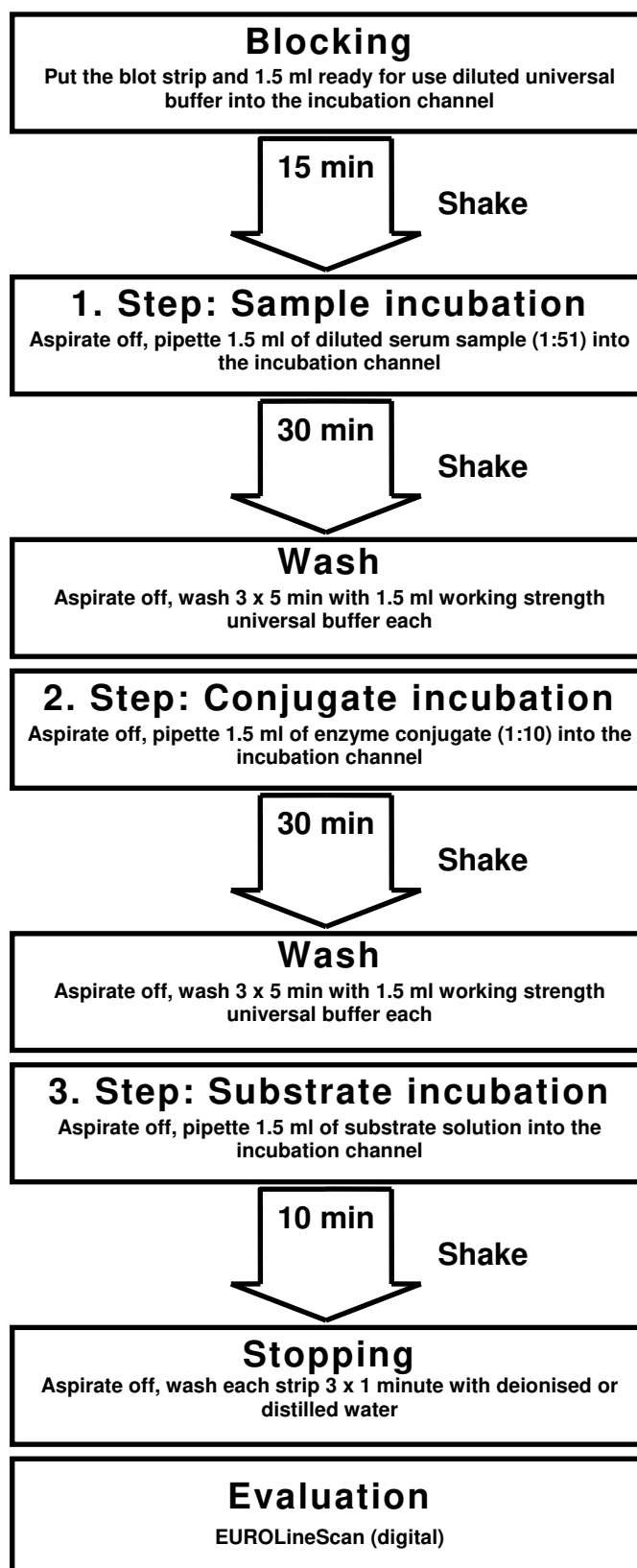
Wash: Aspirate off the liquid from each channel. Wash as described above.

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 monoclonal 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.

Clinically characterised sera	n	Prevalence		
		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%

Serum samples	n	Prevalence	
		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 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 epidemic 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 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 ticks transmitted "treponema-like spirochaetes", which were later authenticated 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 m². 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)

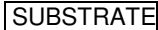
Test instruction

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	
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	
4. Universal buffer 10x concentrate	1 x 100 ml	8 x 100 ml	
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	
6. Adhesive foil	---	8 sheets	---
7. Test instruction	1 booklet	1 booklet	---
 Lot description			 Storage temperature
 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.

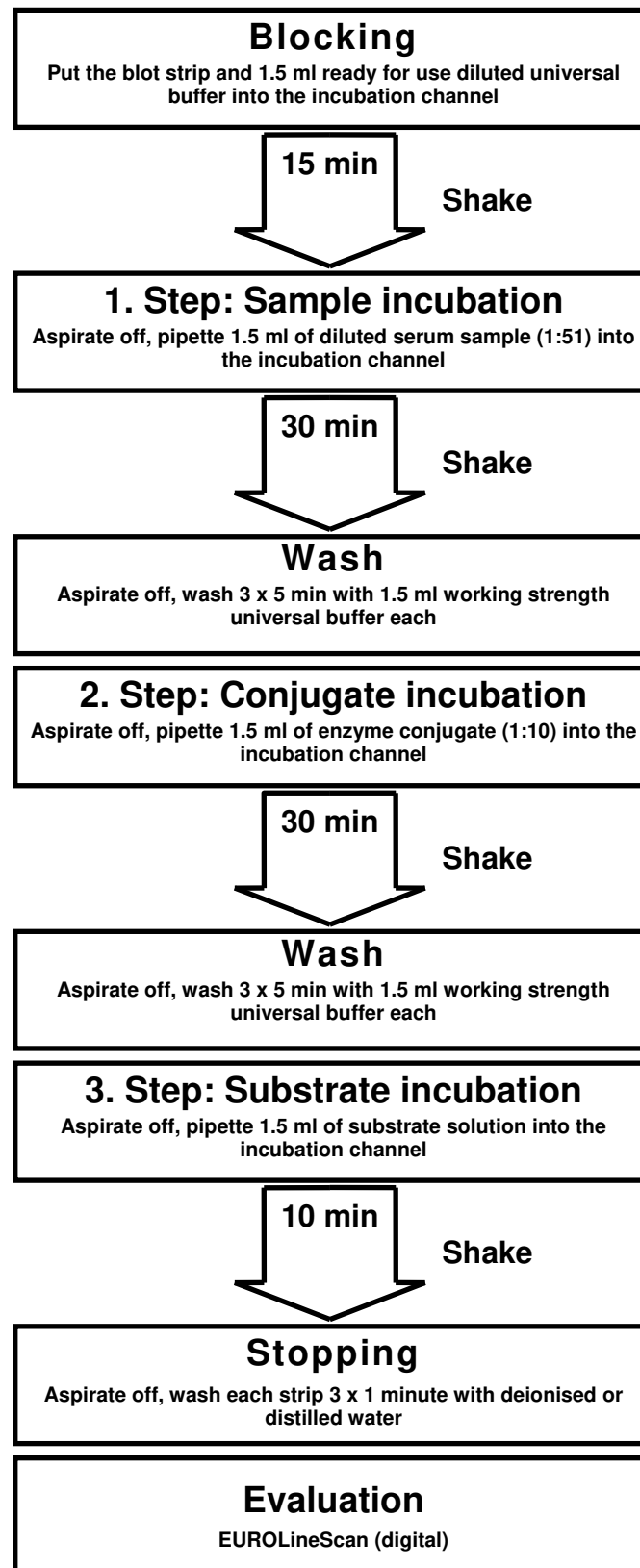
Wash: Aspirate off the liquid from each channel. Wash as described above.

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 monoclonal 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.

Clinically characterised sera	n	Prevalence		
		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%

Serum samples	n	Prevalence	
		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 19th/20th 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 authenticated 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 m² [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 (variable major protein-like sequence, expressed), 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

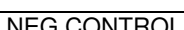
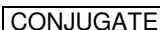
ORDER NO.	ANTIBODIES AGAINST	IG CLASS	SUBSTRATE	FORMAT
EI 2132-9601-2 G	Borrelia burgdorferi, Borrelia afzelii, Borrelia garinii, Borrelia VlsE Antigen	IgG	Ag-coated microplate wells	96 x 01 (96)

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 relevant 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	
2. Calibrator 1 200 RU/ml (IgG, human), ready for use	dark red	1 x 2.0 ml	
3. Calibrator 2 20 RU/ml (IgG, human), ready for use	red	1 x 2.0 ml	
4. Calibrator 3 2 RU/ml (IgG, human), ready for use	light red	1 x 2.0 ml	
5. Positive control (IgG, human), ready for use	blue	1 x 2.0 ml	
6. Negative control (IgG, human), ready for use	green	1 x 2.0 ml	
7. Enzyme conjugate peroxidase-labelled anti-human IgG (rabbit), ready for use	green	1 x 12 ml	
8. Sample buffer ready for use	light blue	1 x 100 ml	
9. Wash buffer 10x concentrate	colourless	1 x 100 ml	
10. Chromogen/substrate solution TMB/H ₂ O ₂ , ready for use	colourless	1 x 12 ml	
11. Stop solution 0.5 M sulphuric acid, ready for use	colourless	1 x 12 ml	
12. Test instruction	---	1 booklet	
13. Quality control certificate	---	1 protocol	
 Lot description			 Storage temperature
 In vitro diagnostic medical device			 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 **semiquantitative 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:

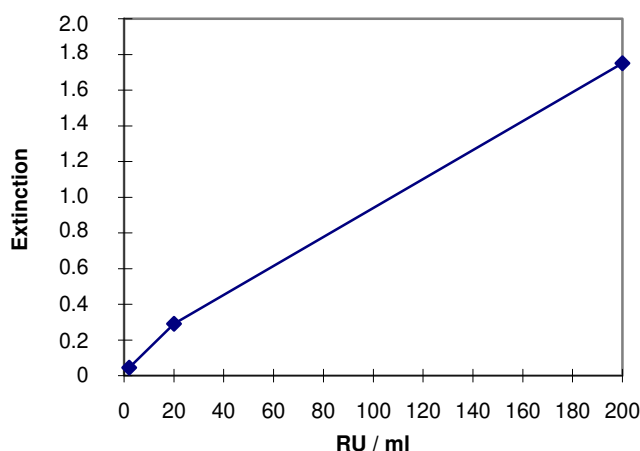
$$\frac{\text{Extinction of the control or patient sample}}{\text{Extinction of calibrator 2}} = \text{Ratio}$$

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 (variable major protein-like sequence, expressed) 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- <i>Borrelia</i> 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- <i>Mycoplasma pneumoniae</i>	12	0%
Anti-Measles virus	12	0%
Anti-Mumps virus	12	0%
Anti-Rubella virus	12	0%
Anti- <i>Toxoplasma gondii</i>	11	0%
Anti- <i>Chlamydia pneumoniae</i>	11	0%
Anti- <i>Helicobacter pylori</i>	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.

<i>Intra-assay variation, n = 20</i>		
Serum	Mean value (RU/ml)	CV (%)
1	52	3.5
2	97	3.5
3	128	3.6

<i>Inter-assay variation, n = 4 x 6</i>		
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 VisE 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 VisE-ELISA (IgG) and Anti-Borrelia-ELISA (IgM). Sensitivities between 89 to 96% were found.

n = 138		EUROIMMUN Anti-Borrelia plus VisE-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), which 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 epidemic 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 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 ticks transmitted "treponema-like spirochaetes", which were later authenticated 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 m². 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 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 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

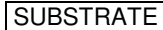
ORDER NO.	ANTIBODIES AGAINST	IG CLASS	SUBSTRATE	FORMAT
EI 2132-9601 M	Borrelia burgdorferi, Borrelia afzelii, Borrelia garinii	IgM	Ag-coated microplate wells	96 x 01 (96)

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 relevant 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	
2. Calibrator 1 , 200 RU/ml (IgM, human), ready for use	dark red	1 x 2.0 ml	
3. Calibrator 2 , 20 RU/ml (IgM, human), ready for use	red	1 x 2.0 ml	
4. Calibrator 3 , 2 RU/ml (IgM, human), ready for use	light red	1 x 2.0 ml	
5. Positive control , (IgM, human), ready for use	blue	1 x 2.0 ml	
6. Negative control , (IgM, human), ready for use	green	1 x 2.0 ml	
7. Enzyme conjugate peroxidase-labelled anti-human IgM (goat), ready for use	red	1 x 12 ml	
8. Sample buffer containing IgG/RF absorbent (anti-human IgG antibody preparation obtained from goat), ready for use	green	1 x 100 ml	
9. Wash buffer 10x concentrate	colourless	1 x 100 ml	
10. Chromogen/substrate solution TMB/H ₂ O ₂ , ready for use	colourless	1 x 12 ml	
11. Stop solution 0.5 M sulphuric acid, ready for use	colourless	1 x 12 ml	
12. Test instruction	---	1 booklet	
13. Quality control certificate	---	1 protocol	
 Lot description			 Storage temperature
 In vitro diagnostic medical device			 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 **semiquantitative 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:

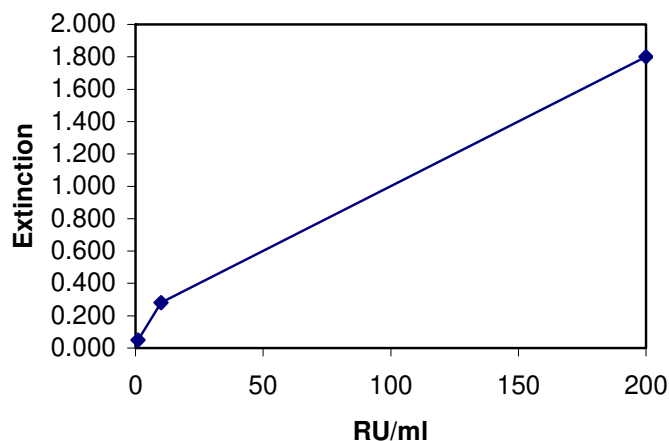
$$\frac{\text{Extinction of the control or patient sample}}{\text{Extinction of calibrator 2}} = \text{Ratio}$$

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- <i>Borrelia</i> ELISA (IgM) positive
Acute EBV infection	27	0%
Anti-CMV	19	0%
Anti-Measles virus	13	0%
Anti-Mumps virus	12	0%
Anti- <i>Toxoplasma gondii</i>	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.

<i>Intra-assay variation, n = 20</i>		
Serum	Mean value (RU/ml)	CV (%)
1	75	4.3
2	103	2.9
3	115	3.4

<i>Inter-assay variation, n = 4 x 6</i>		
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 VisE-ELISA (IgG) and Anti-Borrelia-ELISA (IgM). Sensitivities between 89-96% were found.

n = 138		EUROIMMUN Anti-Borrelia plus VisE-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 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 epidemic 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 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 ticks transmitted "Treponema-like spirochaetes", which were later authenticated 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 m². 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 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 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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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	
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	
4. Universal buffer 10x concentrate	1 x 50 ml	
5. Substrate solution Nitroblue tetrazolium chloride/5-Bromo-4-chloro-3-indolylphosphate (NBT/BCIP), ready for use	1 x 30 ml	
6. Incubation tray	2 x 8 channels	
7. Test instruction	1 booklet	---
 Lot description		 Storage temperature
 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 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.

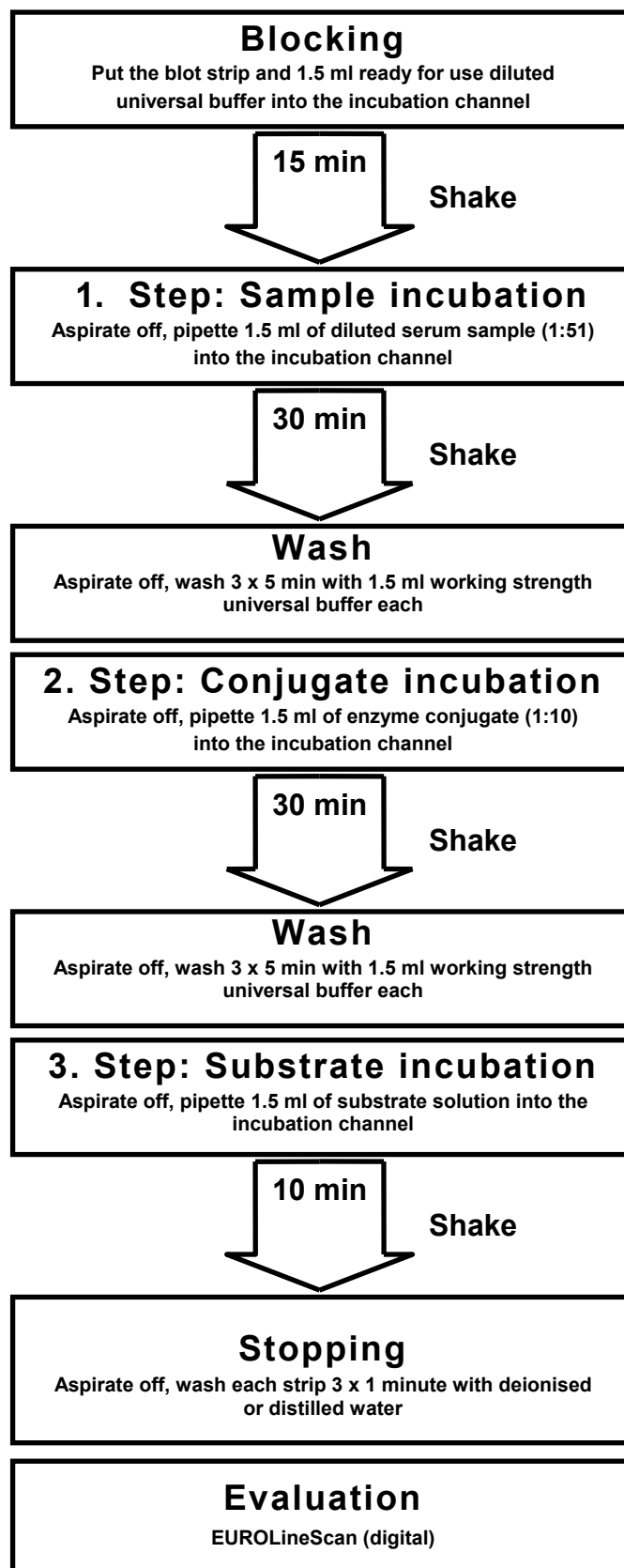
Wash: Aspirate off the liquid from each channel. Wash as described above.

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**.

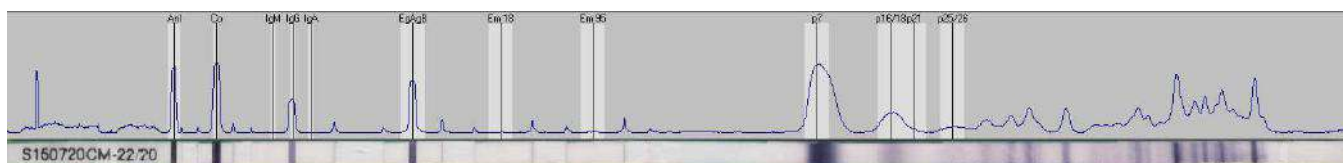
If a visual evaluation must be performed in exceptional cases, hold the evaluation matrix next to the stuck-on blot strips and position it so that the black band above the number on the blot strips lines up with the alignment bar of the evaluation matrix. **The lot number on the evaluation matrix must 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
<i>Ascaris lumbricoides</i>	10	3
<i>Anisakis simplex</i>	16	1
Filarioidea	5	0
<i>Strongyloides stercoralis</i>	10	0
<i>Schistosoma</i> ssp.	9	0
Multi-Helminth infection	2	0
<i>Plasmodium</i> ssp.	7	0
<i>Toxocara canis</i>	10	0
<i>Taenia solium</i>	7	0
<i>Trichinella spiralis</i>	13	0
<i>Fasciola hepatica</i>	17	0
<i>Entamoeba histolytica</i>	11	0
<i>Leishmania</i> 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- <i>Echinococcus</i> 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
<i>Echinococcus granulosus</i>	47
<i>Echinococcus multilocularis</i>	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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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 -

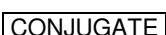
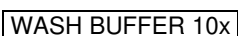
Anti-West Nile Virus ELISA (IgG)

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	
2. Calibrator 1 200 RU/ml (IgG, human), ready for use	dark red	1 x 2.0 ml	
3. Calibrator 2 20 RU/ml (IgG, human), ready for use	red	1 x 2.0 ml	
4. Calibrator 3 2 RU/ml, (IgG, human), ready for use	light red	1 x 2.0 ml	
5. Positive control (IgG, human), ready for use	blue	1 x 2.0 ml	
6. Negative control (IgG, human), ready for use	green	1 x 2.0 ml	
7. Enzyme conjugate peroxidase-labelled anti-human IgG (rabbit), ready for use	green	1 x 12 ml	
8. Sample buffer ready for use	light blue	1 x 100 ml	
9. Wash buffer 10x concentrate	colourless	1 x 100 ml	
10. Chromogen/substrate solution TMB/H ₂ O ₂ , ready for use	colourless	1 x 12 ml	
11. Stop solution 0.5 M sulphuric acid, ready for use	colourless	1 x 12 ml	
12. Test instruction	---	1 booklet	
13. Quality control certificate	---	1 protocol	
14. Protective foil	---	2 pieces	
 Lot description			 Storage temperature
 In vitro diagnostics			 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 **semiquantitative 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:

$$\frac{\text{Extinction of the control or patient sample}}{\text{Extinction of calibrator 2}} = \text{Ratio}$$

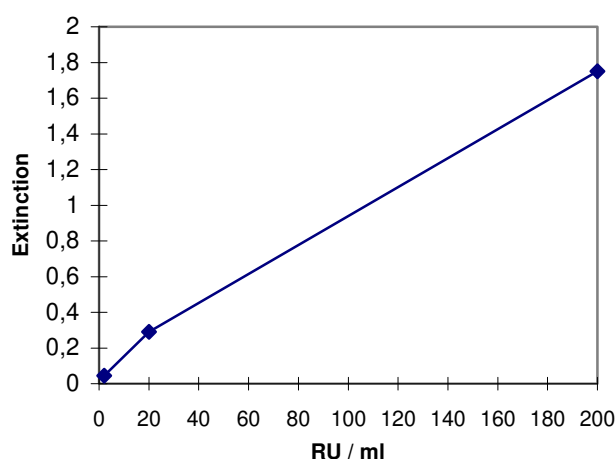
EUROIMMUN recommends interpreting results as follows:

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.

<i>Intra-assay variation, n = 20</i>		
Serum	Mean value (RU/ml)	CV (%)
1	101	6.1
2	104	3.3
3	149	2.7

<i>Inter-assay variation, n = 4 x 6</i>		
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 investigated 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 epidemics 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 prodrome 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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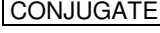
Anti-West Nile Virus ELISA (IgM)

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	
2. Calibrator (IgM, human), ready for use	dark red	1 x 2.0 ml	
3. Positive control (IgM, human), ready for use	blue	1 x 2.0 ml	
4. Negative control (IgM, human), ready for use	green	1 x 2.0 ml	
5. Enzyme conjugate peroxidase-labelled anti-human IgM (goat), ready for use	red	1 x 12 ml	
6. Sample buffer buffer containing IgG/RF-Absorbent (Anti-human IgG antibody preparation obtained from goat), ready for use	green	1 x 100 ml	
7. Wash buffer 10x concentrate	colourless	1 x 100 ml	
8. Chromogen/substrate solution TMB/H ₂ O ₂ , ready for use	colourless	1 x 12 ml	
9. Stop solution 0.5 M sulphuric acid, ready for use	colourless	1 x 12 ml	
10. Test instruction	---	1 booklet	
11. Quality control certificate	---	1 protocol	
12. Protective foil	---	3 pieces	
 Lot description  In vitro diagnostics   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:

$$\frac{\text{Extinction of the control/patient sample}}{\text{Extinction of calibrators}} = \text{Ratio}$$

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.

<i>Intra-assay variation, n = 20</i>		
Serum	Mean value (Ratio)	CV (%)
1	1.9	5.9
2	2.0	2.2
3	2.4	4.0

<i>Inter-assay variation, n = 4 x 6</i>		
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 epidemics 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 prodrome 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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2013 11 0039 EN

según la norma
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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

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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 <i>Change in the description of the method of analysis in the technological scope (infectious immunology and molecular biology techniques).</i> <i>Change in the level of detail of the technological scope description.</i>
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". <i>Extension of technological scope:</i> <i>Immunochemistry and Microbiology</i> <i>Instruments and software for "in vitro" diagnostic</i> <i>Modification of the scope to include the activity of technical servicing of instruments and software for "in vitro" diagnostic</i>

Madrid, 08 de marzo de 2019

DIRECTORA DE LA AGENCIA ESPAÑOLA DE MEDICAMENTOS Y PRODUCTOS SANITARIOS



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**CERTIFICADO CE DE SISTEMA DE GARANTÍA DE CALIDAD TOTAL
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**EC FULL QUALITY ASSURANCE SYSTEM CERTIFICATE
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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

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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 certificate*
2003 12 0390 ED

1.3. HBc Ab

- BCAB.CE (96 tests)

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2003 12 0391 ED

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1.4. HBc IgM

- BCM.CE (96 tests) Descrito en el certificado / Described in the certificate 2004 03 0424 ED

1.5. HBe Ag & Ab

- HBE.CE (96 tests) Descrito en el certificado / Described in the certificate 2004 03 0425 ED

1.6. HBs Ag Confirmation

- SCONF.CE (20 tests) Descrito en el certificado / Described in the certificate 2006 11 0511 ED
- SCONF.CE.40 (40 tests)

1.7. HBs Ag one Version ULTRA

- SAG1ULTRA.CE (192 tests) Descrito en el certificado / Described in the certificate 2008 12 0588 ED
- SAG1ULTRA.CE.96 (96 tests)
- SAG1ULTRA.CE.480 (480 tests)
- SAG1ULTRA.CE.960 (960 tests)
- SAG1ULTRA.CE.DB (192 tests)

1.8. HCV Ab

- CVAB.CE (192 tests) Descrito en el certificado / Described in the certificate 2003 12 0392 ED
- CVAB.CE.96 (96 tests)
- CVAB.CE.480 (480 tests)
- CVAB.CE.960 (960 tests)
- CVAB.CE.DB (192 tests)

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1.9. HCV Ab Confirmation

- CCONF.CE (12 tests) Descrito en el certificado / *Described in the certificate*
2005 09 0485 ED

1.10. HCV IgM

- CVM.CE (96 tests) Descrito en el certificado / *Described in the certificate*
2007 09 0532 ED

1.11. HCV Ab (Format 20)

- CVAB.CE.EG (192 tests) Descrito en el certificado / *Described in the certificate*
- CVAB.CE.EG.96 (96 tests) 2015 10 0842 ED
- CVAB.CE.EG.480 (480 tests)
- CVAB.CE.EG.960 (960 tests)

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 certificate*
2003 12 0394 ED

1.14. HDV IgM

- DIM.CE (96 tests) Descrito en el certificado / *Described in the certificate*
2003 12 0395 ED

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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 certificate* 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 certificate* 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 certificate* 2008 02 0539 ED

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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 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 certificate* 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 certificate* 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 certificate* 2015 01 0834 ED

3.2. DIA.CHEMILUX HBs Ag

- RASAG.CE (100 tests)

Descrito en el certificado / *Described in the certificate* 2015 10 0841 ED

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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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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

Firmado digitalmente por: Agencia Española de Medicamentos y Productos Sanitarios

Localizador: YD6VVGJ021

Fecha de la firma: 23/11/2018

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<u>Fabricante/Manufacturer:</u>
Nombre/Name: Dia. Pro Diagnostic Bioprobes S.r.l.
Dirección/Address: Via G. Carducci, 27 -20099- Sesto San Giovanni – Milano (Italy).
<u>Representante autorizado ante la UE/Authorized EU representative:</u>
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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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)

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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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3.1. 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
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productos sanitarios**

Fdo. M^a Jesús Lamas Díaz

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CMV IgG

**Enzyme Immunoassay for the
quantitative/qualitative determination of
IgG antibodies to Cytomegalovirus
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

Code: CMVG.CE
96 Tests

CMV IgG

A. INTENDED USE

Enzyme ImmunoAssay (ELISA) for the quantitative/qualitative determination of IgG antibodies to Cytomegalovirus in plasma and sera.

For "in vitro" diagnostic use only.

B. INTRODUCTION

Cytomegalovirus or CMV is an ubiquitous human pathogen, whose infection is particularly prevalent among children and young adults. Infections by CMV continue to be an important health problem in certain patient populations, such as newborns, graft recipients of solid organs or bone marrow and AIDS patients. In these groups CMV is a major cause of morbidity and mortality.

The detection of virus-specific IgG and IgM antibodies is of great value in the diagnosis of acute/primary virus infections or reactivation of a latent one, in the absence of typical clinical symptoms. Asymptomatic infections usually happen for CMV in apparently healthy individuals, during pregnancy and several diseases as a coinfective agent.

C. PRINCIPLE OF THE TEST

Microplates are coated with native Cytomegalovirus antigens, highly purified by sucrose gradient centrifugation and inactivated.

The solid phase is first treated with the diluted sample and IgG to Cytomegalovirus are captured, if present, by the antigens.

After washing out all the other components of the sample, in the 2nd incubation bound anti Cytomegalovirus 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 Cytomegalovirus IgG antibodies present in the sample. A Calibration Curve, calibrated against the 1st W.H.O international 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

12 strips x 8 microwells coated with highly purified and UV inactivated Cytomegalovirus 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 CMV IgG and titrated on WHO standard (proposed International Standard) ranging:

4ml CAL 1 = 0 WHO IU/ml
4ml CAL 2 = 0,5 WHO IU/ml
2ml CAL 3 = 1 WHO IU/ml
2ml CAL 4 = 2 WHO IU/ml
2ml CAL 5 = 4 WHO IU/ml
4ml CAL 6 = 8 WHO IU/ml.

Standards are calibrated against W.H.O proposed international standard for anti-CMV IgG (document BS/95.1814).

It contains human serum proteins, 2% casein, 10 mM Tris-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 CMV calibrated at 2 WHO IU/ml $\pm$ 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% 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 H₂O₂).

Note: To be stored protected from light as sensitive to strong illumination.

7. Sulphuric Acid: H₂SO₄ 0.3 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. Specimen Diluent: DILSPE

2x60ml/vial. It contains 2% casein, 10 mM Tris-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

E. MATERIALS REQUIRED BUT NOT PROVIDED

1. Calibrated Micropipettes (1000 μ l, 100 μ l and 10 μ l) 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

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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-borne 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-CMV IgG antibody concentration is expected to be higher than 8 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 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.

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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 350 µl/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 ±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 ≤ 10 nm; (b) absorbance range from 0 to ≥ 2.0; (c) linearity to ≥ 2.0; 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, 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 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 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 Calibrator 0 IU/ml and 100 µl Calibrator 0.5 IU/ml in duplicate, and 100 µl Calibrator 8 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 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/H ₂ O ₂	100 µl
3rd incubation	20 min
Temperature	r.t.
Sulphuric Acid	100 µl
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 and the calibrator 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
Calibrator 0 IU/ml (CAL1)	< 0.150 mean OD450nm value after blanking coefficient of variation < 30%
Calibrator 0.5IU/ml	OD450nm > OD450nm CAL1 + 0.100
Calibrator 8 IU/ml	OD450nm > 1.000
Control Serum	2 WHO 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 > 0.100 OD450nm	1. that the Chromogen/Sustrate solution has not got contaminated during the assay
Calibrator 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 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 0.5 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.
Calibrator 8 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.

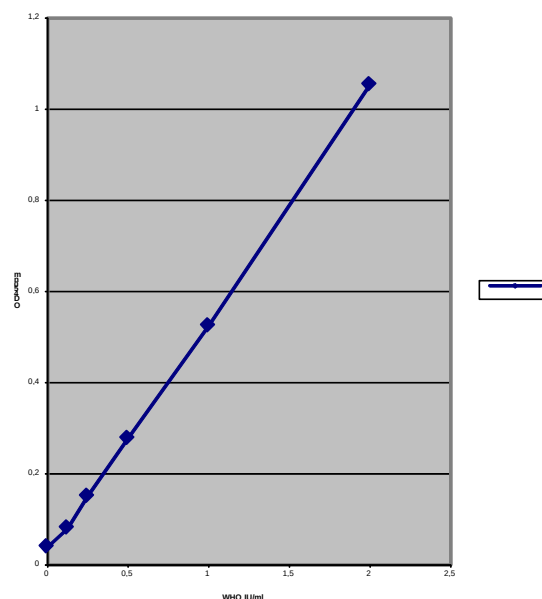
Should one of these problems have happened, after checking, report to the supervisor for further actions.

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 Cytomegalovirus 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 0,5 IU/ml and then check that the assay is valid.

Example of calculation:

The following data must not be used instead of real figures obtained by the user.

Calibrator 0 IU/ml: 0.035 – 0.045 OD450nm
Mean Value: 0.040 OD450nm
Lower than 0.150 – Accepted
Calibrator 0.5 IU/ml: 0.260 – 0.280 OD450nm
Mean Value: 0.270 OD450nm
Higher than Cal 0 + 0.100 – Accepted
Calibrator 8 IU/ml: 2.885 OD450nm
Higher than 1.000 – Accepted

Q. INTERPRETATION OF RESULTS

Samples with a concentration lower than 0.5 WHO IU/ml are considered negative for anti Cytomegalovirus IgG antibody. Samples with a concentration higher than 0.5 WHO IU/ml are considered positive for anti Cytomegalovirus IgG antibody. Particular attention in the interpretation of results has to be used in the follow-up of pregnancy for an infection of Cytomegalovirus 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 Cytomegalovirus infection a positive result (presence of IgG antibody > 0.5 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 with the European standard.

1. Limit of detection

The limit of detection of the assay (or analytical sensitivity) has been calculated by means of the 1st proposed international standard produced by the World Health Organization (WHO) for CMV IgG.

The limit of detection has been calculated as mean OD450nm Calibrator 0 WHO 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.

Mean OD450nm

WHO IU/ml	CMVG.CE Lot # 0303	CMVG.CE Lot # 0203	CMVG.CE Lot # 0103
2	1.053	1.101	1.098
1	0.524	0.498	0.559
0.5	0.277	0.268	0.271
0.25	0.150	0.169	0.161
0.125	0.080	0.091	0.087
Negative	0.039	0.035	0.040

The assay shows a limit of detection far better than 0.5 WHO IU/ml; however the interpretation of results is maintained at that value in order to safely monitor pregnancy and neonatal risk.

2. Diagnostic sensitivity:

The diagnostic sensitivity has been tested in an external performance evaluation study (University Hospital, Microbiology Department, Salamanca, Spain) on panels of samples classified positive by a kit US FDA approved. Positive samples from different stage of CMV infection were tested.

The value, obtained from the analysis of more than 300 specimens, has been > 98%.

In addition the seroconversion panel PT 901 produced by Boston Biomedical Inc., BBI, USA, has been tested. Results are reported below with reference to an European kit.

BBI Panel PTC 901

Member ID	CMVG.CE OD450nm	S/Co	BioMerieux VIDAS
01	0.071	0.2	Negative
02	0.043	0.1	Negative
03	0.057	0.2	Negative
04	0.046	0.1	Negative
05	0.086	0.3	Negative
06	1.002	3.2	Positive
07	1.442	4.6	Positive
08	1.630	5.2	Positive
09	1.770	5.6	Positive

Note: Cut-Off = 0.5 IU/ml = 0.316

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:

CMVG.CE: lot # 0303

Calibrator 0 IU/ml (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	0.073	0.073	0.077	0.074
Std.Deviation	0.010	0.010	0.009	0.010
CV %	13.3	14	12	13.1

Calibrator 0.5 IU/ml (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	0.316	0.292	0.309	0.306
Std.Deviation	0.027	0.015	0.020	0.020
CV %	8.4	5.1	6.3	6.6

Calibrator 8 IU/ml (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	3.262	3.137	3.210	3.203
Std.Deviation	0.126	0.065	0.147	0.113
CV %	3.9	2.1	4.6	3.5

CMVG.CE: lot # 0203

Calibrator 0 IU/ml (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	0.058	0.060	0.063	0.061
Std.Deviation	0.005	0.005	0.005	0.005
CV %	8.8	7.9	8.6	8.4

Calibrator 0.5 IU/ml (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	0.299	0.297	0.300	0.299
Std.Deviation	0.012	0.007	0.011	0.010
CV %	3.9	2.5	3.6	3.3

Calibrator 8 IU/ml (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	3.124	3.062	3.094	3.093
Std.Deviation	0.051	0.068	0.057	0.059
CV %	1.6	2.2	1.9	1.9

CMVG.CE: lot # 0103

Calibrator 0 IU/ml (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	0.064	0.062	0.067	0.064
Std.Deviation	0.005	0.005	0.006	0.005
CV %	7.9	8.3	8.2	8.1

Calibrator 0.5 IU/ml (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	0.314	0.300	0.296	0.303
Std.Deviation	0.031	0.019	0.012	0.021
CV %	10.0	6.5	4.0	6.8

Calibrator 8 IU/ml (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	2.729	2.688	2.700	2.705
Std.Deviation	0.109	0.067	0.109	0.095
CV %	4.0	2.5	4.0	3.5

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.

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
Bio**Probes**

EC DECLARATION OF CONFORMITY

MANUFACTURER	DIA.PRO DIAGNOSTIC BIOPROBES S.R.L. VIA G. CARDUCCI N° 27 – 20099 SESTO SAN GIOVANNI (MILANO) – ITALY
PRODUCT	CMV IgG CODE: CMVG.CE (96 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

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CMV IgM

**“Capture” Enzyme Immuno Assay
(ELISA) for the determination of IgM
antibodies to Cytomegalovirus
in human plasma and sera**

- for “in vitro” diagnostic use only -



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CMV IgM

A. INTENDED USE

Enzyme Immuno Assay (ELISA) for the determination of IgM class antibodies to Cytomegalovirus or CMV in human plasma and sera with the "capture" system.

The kit is intended for the follow-up of CMV infected patients and the monitoring of the risk of neonatal defects due to CMV infection during pregnancy.

For "in vitro" diagnostic use only.

B. INTRODUCTION

Cytomegalovirus or CMV is an ubiquitous human pathogen, whose infection is particular prevalent among children and young adults. Infections by CMV continue to be an important health problem in certain patient populations, such as newborns, graft recipients of solid organs or bone marrow and AIDS patients. In these groups CMV is a major cause of morbidity and mortality.

The detection of virus-specific IgG and IgM antibodies is of great value in the diagnosis of acute/primary virus infections or reactivation of a latent one, in the absence of typical clinical symptoms.

Asymptomatic infections usually happen for CMV in apparently healthy individuals, during pregnancy and several diseases as a co-infective agent.

Recently developed IgM capture ELISA's for CMV of new generation, taking advantage of CMV specific synthetic antigens, provide the clinician with a powerful and reliable diagnostic test, not affected by rheumatoid factor, for the monitoring of "risk" population.

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, in the 2nd incubation bound anti CMV IgM are detected by the addition of a complex composed of biotinylated CMV antigens and Streptavidine, labeled 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 Cytomegalovirus 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

Each kit contains sufficient reagents to carry out 96 tests.

1. Microplate: MICROPLATE

12 strips x 8 microwells coated with an affinity purified antibody mono specific to human IgM, 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 plasma negative for CMV IgM, 2% casein, 10 mM Tris-citrate

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 colorless.

3. Positive Control: CONTROL+

1x4.0 ml/vial. Ready to use control. It contains 1% human plasma positive for CMV IgM, 2% casein, 10 mM Tris-citrate buffer pH 6.0+/-0.1, 0.1% Tween 20, 0.09% sodium azide and 0.1% Kathon GC as preservatives.

Code colored with 0.01% green alimentary dye

4. Calibrator: CAL ...ml

N° 1 lyophilized vial. To be dissolved with EIA grade water as reported in the label. It contains anti CMV IgM positive human plasma calibrated on BBI Accurun # 146, 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 CMV Ag: AG CMV

N° 6 lyophilized vials. The vials contain lyophilized CMV reacting antigens biotinylated. 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 Streptavidine, labeled with HRP and diluted in a protein buffer containing 10 mM Tris HCl 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 HCl 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 colored 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-citrate 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 (H₂O₂) and 4% dimethylsulphoxide.

Note: To be stored protected from light as sensitive to strong illumination.

11. Sulphuric Acid: H₂SO₄ 0.3 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 (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 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.

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.

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 Cytomegalovirus 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 Immucocomplex 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, 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 $\pm 2\%$. Decontamination of spills or residues of kit components should also be carried out regularly.
2. The ELISA incubator has to be set at $+37^{\circ}\text{C}$ (tolerance of $\pm 0.5^{\circ}\text{C}$) 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 350 μl /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 $\leq 10 \text{ nm}$; (b) absorbance range from 0 to ≥ 2.0 ; (c) linearity to ≥ 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 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 $+37^{\circ}\text{C}$ 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.

- Check that the ELISA reader is turned on or ensure it will be turned on at least 20 minutes before reading.
- If using an automated work station, turn on, check settings and be sure to use the right assay protocol.
- Check that the micropipettes are set to the required volume.
- 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.

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:

- Dilute samples 1:101 by dispensing first 10 µl sample and then 1 ml Specimen Diluent into a dilution tube; mix gently on vortex.
- Place the required number of Microwells in the microwell holder. Leave the well in position A1 empty for the operation of blanking.
- 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!
- 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.
- 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.

- Wash the microplate with an automatic washer by delivering and aspirating as reported previously (section I.3).
- Pipette 100 µl Antigen/Conjugate Complex 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

- Incubate the microplate for **60 min at +37°C**.
- Wash microwells as in step 6.
- 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.

- 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.
- 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:

- 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.
- 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.
- 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

Controls&calibrator	100 ul
Samples diluted 1:101	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/H ₂ O ₂ 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	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 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)	< 0.150 OD450nm value after blanking coefficient of variation < 30%
Calibrator	S/Co > 0.75
Positive Control	> 0.750 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	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 < 0.75	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 < 0.750 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. 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:

$$\text{Cut-Off} = \text{NC} + 0.250$$

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 Cytomegalovirus.

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 CMV 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: 0.050 – 0.060 – 0.070 OD450nm
Mean Value: 0.060 OD450nm
Lower than 0.150 – Accepted

Positive Control: 1.850 OD450nm
Higher than 0.750 – 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 0.75 – 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 Cytomegalovirus due to the risk of severe neonatal malformations.
3. 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.
4. 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 Cytomegalovirus IgM detection, before taking any preventive medical action.
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

In absence of an international standard, Dia.Pro Diagnostic BioProbes s.r.l. has defined an internal gold Standard, prepared from a sample positive for CMV IgM. The dilution curves prepared with this material of reference are reported below:

OD450nm values

IGS dilution	CMVM.CE Lot # 0703	CMVM.CE Lot # 0603	CMVM.CE Lot # 0403
3X	1.262	1.155	1.109
6X	0.593	0.642	0.570
12X	0.210	0.277	0.225
24X	0.100	0.115	0.110
negative	0.015	0.029	0.030

In addition the preparation code Accurun n° 146, prepared by Boston Biomedica Inc., USA, for CMV IgM testing, was also used to generate limiting dilution curves, prepared as described above and reported in the next table

OD450nm values

Accurun # 146	CMVM.CE Lot # 0703	CMVM.CE Lot # 0603	CMVM.CE Lot # 0403
1X	0.653	0.596	0.603
2X	0.339	0.312	0.301
4X	0.165	0.159	0.148
8X	0.070	0.075	0.069
16X	0.020	0.031	0.027
Negative	0.013	0.015	0.012

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 Cytomegalovirus infection, confirmed by clinical symptoms and analysis.

An overall value > 98% has been found in the study conducted on a total number of more than 60 samples.

The Performance Panel coded PTC 202 and Seroconversion panel coded PTC 901, supplied by BBI, USA, have been also evaluated. Data are reported below:

Performance Panel PTC 202

Sample	CMVM.CE		Abbott EIA	Abbott IMx	Diamedix
ID	OD450nm	S/Co	S/Co	S/Co	S/Co
1	2.028	6.4	> 3.8	5.1	5.4
2	0.081	0.3	0.2	0.2	0.1
3	0.606	1.9	> 3.8	2.2	2.6
4	0.027	0.0	0.5	0.8	0.2
5	0.792	2.5	> 3.8	4.4	2.6
6	0.044	0.1	0.2	0.2	0.0
7	0.081	0.3	0.4	0.2	0.2
8	0.064	0.2	0.3	0.3	0.2
9	0.074	0.2	0.5	0.3	0.2
10	0.054	0.2	0.2	0.1	0.1
11	0.790	2.5	1.3	3.8	1.7
12	0.459	1.4	0.3	0.5	0.1
13	0.725	2.3	> 3.8	3.7	4.7
14	0.065	0.2	0.5	0.6	0.4
15	0.086	0.3	0.3	0.2	0.1
16	0.146	0.5	0.3	0.6	0.1
17	0.092	0.3	1.3	0.7	0.5
18	0.757	2.4	1.2	1.0	1.1

19	0.169	0.5	0.3	0.3	0.2
20	0.060	0.2	0.3	0.2	0.1
21	0.061	0.2	0.4	0.3	0.2
22	3.614	11.3	> 3.8	5.1	5.9
23	0.094	0.3	0.3	0.4	0.1
24	0.095	0.3	0.1	0.1	0.0
25	0.168	0.5	0.2	0.1	0.1

The table below reports the data obtained with the product against the values presented by BBI in its package insert of the Seroconversion Panel PTC 901 for Abbott EIA and bioMerieux VIDAS.

BBI Panel PTC 901

Member ID	CMVM.CE		REF bioMerieux VIDAS	REF Abbott IMx
	OD450nm	S/Co	S/Co	S/Co
01	0.046	0.1	0.3	0.2
02	0.048	0.2	0.3	0.2
03	0.045	0.1	0.3	0.2
04	0.048	0.2	0.3	0.2
05	0.459	1.4	2.7	4.8
06	2.521	7.9	3.2	6.0
07	2.424	7.6	3.0	5.8
08	1.693	5.3	2.8	5.5
09	1.508	4.7	2.6	5.0

3. Diagnostic specificity:

The diagnostic specificity has been determined 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 study conducted in a qualified external reference center on more than 400 total samples 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:

It has been calculated on three samples, a negative, a low positive and a positive, examined in 16 replicates in three separate runs. Results are reported as follows:

CMVM.CE: lot # 0703

Negative (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	0.036	0.033	0.034	0.034
Std.Deviation	0.003	0.003	0.002	0.003
CV %	9.0	9.8	6.3	8.4

Low reactive (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	0.727	0.723	0.709	0.720
Std.Deviation	0.022	0.029	0.045	0.032
CV %	3.0	3.9	6.3	4.4

High reactive (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	2.279	1.980	2.131	2.130
Std.Deviation	0.220	0.186	0.207	0.204
CV %	9.7	9.4	9.7	9.6

CMVM.CE: lot # 0603

Negative (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	0.027	0.034	0.032	0.031
Std.Deviation	0.005	0.006	0.006	0.006
CV %	17.4	17.8	19.9	18.4

Low reactive (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	0.617	0.619	0.623	0.620
Std.Deviation	0.033	0.040	0.046	0.039
CV %	5.4	6.4	7.3	6.4

High reactive (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	1.913	1.890	1.895	1.899
Std.Deviation	0.051	0.056	0.047	0.051
CV %	2.7	3.0	2.5	2.7

CMVM.CE: lot # 0403

Negative (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	0.037	0.038	0.037	0.037
Std.Deviation	0.003	0.005	0.004	0.004
CV %	8.7	12.8	9.6	10.4

Low reactive (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	0.637	0.644	0.634	0.638
Std.Deviation	0.039	0.029	0.031	0.033
CV %	6.2	4.5	4.9	5.2

High reactive (N = 16)

Mean values	1st run	2nd run	3 rd run	Average value
OD 450nm	1.962	2.061	2.167	2.063
Std.Deviation	0.019	0.034	0.066	0.039
CV %	1.0	1.6	3.0	1.9

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 CMV 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 µl concentrated Enzymatic Conjugate are diluted in 500 µl Antigen Diluent and mixed gently on vortex. Do not use any lyophilized vial of CMV 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, strongly recommended), 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 CMV and a crossreaction with the enzymatic tracer 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 1.2 the sample is considered a **true positive**. The reactivity of the sample is in fact dependent on the specific presence of CMV 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	< 1.2
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 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



0318



Dia.Pro
Diagnostic
Bio**Probes**

EC DECLARATION OF CONFORMITY

MANUFACTURER	DIA.PRO DIAGNOSTIC BIOPROBES S.R.L. VIA G. CARDUCCI N° 27 – 20099 SESTO SAN GIOVANNI (MILANO) – ITALY
PRODUCT	CMV IgM CODE: CMVM.CE (96 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

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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 -



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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 hlgG 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 CAL5 = 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 H₂O₂).

Note: To be stored protected from light as sensitive to strong illumination.

6. Sulphuric Acid: H2SO4 0.3M

1x15ml/vialIt contains 0.3 M H₂SO₄ 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-borne 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 ±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 ≤ 10 nm; (b) absorbance range from 0 to ≥ 2.0; (c) linearity to ≥ 2.0; 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, 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.

- Incubate the microplate for **60 min at +37°C**.
- Wash microwells as in step 5.
- 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.

- 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.
- 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:

- 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.
- Place the required number of Microwells in the microwell holder. Leave A1 well empty for the operation of blanking.
- 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.
- 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.

- Wash the microplate with an automatic washer as reported previously (section I.3).
- 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.

- Incubate the microplate for **60 min at +37°C**.
- Wash microwells as in step 5.
- 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.

- 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.
- 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:

- 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.
- 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/H ₂ O ₂	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.

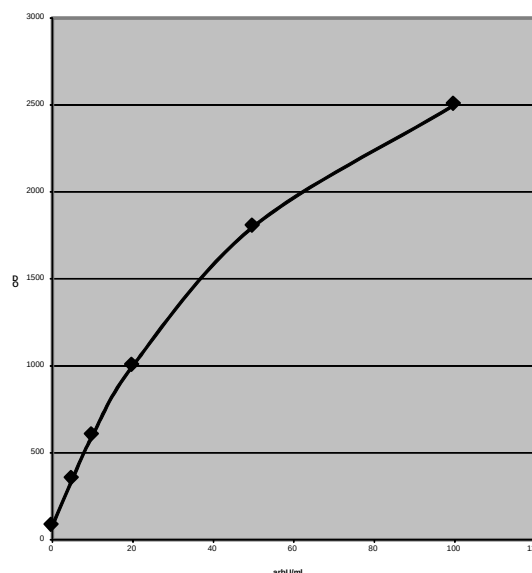
P. RESULTS

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 of 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





Dia.Pro
Diagnostic
Bio**Probes**

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
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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 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 (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 H₂O₂).

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 H₂SO₄ 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-borne 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-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 sévère 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 réutilisation.

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 ±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 ≤ 10 nm; (b) absorbance range from 0 to ≥ 2.0; (c) linearity to ≥ 2.0; 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, 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.

- Place the required number of Microwells in the microwell holder. Leave the A1 and B1 empty for the operation of blanking.
- Dispense 100 µl of Calibrators and 100 µl Control Serum in duplicate. Then dispense 100 µl of diluted samples in each properly identified well.
- 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.

- Wash the microplate with an automatic washer as reported previously (section I.3).
- 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.

- Incubate the microplate for **60 min at +37°C**.
- Wash microwells as in step 5.
- 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.

- 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.
- 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:

- 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.
- Place the required number of Microwells in the microwell holder. Leave A1 well empty for the operation of blanking.
- 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.
- 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.

- Wash the microplate with an automatic washer as reported previously (section I.3).
- 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.

- Incubate the microplate for **60 min at +37°C**.
- Wash microwells as in step 5.
- 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.

- 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.
- 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:

- 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.
- 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(*)	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/H ₂ O ₂	100 µl
3rd incubation	20 min
Temperature	r.t.
Sulphuric Acid	100 µl
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/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 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.

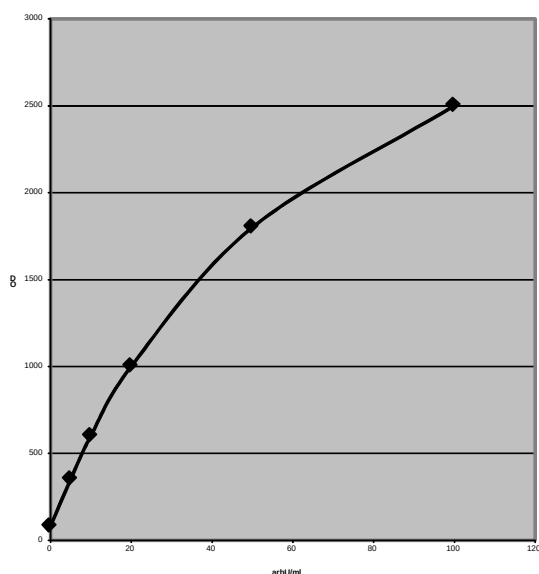
P. RESULTS

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 of 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 specificity 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





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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	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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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 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 (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 H₂O₂).

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 H₂SO₄ 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-borne 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 freeze/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 ≤ 10 nm; (b) absorbance range from 0 to ≥ 2.0 ; (c) linearity to ≥ 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 μ l Sample Diluent + 10 μ l 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 μ l of Calibrators and 100 μ l Control Serum in duplicate. Then dispense 100 μ l 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.

- Wash the microplate with an automatic washer as reported previously (section I.3).
- 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.

- Incubate the microplate for **60 min at +37°C**.
- Wash microwells as in step 5.
- 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.

- 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.
- 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:

- 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.
- Place the required number of Microwells in the microwell holder. Leave A1 well empty for the operation of blanking.
- 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.
- 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.

- Wash the microplate with an automatic washer as reported previously (section I.3).
- 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.

- Incubate the microplate for **60 min at +37°C**.
- Wash microwells as in step 5.
- 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.

- 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.
- 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:

- 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.
- 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/H ₂ O ₂	100 µl
3rd incubation	20 min
Temperature	r.t.
Sulphuric Acid	100 µl
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
S = Sample
CAL = Calibrators

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.

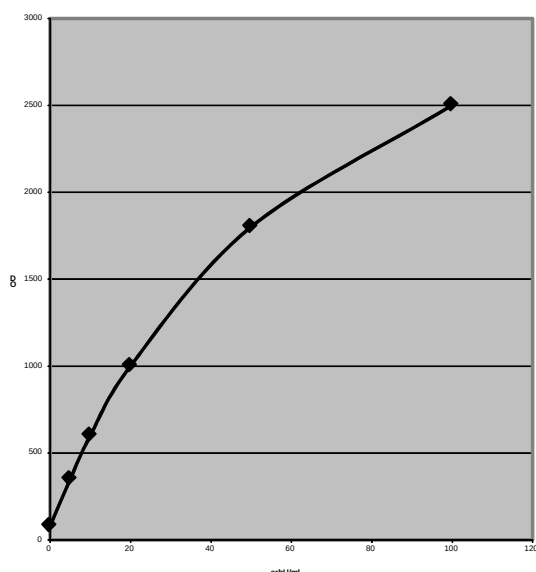
P. RESULTS

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 of 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 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
Via G. Carducci n° 27 – Sesto San Giovanni (MI) – Italy



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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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 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 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-IgM 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 biotinylated 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 ...m

1 vial. Lyophilized. Contains fetal bovine serum proteins, human anti EBV VCA IgM antibodies at $20 \pm 20\%$ 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 biotinylated 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 H₂O₂.

Note: To be stored protected from light as sensitive to strong illumination.

9. Sulphuric Acid: H₂SO₄ 0.3 M

1x15ml/vial. Contains 0.3 M H₂SO₄ 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/H₂O₂) 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 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.
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 ±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/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 ±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 ≤ 10 nm; (b) absorbance range from 0 to ≥ 2.0; (c) linearity to ≥ 2.0; 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 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/H₂O₂ 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/H₂O₂ 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	100 ul
Control Serum (*)	100 ul
Samples diluted 1:101	100 ul
1st incubation	60 min
Temperature	+37°C
Enzyme Conjugate	100 ul
2nd incubation	60 min
Temperature	+37°C
TMB/H ₂ O ₂ 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.

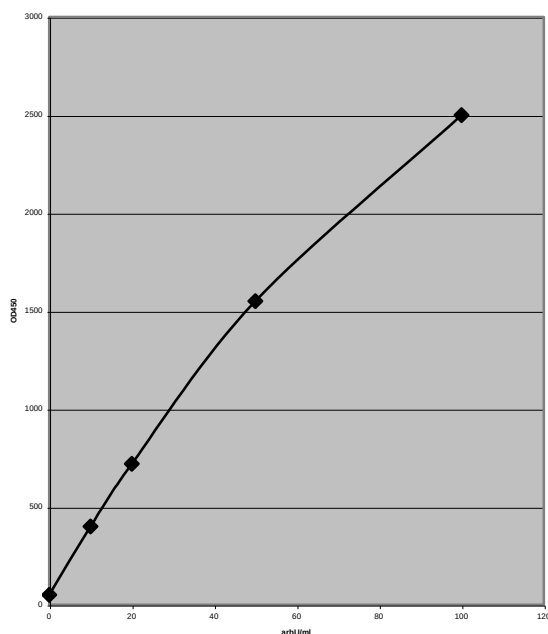
Anyway, if all other parameters (Blank, CAL1, CAL2, CAL 5), match the established requirements, the test may be considered valid.

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 of 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 arbU/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 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 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 Community.
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 than 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
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Dia.Pro
Diagnostic
Bio**Probes**

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
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SIGNATURE Legal Representative Dr.ssa Fiorenza Scozzesi	

Rev: 05/2018



Dia.Pro
Diagnostic
Bio**P**robes

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
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HP Ag

**Enzyme Immunoassay for the
qualitative/quantitative determination
of Helicobacter pylori Antigen
in human stools**

- for “in vitro” diagnostic use only -



DIA.PRO

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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 (H₂O₂).

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 H₂SO₄ 0.3 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-borne 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 $\pm 0.5^\circ\text{C}$) 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 ≤ 10 nm; (b) absorbance range from 0 to ≥ 2.0 ; (c) linearity to ≥ 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.

- Dilute all the content of the 20x concentrated Wash Solution as described above.
- Dissolve the Calibrator Set as described above.
- Allow all the other components to reach room temperature (about 1 hr) and then mix as described.
- 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.
- Check that the ELISA reader has been turned on at least 20 minutes before reading.
- If using an automated workstation, turn it on, check settings and be sure to use the right assay protocol.
- Check that the micropipettes are set to the required volume.
- 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 are available: a quantitative method able to provide a quantification of HP Ag in the specimen and a qualitative method.

A. Quantitative Assay

- 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.
- Pipette 100 µl Calibrators in duplicate into the calibration wells (see the example of dispensation reported below).
- 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).
- 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.

- 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.

- When the first incubation is over, wash the microwells as previously described (section I.3)
- 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.

- Pipette 100 µl Sulphuric Acid into all the wells to stop the enzymatic reaction, using the same pipetting sequence as in step 6.

- 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

- 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.
- 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.
- 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.

- 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.

- When the first incubation is over, wash the microwells as previously described (section I.3)
- 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.

- Pipette 100 µl Sulphuric Acid into all the wells to stop the enzymatic reaction, using the same pipetting sequence as in step 6.
- 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 OD450nm/ 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:

$$\text{Cut-Off} = (\text{CAL 0} + \text{CAL 0.1}) / 2$$

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):

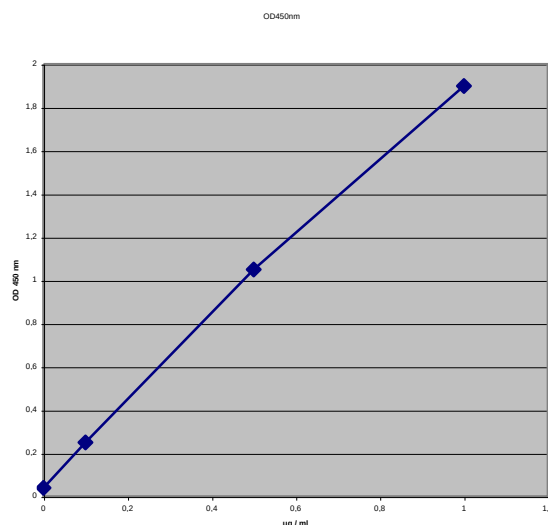
Note: The following data must not be used instead of real figures obtained by the user.

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

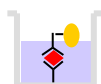
Add 100 µl Calibrators, samples and conjugate to the plate and then incubate for 120 min at +37°C



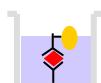
Wash as described in the proper section



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





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

Localizador: 6XZPBYWEFC

Fecha de la firma: 19/11/2018

Puede comprobar la autenticidad del documento en la aplicación Localizador de la Web de la AEMPS

CORREO ELECTRÓNICO

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Página 1 de 2

ORGANISMO NOTIFICADO 0318

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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

Localizador: 6XZPBYWEFC

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Dia.Pro
Diagnostic
Bio**Probes**

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	

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

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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 H₂SO₄ 0.3 M

1x25ml/bottle It contains 0.3 M H₂SO₄ 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-borne 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 0.2-0.8µ 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 350µl/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 ±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 ≤ 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.

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 lyophilized 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.

- Pipette 100 µl 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.
- 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:

- 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.
- 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.
- 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 µl
Controls and calibrator	150 µl
Samples	150 µl
1st incubation	60 min
Temperature	+37°C
Wash step	4-5 cycles
Conjugate # 1	150 µl
2nd incubation	30 min
Temperature	+37°C
Conjugate # 2	100 µl
3rd incubation	30 min
Temperature	+37°C
Wash step	4-5 cycles
TMB/H ₂ O ₂	200 µl
4th incubation	30 min
Temperature	r.t.
Sulphuric Acid	100 µl
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):

$$NC + 0.125 = \text{Cut-Off (Co)}$$

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. Repeatedly 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 of 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 device 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 device 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 device 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 device shows a sensitivity ≤ 2 IU/ml as required by CTS:2009.

**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

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 S/Co	Lot # 0706 S/Co	Lot # 0906 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 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 O), 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 4 th Generation HIV Ab&Ag	IVAB.CE 3 rd 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 repeatedly 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.

Manufacturer:
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Via G. Carducci n° 27 – Sesto San Giovanni (MI) – Italy



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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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
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 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 $\pm$ 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 H₂O₂).

Note: To be stored protected from light as sensitive to strong illumination.

7. Sulphuric Acid: H₂SO₄ 0.3 M

1x15ml/vial. It contains 0.3 M H₂SO₄ 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 ≤ 10 nm; (b) absorbance range from 0 to ≥ 2.0 ; (c) linearity to ≥ 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 μ l Sample Diluent and then 10 μ l 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 (*)	100 µl
Samples diluted 1:101	100 µl
1 st 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
2 nd incubation	60 min
Temperature	+37°C
Wash step	n° 5 cycles with 20" of soaking OR n° 6 cycles without soaking
TMB/H ₂ O ₂	100 µl
3 rd incubation	20 min
Temperature	r.t.
Sulphuric Acid	100 µl
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 $\pm$ 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

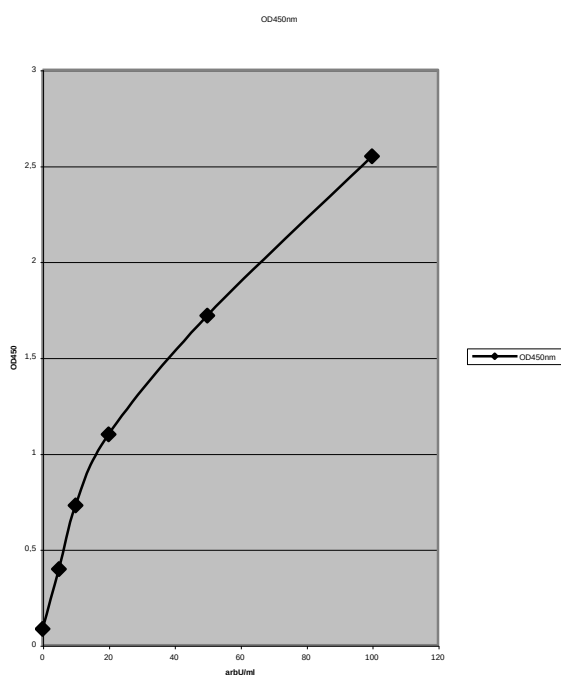
P.1 Quantitative method

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 of 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	3 rd 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	3 rd 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	3 rd 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





Dia.Pro
Diagnostic
Bio**Probes**

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
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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 -



DIA.PRO

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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 (H₂O₂) and 4% dimethylsulphoxide.

Note: To be stored protected from light as sensitive to strong illumination.

11. Sulphuric Acid: H₂SO₄ 0.3 M

1x15ml/vial. It contains 0.3 M H₂SO₄ 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 Immucplex 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 ≤ 10 nm; (b) absorbance range from 0 to ≥ 2.0 ; (c) linearity to ≥ 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.

- 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.

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:

- Dilute samples 1:101 by dispensing first 10 µl sample and then 1 ml Specimen Diluent into a dilution tube; mix gently on vortex.
- Place the required number of Microwells in the microwell holder. Leave the well in position A1 empty for the operation of blanking.
- 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!
- 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.
- 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.

- Wash the microplate with an automatic washer as reported previously (section I.3).
- 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.

- Incubate the microplate for **60 min at +37°C**.
- Wash microwells as in step 6.
- 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.

- 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.
- 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:

- 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.
- 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(*)	100 ul
Samples diluted 1:101	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:

$$\text{Cut-Off} = \text{NC} + 0.250$$

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

$$\text{Cut-Off} = 0.110 + 0.250 = 0.360$$

Calibrator: 1.000 - 0.900 OD450nm

Mean value: 0.950 OD450nm

S/Co higher than 1.2 – Accepted S/Co = 2.6

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.

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	3 rd 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	3 rd 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	3 rd 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	3 rd 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	3 rd 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	3 rd 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	3 rd 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	3 rd 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

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%.

High reactive (N = 16)

Mean values	1st run	2nd run	3 rd 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





Dia.Pro
Diagnostic
Bio**Probes**

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)

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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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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 H₂O₂).

Note: To be stored protected from light as sensitive to strong illumination.

7. Sulphuric Acid: H₂SO₄ 0.3 M

1x15ml/vial. It contains 0.3 M H₂SO₄ 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-borne 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 ±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 ≤ 10 nm; (b) absorbance range from 0 to ≥ 2.0; (c) linearity to ≥ 2.0; 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, 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 (*)	100 µl
Samples diluted 1:101	100 µl
1 st 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
2 nd incubation	60 min
Temperature	+37°C
Wash step	n° 5 cycles with 20" of soaking OR n° 6 cycles without soaking
TMB/H ₂ O ₂	100 µl
3 rd incubation	20 min
Temperature	r.t.
Sulphuric Acid	100 µl
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	S 1	S 8	S 16									
H	S 2	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 $\pm$ 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

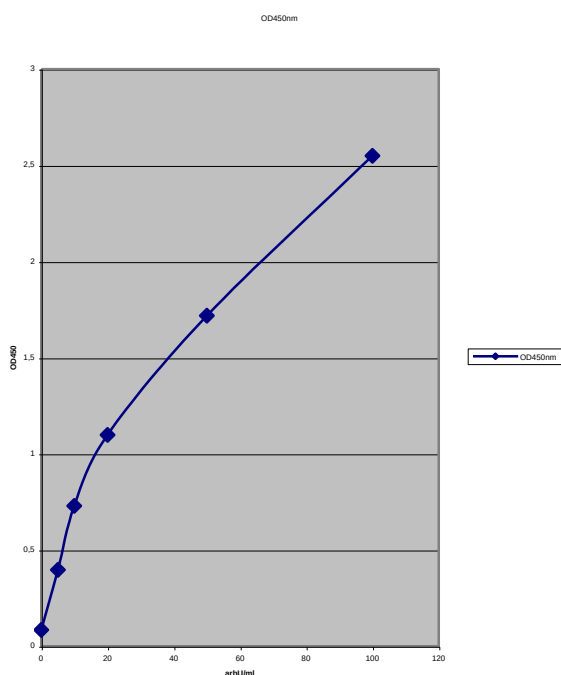
P.1 Quantitative method

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 of 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	3 rd 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	3 rd 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	3 rd 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





Dia.Pro
Diagnostic
Bio**P**robes

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
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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 -



DIA.PRO

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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 device 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 (H₂O₂) and 4% dimethylsulphoxide.

Note: To be stored protected from light as sensitive to strong illumination.

11. Sulphuric Acid: H₂SO₄ 0.3 M

1x15ml/vial. It contains 0.3 M H₂SO₄ 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 Immunocomplex 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 ±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 ≤ 10 nm; (b) absorbance range from 0 to ≥ 2.0; (c) linearity to ≥ 2.0; 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, 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.

- Dissolve the Calibrator as described above and gently mix.
- Allow all the other components to reach room temperature (about 1 hr) and then mix gently on vortex all liquid reagents.
- 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.
- Check that the ELISA reader is turned on or ensure it will be turned on at least 20 minutes before reading.
- If using an automated work station, turn on, check settings and be sure to use the right assay protocol.
- Check that the micropipettes are set to the required volume.
- 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.

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:

- Dilute samples 1:101 by dispensing first 10 µl sample and then 1 ml Specimen Diluent into a dilution tube; mix gently on vortex.
- Place the required number of Microwells in the microwell holder. Leave the well in position A1 empty for the operation of blanking.
- 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 !
- 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.
- 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.

- Wash the microplate with an automatic as reported previously (section I.3).
- 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.

- Incubate the microplate for **60 min at +37°C**.
- Wash microwells as in step 6.
- 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.

- 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.
- 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:

- Ensure that no finger prints are present on the bottom of the microwell before reading. Finger prints could generate false positive results on reading.
- 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 (*)	100 ul
Samples diluted 1:101	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:

$$\text{Cut-Off} = \text{NC} + 0.250$$

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	3 rd 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	3 rd 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	3 rd 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	3 rd 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	3 rd 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	3 rd 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	3 rd 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	3 rd 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	3 rd 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 µl concentrated Enzymatic Conjugate are diluted in 500 µl 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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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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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 proteínas 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 sustrato cromogénico. En presencia del conjugado, el sustrato 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 proteínas 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 8°C.

2. Control Negativo: CONTROL

1x4.0 ml/vial. Listo para el uso. Contiene 1% de proteínas del suero humano, 2% de caseína, tampón Tris 10 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 Tris 10 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 proteínas 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 Diluyente 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 (HRP) diluido 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. Diluyente 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. Diluyente 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 (H₂O₂) 0.02% así como dimetilsulfóxido al 4%.

Nota: Evitar la exposición a la luz, la sustancia es fotosensible.

11. Ácido Sulfúrico: H₂SO₄ 0.3M

1x15ml/vial. Contiene solución de H₂SO₄ 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 37°C (+/-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 sustrato a la luz y las vibraciones de la mesa de trabajo durante el ensayo.
5. Conservar el estuche a temperaturas entre 2-8 °C, 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 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.
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 conservación. 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 2-8°C. 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 cuidadosamente con el vórtex antes de usar.

Nota: Para preservar la reactividad se recomienda mantenerla congelada en alícuotas a -20°C . 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 8°C .

Inmunocomplejo Ag/Ab:

Proceder cuidadosamente según se indica:

1. Disolver el contenido de un vial liofilizado utilizando 1.9 ml de Diluyente 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.

Diluyente 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 $\pm 2\%$. Deben descontaminarse periódicamente los residuos de los componentes del estuche.
2. La incubadora de ELISA debe ser ajustada a 37°C ($\pm 0.5^{\circ}\text{C}$ 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 $\pm 5\%$.
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 $\leq 10\text{nm}$ b) Rango de absorbancia de 0 a ≥ 2.0 , c) Linealidad ≥ 2.0 , reproducibilidad $\geq 1\%$. 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 pesquaje 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 37°C 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 Diluyente 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 diluidas, 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 Diluyente de Muestras + 10 µl de muestra) en una segunda plataforma de dilución. Programar el equipo para aspirar primeramente 100 µl de Diluyente 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 Diluyente 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 diluidas 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 sección 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 (*)	100 ul
Muestras diluidas 1:101	100 ul
1^{ra} incubación	60 min
Temperatura	+37°C
Lavado	5 ciclos con 20"de remojo o 6 ciclos sin remojo
Inmunocomplejo	100 ul
2^{da} incubación	60 min

Temperatura	+37°C
Lavado	5 ciclos con 20"de remojo o 6 ciclos sin remojo
Mezcla TMB/H ₂ O ₂	100 ul
3 ^{ra} 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 Obligatorio 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 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 (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:

$$\text{Valor de corte} = \text{CN} + 0.250$$

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:

- 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.
- 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.
- 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.
- Antes de emitir un resultado positivo, cada muestra reactiva debe someterse al examen de confirmación reportado en la sección T, lo cual permite una correcta interpretación de los resultados ya que descarta los falsos positivos.
- Cuando se transmiten los resultados de la prueba, del laboratorio a otras instalaciones, debe ponerse mucha atención para evitar el traslado de datos erróneos.
- 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 aprobado 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 (citrate, EDTA y heparina) y suero humano 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 débilmente 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	1ª corrida	2ª corrida	3ª 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	1ª corrida	2ª corrida	3ª 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	1ª corrida	2ª corrida	3ª 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	1ª corrida	2ª corrida	3ª 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	1ª corrida	2ª corrida	3ª 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	1ª corrida	2ª corrida	3ª 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	1ª corrida	2ª corrida	3ª 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	1ª corrida	2ª corrida	3ª 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	1ª corrida	2ª corrida	3ª 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 precisión 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 Diluyente de Antígeno (ej: 25 µl de Conjugado concentrado en 500 µl de Diluyente 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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Fabricante:
Dia.Pro Diagnostic Bioprobes S.r.l.
Via G. Carducci n° 27 – Sesto San Giovanni (Milán) – Italia





Dia.Pro
Diagnostic
Bio**Probes**

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)

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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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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 Togaviridae.

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 $\pm$ 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/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.

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 H₂O₂) and 4% dimethylsulphoxide.

Note: To be stored protected from light as sensitive to strong illumination.

7. Sulphuric Acid: H₂SO₄ 0.3 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. 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 ±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 ≤ 10 nm; (b) absorbance range from 0 to ≥ 2.0; (c) linearity to ≥ 2.0; 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, 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.

- 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.
- 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:

- 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.
- 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.
- 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.

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%

N. ASSAY SCHEME

Method	Operations
Calibrators & Control	100 µl
Samples diluted 1:101	100 µl
1 st incubation	60 min
Temperature	+37°C
Wash step	4-5 cycles
Enzyme conjugate	100 µl
2 nd incubation	60 min
Temperature	+37°C
Wash step	4-5 cycles
TMB/H ₂ O ₂	100 µl
3 rd incubation	20 min
Temperature	r.t.
Sulphuric Acid	100 µl
Reading OD	450nm

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 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.

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:

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.

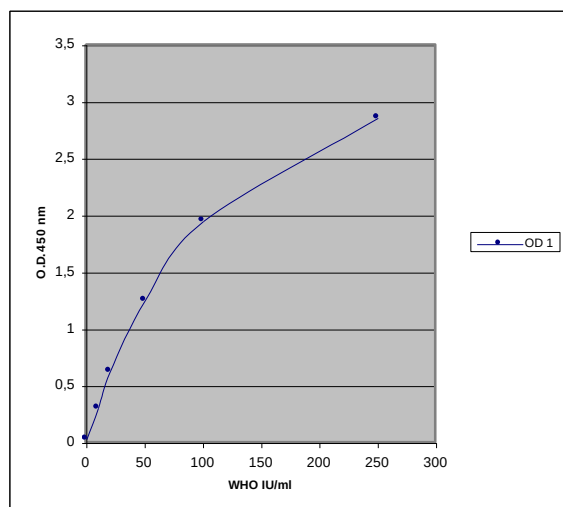
Should one of these problems have happened, after checking, report to the supervisor for further actions.

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 of 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	3 rd 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	3 rd 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	3 rd 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	3 rd 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	3 rd 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	3 rd 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	3 rd 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	3 rd 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	3 rd 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 essential 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



0318



Dia.Pro
Diagnostic
Bio**Probes**

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



Dia.Pro
Diagnostic
Bio**Probes**

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
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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 -



DIA.PRO

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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 infectiosum. It is typical 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 hlgG 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 H₂O₂) and 4% dimethylsulphoxide.

Note: To be stored protected from light as sensitive to strong illumination.

7. Sulphuric Acid: H₂SO₄ 0.3 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. 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.

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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.

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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 ≤ 10 nm; (b) absorbance range from 0 to ≥ 2.0 ; (c) linearity to ≥ 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 µl 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(*)	100 µl
Samples diluted 1:101	100 µl
1 st 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
2 nd incubation	60 min
Temperature	+37°C
Wash step	n° 5 cycles with 20" of soaking OR n° 6 cycles without soaking
TMB/H ₂ O ₂	100 µl
3 rd 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	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;
coefficient of variation > 30%	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 $\pm$ 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.

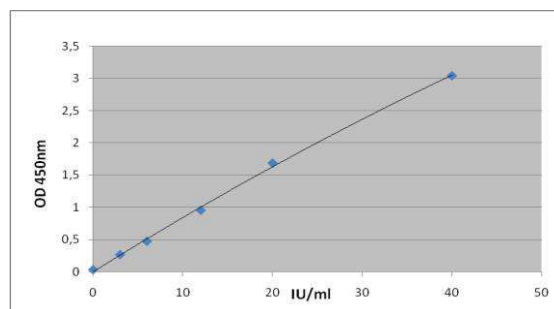
P. RESULTS

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:

$$\text{Cut-Off (Co)} = \text{CAL5/5}$$

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 of 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 $\geq 98\%$ 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 $\geq 98\%$ was observed.

These findings are summarized in the following table.

Sensitivity	$\geq 98\%$
Specificity	$\geq 98\%$

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	3 rd 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	3 rd 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	3 rd 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	3 rd 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	3 rd 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	3 rd 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





Dia.Pro
Diagnostic
Bio**Probes**

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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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 infectiosum. It is typical 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 H₂O₂).

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 H₂SO₄ 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

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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.

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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 ≤ 10 nm; (b) absorbance range from 0 to ≥ 2.0 ; (c) linearity to ≥ 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.

- 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.

- 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.
- Place the required number of Microwells in the microwell holder. Leave A1 well empty for the operation of blanking.
- Not dispense Neutralizing Reagent in A1 used for blanking operations and in the wells used for the Controls and the Calibrator.
- Dispense 50 µl Neutralizing Reagent in all the samples wells.
- Dispense 100 µl of Negative Control in duplicate, 100 µl of Positive Control in single, 100 µl of Calibrator in duplicate and 100 µl of diluted samples in each properly identified well.
- 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.

- Wash the microplate with an automatic as reported previously (section I.3).
- 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.

- Incubate the microplate for **60 min at +37°C**.
- Wash microwells as in step 6.
- 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.

- 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.
- 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:

- 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.

- 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 µl
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/Substrate 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:

$$\text{Cut-Off} = \text{NC} + 0.250$$

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 of 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

$$\text{Cut-Off} = 0.100 + 0.250 = 0.350$$

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

Positive Control (N = 16)				
Mean values	1st run	2nd run	3 rd 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.

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 $\geq 98\%$ 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 $\geq 98\%$ was observed.

These findings are summarized in the following table.

Sensitivity	$\geq 98\%$
Specificity	$\geq 98\%$

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	3 rd 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	3 rd 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	3 rd 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	3 rd 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	3 rd 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

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
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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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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°C.

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 grade 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 (H₂O₂) 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 H₂SO₄ 0.3 M

1x15ml/vial. Contains 0.3 M H₂SO₄ 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 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.
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 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.
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 0.2-0.8µ 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 ±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/calibrator and reference panels, before using the kit for routine laboratory tests. Usually 4-5 washing cycles (aspiration + dispensation of 350 µl/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 ±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 ≤ 10 nm; (b) absorbance range from 0 to ≥ 2.0; (c) linearity to ≥ 2.0; 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.

- 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.
- 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

- Check the expiration date of the kit printed on the external label (primary container). Do not use if expired.
- 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.
- Dilute all the content of the 20x concentrated Wash Solution as described above.
- Dissolve the Calibrator as described above and gently mix.
- Allow all the other components to reach room temperature (about 1 hr) and then mix gently on vortex all liquid reagents.
- 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.
- Check that the ELISA reader is turned on or ensure it will be turned on at least 20 minutes before reading.
- If using an automated work station, turn on, check settings and be sure to use the right assay protocol.
- Check that the micropipettes are set to the required volume.
- 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.

- Place the required number of strips in the plastic holder and carefully identify the wells for controls, calibrator and samples.
- Leave the A1 well empty for blanking purposes.
- Dispense 50 µl Specimen Diluent into all the control and sample wells.
- Pipette 50 µl of the Negative Control in triplicate, 50 µl of the Calibrator in duplicate and then 50 µl of the Positive Control in single. Then dispense 50 µl of each of the samples.
- 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.

- When the first incubation is finished, wash the microwells as previously described (section I.3)
- Pipette 100 µl Enzyme Conjugate in all the wells, except A1; incubate the microplate for **60 min at +37°C**.

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.

- When the second incubation is finished, wash the microwells as previously described (section I.3)
- 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.

- Incubate the microplate protected from light at **room temperature (18-24°C) for 20 minutes**. Wells dispensed with negative control and negative samples will turn from clear to blue (competitive method).
- 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.
- 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:

- 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.
- 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 µl
Controls&calibrator and samples	50 µl
1st incubation	60 min
Temperature	+37°C
Wash	n° 4-5
Enzyme Conjugate	100 µl
2nd incubation	60 min
Temperature	+37°C
Wash	n° 4-5
TMB/H2O2 mix	100 µl
3rd incubation	20 min
Temperature	r.t.
Sulphuric Acid	100 µl
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:

$$\text{Cut-Off} = (\text{NC} + \text{PC}) / 5$$

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 of 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

$$\text{Cut-Off} = (2.100 + 0.100) / 5 = 0.440$$

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, hemolyzed, 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	3 rd 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	3 rd 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	3 rd 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	3 rd 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	3 rd 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	3 rd run	Average value
OD 450nm	0.193	0.190	0.199	0.194
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.

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CE
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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 -



DIA.PRO

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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 hepatocytes 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 H₂O₂.

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 H₂SO₄ 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-borne 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 $\pm 2\%$.
2. The ELISA incubator has to be set at $+37^{\circ}\text{C}$ (tolerance of $\pm 0.5^{\circ}\text{C}$) 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 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+H₂O₂) 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 $+37^{\circ}\text{C}$ 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.

- Dilute samples **1:101** dispensing 1 ml Sample Diluent into a disposable tube and then 10 µl sample; mix on vortex before use. Do not dilute the Calibrators and the dissolved Control Serum as they are ready-to-use.
- Leave the A1+B1 wells empty for blanking purposes.
- 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.
- 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.

- When the first incubation is finished, wash the microwells as previously described (section I.3)
- 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.

- When the second incubation is finished, wash the microwells as previously described (section I.3)
- 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.

- 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.
- 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.
- 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

- Place the required number of strips in the plastic holder and carefully identify the wells for standards and samples.
- Dilute samples **1:101** dispensing 1 ml Sample Diluent into a disposable tube and then 10 µl sample; mix on vortex before use. Do not dilute the Calibrators as they are ready-to-use.
- Leave the A1 well empty for blanking purposes.
- 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.
- 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.

- When the first incubation is finished, wash the microwells as previously described (section I.3)
- 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.

- When the second incubation is finished, wash the microwells as previously described (section I.3)
- 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.

- 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.
- 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.
- 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:

- Ensure that no finger prints are present on the bottom of the microwell before reading. Finger prints could generate false positive results on reading.
- 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.
- 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 µl
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 µl
2nd incubation	60 min
Temperature	+37°C
Washing steps	n° 5 cycles with 20" of soaking OR n° 6 cycles without soaking
Chromogen/Substrate	100µl
3rd incubation	20 min
Temperature	room
Sulphuric Acid	100 µl
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

	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.

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	< 0.150 OD450nm after blanking
coefficient of variation	< 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 0, 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

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.

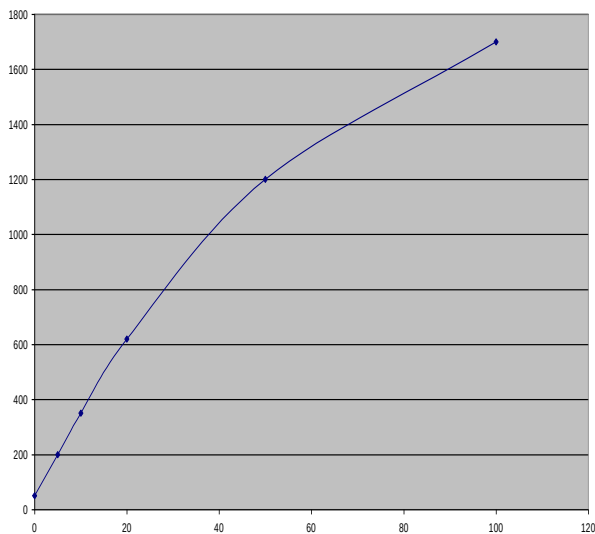
P. RESULTS

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 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:

- 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.
- Interpretation of results should be done under the supervision of the laboratory supervisor to reduce the risk of judgement errors and misinterpretations.
- A positive result is indicative of HBV infection and therefore the patient should be treated accordingly.
- When test results are transmitted from the laboratory to another facility, attention must be paid to avoid erroneous data transfer.
- 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 :

- the HBcIgM reference preparation supplied by Paul Erlich Institute, Germany (HBc-Referenzserum-IgM 84), on which the Standard Curve has been calibrated.
- 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, has 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, hemolyzed, 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	3 rd 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	3 rd 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	3 rd 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	3 rd 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	3 rd 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	3 rd 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	3 rd 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	3 rd 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	3 rd 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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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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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 molécula 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 convalescentes. 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 4°C.

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. Diluyente de muestras :DILSPE

2x60.0 ml/vial. Solución tamponada para disolver las muestras. Contiene tampón Tris 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 (H₂O₂) 0.02%.

Nota: Evitar la exposición a la luz, ya que la sustancia es fotosensible.

8. Ácido Sulfúrico: H₂SO₄ 0.3 M

1x15ml/vial. Contiene solución de H₂SO₄ 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 37°C (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 (aguja). 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 sustrato a la luz y las vibraciones de la mesa de trabajo durante el ensayo.
5. Conservar el equipo a temperaturas entre 2-8 °C, 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 negativos.
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 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 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 2-8°C. 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 8°C.

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.

Diluyente 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 -20°C.

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 37°C (+/- 0.5°C) 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 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 pesquaje 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 37°C 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 Diluyente 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 diluidas. 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 +37°C**.

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 Diluyente 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 diluidas en los pocillos correspondientes. Comprobar que el Suero Control, los Calibradores y las muestras han sido añadidos adecuadamente.
5. Incubar la microplaca durante **60 minutos a +37°C**.

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 diluidas & Suero Control Disuelto	100 µl
1ª incubación	60 min
Temperatura	+37°C
Lavados	n° 5 cycles with 20" of soaking OR n° 6 cycles without soaking
Conjugado	100 µl
2ª incubación	60 min
Temperatura	+37°C
Lavados	n° 5 cycles with 20" of soaking OR n° 6 cycles without soaking
Cromógeno/Substrato	100µl
3ª incubación	20 min
Temperatura	t.a.*
Acido Sulfúrico	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	< 0.150 DO450nm después de leer el blanco
Coeficiente de variación	< 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, procedase 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:

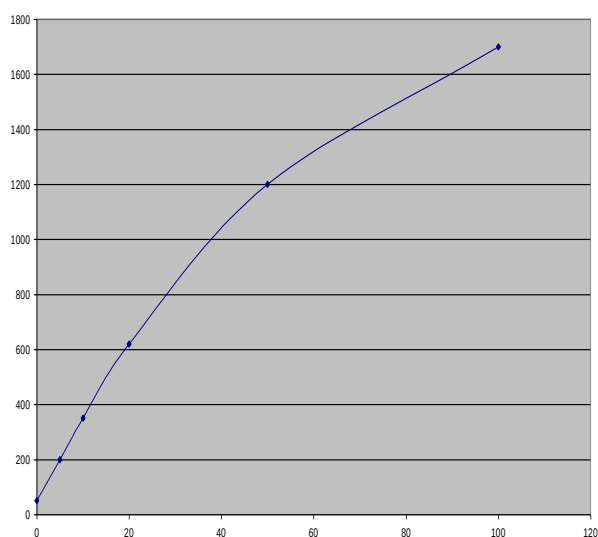
El análisis debe seguir el paso de lectura descrito en la sección M, punto 12.

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 ≥ 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	1ª corrida	2ª corrida	3ª 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	1ª corrida	2ª corrida	3ª 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	1ª corrida	2ª corrida	3ª 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	1ª corrida	2ª corrida	3ª 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	1ª corrida	2ª corrida	3ª 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	1ª corrida	2ª corrida	3ª 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	1ª corrida	2ª corrida	3ª 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	1ª corrida	2ª corrida	3ª 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	1ª corrida	2ª corrida	3ª 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 mercado 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:
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Via G. Carducci n° 27 – Sesto San Giovanni
Milán – Italia


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 -



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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 hepatocytes, 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. Lyophilized 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 H₂O₂.

Note: To be stored protected from light as sensitive to strong illumination.

11. Sulphuric Acid: H₂SO₄ 0.3 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-borne 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 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; 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 $\pm 0.5^\circ\text{C}$) 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+H₂O₂) 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	100 ul
Samples	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/H ₂ O ₂ 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	50 ul
Samples	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:

$$NC + 0.100 = \text{Cut-Off (Co)}$$

The value found for the test is used for the interpretation of results as described in the next paragraph.

HBeAb:

$$(NC + PC) / 3 = \text{Cut-Off (Co)}$$

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 of 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 higher than 2.0 – Accepted S/Co = 4.2

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 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 = $(2.100 + 0.100) / 3 = 0.733$

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	3 rd 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	3 rd 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	3 rd 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	3 rd 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	3 rd 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	3 rd 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	3 rd 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	3 rd 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	3 rd 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	3 rd 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

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.

HBE.CE: lot #0303

Negative Control (N = 16)

Mean values	1st run	2nd run	3 rd 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

Manufacturer:
Dia.Pro Diagnostic Bioprobes S.r.l.
Via G. Carducci n° 27 – Sesto San Giovanni (MI) – Italy

CE
0318

PEI 0.25 U/ml (N = 16)

Mean values	1st run	2nd run	3 rd 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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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 -



DIA.PRO

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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 H₂SO₄ 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-borne 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 freeze/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 0.2-0.8µ 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 µl of each specimen with 450 µl of Cal 0 (1:10). Then 50 µl of the 1:10 dilution are diluted with 450 µl 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 ±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. 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 350µl/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 ±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 ≤ 10 nm; (b) absorbance range from 0 to ≥ 2.0; (c) linearity to ≥ 2.0; 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 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/H₂O₂ 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/H₂O₂ 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	50 ul
Calibrators	100 ul
Control Serum	100 ul
Samples	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/H ₂ O ₂ 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	S3	S11									
B	CAL1	S4	S12									
C	CAL1	S5	S13									
D	CAL2	S6	S14									
E	CAL2	S7	S15									
F	CAL5	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 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

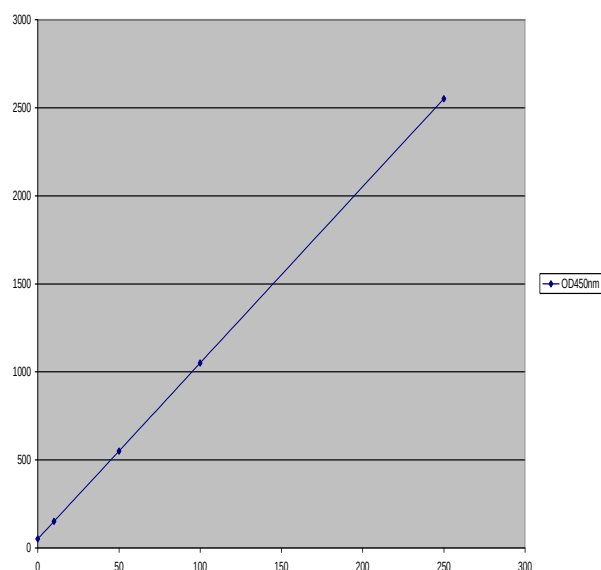
If the results of the test match the requirements stated above, proceed to the next section.

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	1. that the washing procedure and the washer settings are as validated in the pre qualification study;
coefficient of variation > 30%	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.

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 of 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

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.

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	3 rd 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	3 rd 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	3 rd 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	3 rd 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	3 rd 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	3 rd 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	3 rd 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	3 rd 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	3 rd 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.

5. 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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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.

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6. Enzyme Conjugate Diluent CONJ DIL

2x16ml/vial. Ready to use and pink/red color coded reagent. 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 H₂SO₄ 0.3 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.

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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 0.2-0.8µ 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 350µl/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

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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 $\pm 5\%$.
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 $\geq 1\%$. 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.
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 $2\pm 0.8^\circ\text{C}$, 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 $+37^\circ\text{C}$ 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 μl controls & calibrator, then all the samples and finally 100 μl 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 350 μl /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 150 μl of the Negative Control in triplicate, 150 μl of the Calibrator in duplicate and then 150 μl of the Positive Control in single followed by 150 μl 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 100 μl 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 $+37^\circ\text{C}$** .

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:

- 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.
- 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.
- 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 µl of controls, calibrator and samples
 - incubate 60 min at +37°C on shaking
 - wash according to instructions (section I.4)
 - dispense 100 µl diluted enzyme tracer
 - incubate 30 min at +37°C on shaking
 - wash
 - dispense 100 µl 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	150 µl
Diluted Enzyme Conjugate	100 µl
1st incubation	120 min
Temperature	+37°C
Washing steps	n° 4-5
Chromogen/Substrate	200µl
2nd incubation	30 min
Temperature	room
Sulphuric Acid	100 µl
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 $\geq$ 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:

$$NC + 0.050 = \text{Cut-Off (Co)}$$

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:

- Interpretation of results should be done under the supervision of the laboratory supervisor to reduce the risk of judgment errors and misinterpretations.
- Any positive result must be confirmed first by repeating the test on the sample, after having filtered it on 0.2-0.8 µm 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.

- When test results are transmitted from the laboratory to another department, attention must be paid to avoid erroneous data transfer.
- 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 of 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	1 st 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	Negative Sample	Calibrator 0.5 IU/ml
Total n = 144		
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, cryoglobulins, 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.
Via G. Carducci n° 27 – Sesto San Giovanni (MI) – Italy

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 hlgG 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 **H₂SO₄ 0.3 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).

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-borne 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.
- 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.
- 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 $\pm 5\%$.
- 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 ≤ 10 nm; (b) absorbance range from 0 to ≥ 2.0 ; (c) linearity to ≥ 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.
- 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.
- 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

- Check the expiration date of the kit printed on the external label of the kit box. Do not use if expired.
- 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.
- Dilute all the content of the 20x concentrated Wash Solution as described above.
- Dissolve the Calibrator as described above.
- Allow all the other components to reach room temperature (about 1 hr) and then mix as described.
- 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.
- Check that the ELISA reader has been turned on at least 20 minutes before reading.
- If using an automated workstation, turn it on, check settings and be sure to use the right assay protocol.
- Check that the micropipettes are set to the required volume.
- 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.

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:

- Place the required number of Microwells in the microwell holder. Leave the 1st well empty for the operation of blanking.
- 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 !
- 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.

- 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.
- 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.

- Wash the microplate with an automatic washer by delivering and aspirating 300ul/well of diluted washing solution as reported previously (section I.3).
- 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.

- Incubate the microplate for **45 min at +37°C**.
- Wash microwells as in step 6.

- 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.

- 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.
- 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:

- Ensure that no finger prints are present on the bottom of the microwell before reading. Finger prints could generate false positive results on reading.
- 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(*)	200 ul
Samples	200ul dil.+10ul
Assay Diluent (DILAS)	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/Substrate 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:

$$\text{Cut-Off} = \text{NC mean OD450nm/620-630nm} + 0.350$$

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 of 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
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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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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 sustrato/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 contiene 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

sódico 10mM 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, cuyo 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 enzimático **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. Cromógeno/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 (H₂O₂) 0.02%.

Nota: Evitar la exposición a la luz, la sustancia es fotosensible.

8. Diluyente 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 **H₂SO₄ 0.3 M**

1x15ml/vial. Contiene solución de H₂SO₄ 0.3M

Atención: Irritante (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

10. Diluyente 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 diluyente 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 termostatzado de microplacas ELISA, calibrado (en seco o húmedo) fijo a 37°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 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 pesquiasaje 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 sustrato a la luz y las vibraciones de la mesa de trabajo durante el ensayo.
6. Conservar el estuche a temperaturas entre 2-8 °C, 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 pesquiasaje 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, 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 2-8°C.

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 -20°C .

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 8°C .

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. Diluyente 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. Diluyente 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 $\pm 2\%$. Deben descontaminarse periódicamente los residuos de los componentes del estuche.

- La incubadora de ELISA debe ser ajustada a 37°C ($\pm 0.5^{\circ}\text{C}$) 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.
- 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.
- Los tiempos de incubación deben tener un margen de $\pm 5\%$.
- 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 $\leq 10\text{nm}$ b) Rango de absorbancia de 0 a ≥ 2.0 , c) Linealidad ≥ 2.0 , 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 la correcta medición de la densidad óptica, según las normas del fabricante.
- 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 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 pesaje en unidades de sangre y cuando la cantidad de muestras supera las 20-30 unidades por ensayo.
- 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.

- Compruebe la fecha de caducidad indicada en la parte externa del estuche (envase primario). No usar si ha caducado.
- 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 37°C 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 Diluyente 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 Diluyente 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 Diluyente de muestras cambia de verde a verde azul oscuro, una vez adicionada la muestra..

4. Dispensar 50 ul de Diluyente 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 diluida, 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(*)	200 ul
Muestras	200ul dil.+10ul
Diluyente de ensayo (DILAS)	50 ul
1ª incubación	45 min
Temperatura	+37°C
Lavado	5 ciclos con 20"de remojo o 6 ciclos sin remojo
Conjugado	100 ul
2ª incubación	45 min
Temperatura	+37°C
Lavado	5 ciclos con 20"de remojo o 6 ciclos sin remojo

TMB/H ₂ O ₂	100 ul
3^{ra} 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	- el proceso de lavado y los parámetros del lavador estén validados según los estudios previos de calificación. - se ha usado la solución de lavado apropiada y que el lavador ha sido cebado con la misma antes del uso. - no se han cometido errores en el procedimiento (dispensar el control positivo en lugar del negativo). - no ha existido contaminación del control negativo o de sus pocillos debido a muestras positivas derramadas, o al conjugado. - las micropipetas no se han contaminado con muestras positivas o con el conjugado. - las agujas del lavador no estén parcial o totalmente obstruidas.

Control Positivo < 1.000 DO450nm	- el procedimiento ha sido realizado correctamente. - 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. - el proceso de lavado y los parámetros del lavador estén validados según los estudios previos de calificación. - 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	- el procedimiento ha sido realizado correctamente. - no ha habido errores durante su distribución (dispensar el control negativo en lugar del calibrador). - el proceso de lavado y los parámetros del lavador estén validados según los estudios previos de calificación. - 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:

$$\text{Valor de corte} = \text{CN medio DO450nm/620-630nm} + 0.350$$

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 siguiente tabla.

(M/Co)	Interpretación
< 0.9	Negativo
0.9 – 1.1	Equivoco
> 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 UI/ml.

Asimismo, siempre en la sensibilidad de 0.25 WHO UI/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. PRECISIÓN:

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





Dia.Pro
Diagnostic
Bio**P**robes

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
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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 -



DIA.PRO

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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 H₂SO₄ 0.3 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. 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 ±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 ≤ 10 nm; (b) absorbance range from 0 to ≥ 2.0; (c) linearity to ≥ 2.0; 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, 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.

- Wash the microplate with an automatic washer by delivering and aspirating 300 µl/well of diluted washing solution as reported previously (section I.3).
- 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.

- Incubate the microplate for **60 min at +37°C**.
- Wash microwells as in step 6.
- 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.

- 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.
- 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:

- Ensure that no finger prints are present on the bottom of the microwell before reading. Finger prints could generate false positive results on reading.
- 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 !)	50 µl
Negative and Positive Controls	100 µl
Samples diluted 1:101	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/H ₂ O ₂	100 µl
3rd incubation	20 min
Temperature	r.t.
Sulphuric Acid	100 µl
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/Substrate 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:

$$\text{Cut-Off} = \text{NC mean OD}_{450\text{nm}/620-630\text{nm}} + 0.250$$

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 OD_{450nm/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 of real figures obtained by the user.

Negative Control: 0.060 – 0.080 – 0.070 DO 450nm
Mean Value: 0.070 OD_{450nm}
Lower than 0.100 – Accepted
Positive Control: 1.589 OD_{450nm}
Higher than 0.500 – Accepted
Cut-Off = 0.070+0.250 = 0.320

Sample 1: 0.070 OD_{450nm}
Sample 2: 1.690 OD_{450nm}
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 ≥ 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 OD_{450nm} 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.
Via G. Carducci n° 27 – Sesto San Giovanni (MI) – Italy



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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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 Código 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 tampón 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 tampón 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 **H₂SO₄ 0.3 M**

1x15ml/vial.

Contiene: solución 0.3 M de H₂SO₄.

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% caseína, 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. Usar para diluir las muestras.

9. Reactivo Neutralizante **SOLN NTR**

1x8ml/vial.

Listo para el uso. Contiene: anticuerpos de cabra anti-IgG humana, 2% caseína, 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 37°C.
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 entrenado 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 °C, 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 pesquaje 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 al menos 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 2-8°C. 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 químicamente.

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 2-8°C

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 8°C.

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 37°C (+/- 0.5°C) 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 $\geq 1\%$. 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 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 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 número 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 37°C 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 µl/pocillo de solución de lavado diluida, según se indica (sección I.3).
7. Dispensar 100 µl 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 100 µl 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 100 µl 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 Neutralizante (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
Enzima conjugada	100 µl
2ª incubación	60 min
Temperatura	+37°C
Lavado	5 ciclos con 20"de remojo o 6 ciclos sin remojo
TMB/H ₂ O ₂	100 µl
3ª incubación	20 min
Temperatura	18-24°C
Ácido sulfúrico	100 µl
Lectura DO	450nm/620-630nm

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	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 después 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	Compruebe 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:

$$\text{Valor de corte} = \text{Media CN DO450nm/620-630nm} + 0.250$$

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:

- 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.
- Antes de formular un diagnóstico de hepatitis viral, los resultados positivos deben comprobarse a través de un método alternativo.
- Cuando se transmiten los resultados de la prueba, del laboratorio a otras instalaciones, debe ponerse mucha atención para evitar el traslado de datos erróneos.
- 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

$$\text{Valor de corte} = 0.070 + 0.250 = 0.320$$

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 fueron testados sobre 700 muestras procedentes 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 más 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 diagnóstica $\geq 95\%$.

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.

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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 H₂O₂.

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 H₂SO₄ 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-borne microbial agents, when opening kit vials and microplates and when performing the test. Protect the Chromogen/Substrate (TMB/H₂O₂) 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 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.
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/H₂O₂ 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 µl of it, possibly in duplicate.

N. ASSAY SCHEME

Controls/Calibrator	100 µl
Samples	100 µl
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 µl
2nd incubation	60 min
Temperature	+37°C
Washing step	n° 5 cycles with 20" of soaking OR n° 6 cycles without soaking
TMB/H ₂ O ₂ mix	100 µl
3rd incubation	20 min
Temperature	r.t.
Sulphuric Acid	100 µl
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 ≤ 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:

$$\text{Cut-Off} = (\text{NC} + \text{PC}) / 5$$

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

$$\text{Cut-Off} = (2.100 + 0.100) / 5 = 0.440$$

Calibrator: 0.300-0.260 OD450nm

Mean value: 0.280 OD450nm

Within the range $\text{PC} \leq \text{OD450nm} < (\text{NC} + \text{PC})/5$ – 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 #	DAB.CE	Lot #	DAB.CE	Lot #
	OD450 nm	Co/S value	OD450 nm	Co/S value	OD450 nm	Co/S value
Accurun # 127						
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	3 rd 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	3 rd 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	3 rd 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	3 rd 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	3 rd 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	3 rd 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.

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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"



DIA.PRO

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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 prácticamente 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 exacerbadión 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 recombinante-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 sustrato 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 sustrato 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 4°C.

2. Control Negativo: CONTROL -

1x2.0ml/vial. Listo para el uso. Contiene proteínas 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 proteínas 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 (HRP) 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 (H₂O₂) 0.02% así como dimetilsulfóxido 4%.

Nota: Evitar la exposición a la luz, ya que la sustancia es fotosensible.

8. Ácido Sulfúrico: H₂SO₄ 0.3M

1x15ml/vial. Contiene solución de H₂SO₄ 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 37°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 (TMB/H₂O₂) a la luz y las vibraciones de la mesa de trabajo durante el ensayo.
5. Conservar el equipo a temperaturas entre 2-8 °C, 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 pesquaje 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 2-8°C. 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 -20°C. 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 8°C.

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 37°C (+/- 0.5°C) 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 $\leq 10\text{nm}$ 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 pesquaje 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 37°C 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 +37°C**.

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:

- Asegurarse de que no hay impresiones digitales en el fondo de los pocillos antes de leer. Podrían generarse falsos positivos en la lectura.
- 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.
- 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	100 µl
Muestras	100 µl
1ª incubación	60 min
Temperatura	+37°C
Lavado	5 ciclos con 20"de remojo o 6 ciclos sin remojo
Conjugado	100 µl
2ª incubación	60 min
Temperatura	+37°C
Lavado	5 ciclos con 20"de remojo o 6 ciclos sin remojo
Mezcla TMB/H2O2	100 µl
3ª 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) < 1.000 DO450nm 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:

$$\text{Valor de corte} = (\text{CN} + \text{CP}) / 5$$

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

$$\text{Valor de corte} = (2.100 + 0.100) / 5 = 0.440$$

Calibrador: 0.300-0.260 DO450nm

Valor medio: 0.280 DO450nm

Dentro del rango $CP \leq DO450nm < (CN+CP)/5$ – 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 #	DAB.CE	Lote #	DAB.CE	Lote #
	DO450	Co/M	DO450	Co/M	DO450	Co/M
Accurun	nm	valor	nm	valor	nm	valor
# 127						
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 (citrate, 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	1ª tanda	2ª tanda	3ª 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	1ª tanda	2ª tanda	3ª 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	1ª tanda	2ª tanda	3ª 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	1ª tanda	2ª tanda	3ª 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	1ª tanda	2ª tanda	3ª 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	1ª tanda	2ª tanda	3ª 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 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 (Mi) – Italia



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 immunoassay (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 hlgG&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 **H₂SO₄ 0.3 M**

1x32ml/bottle. It contains 0.3 M H₂SO₄ 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 RECOMMENDATIONS

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 $+2..8^{\circ}\text{C}$.

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 $\pm 2\%$. Decontamination of spills or residues of kit components should also be carried out regularly.
2. The ELISA incubator has to be set at $+37^{\circ}\text{C}$ (tolerance of $\pm 0.5^{\circ}\text{C}$) 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 $\leq 10\text{ nm}$; (b) absorbance range from 0 to ≥ 2.0 ; (c) linearity to ≥ 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 $2..8^{\circ}\text{C}$, 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 100ul 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 100ul 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 100ul 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	200 ul 200ul dil.+10ul
Assay Diluent (DILAS)	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/H ₂ O ₂	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):

$$NC + 0.350 = \text{Cut-Off (Co)}$$

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 of 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	3 rd 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	3 rd 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	3 rd 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	3 rd 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	3 rd 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	3 rd 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:
Dia.Pro Diagnostic Bioprobes Srl.
Via G. Carducci n° 27 – Sesto San Giovanni (MI) - Italy



HCV Ab

**Versión 4.0 del Ensayo
Inmunoenzimático para la determinación
de anticuerpos frente Virus de la
Hepatitis C
en plasma y suero humanos.**

Uso exclusivo para diagnóstico "in vitro"



DIA.PRO

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HCV Ab

A. OBJETIVO DEL EQUIPO.

Versión 4.0 del Ensayo Inmunoenzimático (ELISA) para la determinación de anticuerpos al virus de la Hepatitis C en plasma y suero humanos.

El equipo está diseñado para el cribado en unidades de sangre así como para el seguimiento de pacientes infectados con HCV. Uso exclusivo para diagnóstico "in vitro".

B. INTRODUCCIÓN.

La Organización Mundial de la Salud (OMS) define la infección por el virus de la Hepatitis C como:

"La Hepatitis C es una infección viral del hígado, definida como hepatitis de transmisión parenteral "no A no B" hasta el descubrimiento del agente causal en 1989. El descubrimiento y la caracterización del virus de la hepatitis C (HCV) ha permitido comprender su papel primario en la hepatitis post-transfusional y su tendencia a inducir la infección persistente. El virus de la hepatitis C es la causa principal de hepatitis aguda y enfermedad hepática crónica, incluyendo cirrosis y cáncer de hígado. A nivel mundial se estima que 170 millones de personas estén infectadas de forma crónica con HCV y que de 3 a 4 millones se infecten cada año.

El virus se transmite por contacto directo con sangre humana. Las causas principales de infección por HCV en el mundo son las transfusiones sanguíneas no controladas y la reutilización de jeringuillas y agujas sin una correcta esterilización previa. En la actualidad aún no existe una vacuna eficaz contra el virus y el tratamiento para la hepatitis C crónica es demasiado costoso para la mayoría de las personas en países en vías de desarrollo. Desde una perspectiva global, el mayor impacto contra la hepatitis C puede lograrse a través de esfuerzos orientados hacia la prevención y el control de la transmisión por exposiciones nosocomiales (como las transfusiones sanguíneas y las prácticas invasoras inseguras) y los comportamientos que conllevan alto riesgo (como el consumo de drogas inyectables).

El virus de la hepatitis C aparece en la mayoría de los casos de hepatitis viral. Es un virus RNA envuelto, perteneciente a la familia Flaviviridae y que parece tener un estrecho margen de huéspedes. Humanos y chimpancés son las únicas especies susceptibles conocidas y ambas desarrollan una enfermedad similar. Una característica importante del virus es su variabilidad genómica, la cual pudiera estar relacionada a su elevada capacidad (80%) de inducir infección crónica. El HCV ha sido agrupado por genotipos, lo cual puede ser útil para determinar la gravedad de la enfermedad y la respuesta al tratamiento.

El periodo de incubación varía desde 15 hasta 150 días. En la infección aguda los síntomas más comunes son fatiga e ictericia, sin embargo la mayoría de los casos (entre el 60% y el 70%), incluso aquellos que desarrollan la infección crónica, son asintomáticos. Cerca del 80% de los nuevos pacientes infectados progresan a la infección crónica. Del 10 al 20% de las personas con infección crónica desarrollan cirrosis, mientras que el cáncer de hígado lo presentan entre el 1 y el 5% de las personas con este tipo de infección, en un periodo de 20 a 30 años. Muchos pacientes que padecen cáncer de hígado y no están infectados por el virus de la hepatitis B, presentan evidencias de infección por el virus de la hepatitis C. Los mecanismos que relacionan la infección por HCV y el desarrollo de cáncer hepático no han sido aún esclarecidos. La hepatitis C puede exacerbar la gravedad de una enfermedad subyacente

del hígado cuando coexiste con otras disfunciones hepáticas; particularmente la enfermedad progresa más rápidamente en personas alcohólicas e infectadas por HCV. Las formas de transmisión más frecuentes son a través de transfusiones sanguíneas sin controlar y por la reutilización de agujas, jeringuillas y material médico contaminados. La transmisión sexual y perinatal puede suceder aunque es menos frecuente. Determinadas prácticas y comportamientos sociales y culturales (perforaciones en orejas y otras partes del cuerpo (piercing), circuncisiones y tatuajes) pueden constituir modos de transmisión si existe una inadecuada esterilización de los instrumentos usados. El HCV no se transmite por estornudos, tos, abrazos, agua o alimentos, estrechar la mano, compartir cubiertos o en general por contactos casuales. Tanto en países desarrollados como en aquellos en vías de desarrollo, los grupos de alto riesgo incluyen drogadictos, receptores de transfusiones sin analizar, hemofílicos, pacientes sometidos a diálisis y personas con actividad sexual promiscua y sin la debida protección. En los países desarrollados, se ha estimado que el 90% de las personas con infección crónica por HCV son o han sido drogadictos o han recibido donaciones de sangre o hemoderivados contaminados. En muchos países en vías de desarrollo, donde aún se utilizan transfusiones o hemoderivados sin analizar, los principales medios de transmisión son los instrumentos para inyecciones y las transfusiones sin analizar.

La OMS estima que cerca de 170 millones de personas, es decir el 3% de la población mundial, están infectadas por el HCV y bajo riesgo de desarrollar cirrosis y/o cáncer hepático. La prevalencia de la infección por HCV en países de África, el Mediterráneo oriental, Sudeste Asiático y el Pacífico Occidental es alta, comparada con países de Norteamérica y Europa.

Las pruebas de diagnóstico para el HCV contribuyen a prevenir la infección mediante el cribado de la sangre y plasma del donante, son útiles para establecer un diagnóstico clínico y en el seguimiento de los pacientes. Las pruebas de diagnóstico comerciales disponibles en la actualidad, se basan en ensayos enzimáticos de inmunoabsorción (EIA) para la detección de anticuerpos específicos contra HCV. Estos métodos pueden detectar más del 95% de los pacientes con infección crónica, pero solo entre el 50 y el 70% de las infecciones agudas. Para confirmar los resultados positivos por EIA se usa frecuentemente el sistema inmunoblot recombinante (RIBA), el cual identifica anticuerpos contra los antígenos individuales del HCV. Por otra parte, algunas técnicas de biología molecular (amplificación de ácidos nucleicos: Reacción en Cadena de la Polimerasa (PCR) y DNA ramificado) han sido utilizadas para confirmar los resultados serológicos así como para determinar la efectividad de la terapia antiviral. Un resultado positivo indica la presencia de una infección activa, de una fuente potencial de transmisión y/o del desarrollo de una enfermedad hepática crónica.

Para el tratamiento de personas con hepatitis C crónica se emplean fármacos antivirales como el interferón (administrado solo o en combinación con la ribavirina), pero el costo del tratamiento es elevado. Si se emplea solo el tratamiento con interferón, la eficacia en los pacientes es de 10 a 20%, mientras que en combinación con la ribavirina es eficaz en cerca del 30-50% de los casos. El tratamiento solo con ribavirina no parece ser efectivo.

No existe en la actualidad una vacuna contra HCV, debido en parte, a la alta frecuencia de mutaciones del virus. El escaso conocimiento de la respuesta inmune protectora que sigue a la infección por HCV ha dificultado el desarrollo de la vacuna. No se conoce tampoco acerca de los mecanismos del sistema inmune para la eliminación del virus. Algunos estudios, sin embargo, han demostrado la aparición de anticuerpos neutralizantes en pacientes con infección HCV. En ausencia de la vacuna, es conveniente tomar todas las medidas posibles para prevenir la infección (a) cribado y análisis de sangre y órganos de donantes; (b) inactivación del virus en productos derivados del plasma; (c) implementación y mantenimiento de las prácticas para el control de la infección incluyendo la

esterilización del material médico y dental; (d) promover cambios en la conducta entre el público en general y el personal sanitario para evitar las prácticas incorrectas y (e) vigilancia de los grupos de riesgo (personas con promiscuidad sexual y drogadictos)."

El genoma codifica para componentes estructurales: una proteína de la nucleocápside y dos glicoproteínas de la envoltura, así como para proteínas funcionales involucradas en la replicación viral y la síntesis de proteínas. La región que codifica para la nucleocápside parece estar altamente conservada entre los aislamientos obtenidos en todo el mundo.

C. PRINCIPIOS DEL ENSAYO.

Las microplacas están recubiertas con antígenos específicos del HCV correspondientes a las regiones del "core" y "ns" que codifican para determinantes antígenicos inmunodominantes y conservados (péptido del core y péptidos recombinantes NS3, NS4 y NS5).

Se añade la muestra diluida y los anticuerpos contra HCV, presentes en la muestra, son capturados por los antígenos de la fase sólida.

Después del lavado, en la 2ª incubación, los anticuerpos IgG e IgM son detectados mediante anticuerpos policlonales específicos anti-IgG/IgM humanos, conjugados con Peroxidasa (HPR).

La enzima capturada en la fase sólida, combinada con la mezcla sustrato/cromógeno, genera una señal óptica proporcional a la cantidad de anticuerpos anti-HCV presentes 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 al HCV.

D. COMPONENTES.

Cada equipo (Código CVAB.CE) contiene reactivos suficientes para realizar 192 pruebas.

1. Microplaca: MICROPLATE

n° 2 microplacas

12 tiras de 8 pocillos recubiertos con péptidos recombinantes para el "core" y para NS3, NS4 y NS5. Las placas están empaquetadas 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 al 0,045% como conservantes. 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-HCV, tampón Citrato sódico 10mM pH 6.0 +/-0.1, 0.5% de Tween 20, así como azida sódica 0.09% y ProClin 300 al 0,045% como conservantes. El control positivo está codificado con el color azul.

4. Calibrador CAL

n° 2 viales

Liofilizado. Para disolver en agua calidad EIA como se indica en la etiqueta. Contiene suero fetal bovino, anticuerpos humanos al HCV, calibrados según el código Estándar de Trabajo de NIBSC 99/588-003-VI, tampón Citrato sódico 10mM pH 6.0 +/-0.1, además de sulfato de gentamicina 0.3 mg/ml y ProClin 300 al 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

2x60ml/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

2x16ml/vial. Solución lista para el uso. Contiene 5% de albúmina de suero bovino, tampón Tris 10mM a pH 6.8 +/- 0.1, anticuerpo policlonal de cabra anti-IgM/IgG humanos conjugado con peroxidasa (HPR) en presencia de 0.2 % de sulfato de gentamicina y ProClin 300 al 0,045% como conservantes. El conjugado está codificado con el color rosa/rojo.

7. Cromógeno/Substrato SUBS TMB

2x16ml/vial. Contiene una solución tamponada citrato-fosfato 50mM pH 3.5-3.8, tetra-metil-benzidina (TMB) 0.03% y peróxido de hidrógeno (H₂O₂) 0.02% así como dimetilsulfóxido 4%.

Nota: Evitar la exposición a la luz, la sustancia es fotosensible.

8. Diluyente de ensayo: DILAS

1x15ml/vial. Contiene una solución tamponada Tris 10 mM pH 8.0 +/- 0.1 y 0.1% de ProClin 300 al 0,045% para el pre-tratamiento de muestras y controles, bloquea posibles interferencias.

Nota: Usar todo el contenido del vial antes de abrir un segundo. El reactivo es sensible a oxidación.

9. Ácido Sulfúrico: H₂SO₄ 0.3M

1x32ml/vial. Contiene solución de H₂SO₄ 0.3M

Atención: Irritante (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

10. Diluyente de muestras DILSPE

2x50ml. Contiene una solución tamponada citrato sódico 10 mM pH 6.0 +/- 0.1, 1% de proteínas del suero de cabra, 0.5% de Tween 20, azida sódica 0.09% y ProClin 300 al 0,045% como conservantes. Se usa para diluir las muestras.

11. Sellador adhesivo, n° 4

12. Manual de instrucciones, n° 1

Nota importante: A solicitud del cliente, Dia.Pro puede suministrar reactivos para realizar 96, 480 ó 960 pruebas, según se reporta a continuación:

1. Microplaca	n°1	n°5	n°10
2. Control Negativo	1x2.0ml/vial	1x10ml/vial	1x20. ml/vial
3. Control Positivo	1x2.0ml/vial	1x10ml/vial	1x20. ml/vial
4. Calibrador	n° 1 vial	n° 5 vials	n° 10 vials
5. Soluc. Lav. conc	1x60ml/bot.	5x60ml/frasc.	4x150ml/frasc.
6. Conjugado	1x16ml/vial	2x40ml/frasc.	4x40ml/frasc.
7. Cromóg/Subs	1x16ml/vial	2x40ml/frasc.	4x40ml/frasc.
8. Diluent. ensayo	1x8ml/vial	1x40ml/ frasc.	1x80ml/frasc.
9. Ácido Sulfúrico	1x15ml/vial	2x40ml/ frasc.	2x80ml/frasc.
10. Diluent. muestr.	1x50ml/vial	5x50ml/frasc.	4x125ml/frasc.
11. Sellador adhes.	n° 2	n° 10	n° 20
12. Manual de instrucciones	n° 1	n° 1	n° 1
Número de pruebas	96	480	960
Código	CVAB.CE.96	CVAB.CE.480	CVAB.CE.960

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 termostático de microplacas ELISA, calibrado (en seco o húmedo) fijo a 37°C.
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. 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. Cuando el equipo es usado para cribado 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 equipos, así como durante la realización del ensayo. Evitar la exposición del sustrato a la luz y las vibraciones de la mesa de trabajo durante el ensayo.
6. Conservar el equipo a temperaturas entre 2-8 °C, en un refrigerador con temperatura regulada o en cámara fría.
7. No intercambiar reactivos de diferentes lotes ni tampoco de diferentes equipos.
8. 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 y reemplazar el equipo.
9. Evitar contaminación cruzada entre muestras de suero/plasma usando puntas desechables y cambiándolas después de cada uso. No reutilizar puntas desechables.
10. Evitar contaminación cruzada entre los reactivos del equipo usando puntas desechables y cambiándolas después de cada uso. No reutilizar puntas desechables.
11. No usar el producto después de la fecha de caducidad indicada en el equipo e internamente en los reactivos. Según estudios realizados, no se ha detectado pérdida relevante de actividad en equipos 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 equipo 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 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, 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 equipo se emplea para el cribado 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 varios 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 equipos 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 2-8°C. 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 -20°C.

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.

Por que en los frascos pueden estar presente los cristales, cuando se prepara la solución prestar mucha atención en diluir todo el contenido. 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 8°C.

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. Diluyente 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. Diluyente de muestras :

Listo para el uso. Mezclar bien con un vórtex antes de usar.

I. INSTRUMENTOS Y EQUIPAMIENTO UTILIZADOS EN COMBINACIÓN CON EL EQUIPO.

- 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 equipo.
- La incubadora de ELISA debe ser ajustada a 37°C (+/- 0.5°C) 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.
- 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.
- Los tiempos de incubación deben tener un margen de ±5%.
- El lector de microplacas ELISA debe estar provisto de un filtro de lectura de 450 nm y de un segundo filtro de 620-630 nm, obligatorio para el blanco. El procedimiento estándar debe contemplar: a) Ancho de banda ≤ 10 nm; b) Rango de absorbancia de 0 a ≥ 2,0; c) Linealidad ≥ 2,0; d) Reproducibilidad ≥ 1%. El blanco se prueba en el pocillo indicado en la sección "Procedimiento del ensayo". El sistema óptico del lector debe calibrarse periódicamente para garantizar que se mide la densidad óptica correcta. Periódicamente se debe proceder al mantenimiento según las instrucciones del fabricante.
- 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 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 cribado en unidades de sangre y cuando la cantidad de muestras supera las 20-30 unidades por ensayo.

7. Cuando se utilizan instrumentos automáticos, en el caso en que los contenedores para los frascos del instrumento no sean adecuados a los frascos del kit, transferir la solución en ellos contenida en frascos idóneos al instrumento y etiquetarlos con la misma etiqueta utilizada en el frasco original. Esta operación es importante para evitar el cambio del contenido de los frascos durante el transferimiento. Cuando el test a terminado colocar los contenedores secundarios etiquetados y tapados a 2..8°C.
8. 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 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 37°C 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.

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 Diluyente 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 200µl 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 Diluyente de muestras (DILSPE) a todos los pocillos de muestras, después dispensar 10 µl 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 Diluyente de muestras, después de adicionada la misma, cambia de verde a verde azul oscuro.

4. Dispensar 50 µl de Diluyente 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 350 µl/pocillo de solución de lavado diluida, según según se indica (sección 1.3).
7. Dispensar 100µl del Conjugado en todos los pocillos, excepto en el A1 y cubrir con el sellador. Compruebe que este reactivo de color rosa/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/marrón.

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), calibrando el instrumento con el pocillo A1 (blanco, obligatorio).

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.
3. Se ha probado que la agitación a 350 +/- 150 rpm, durante la incubación, aumenta en un 20% la sensibilidad del ensayo.
4. 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.

N. ESQUEMA DEL ENSAYO.

Método	Operaciones
Controles & Calibrador	200 µl
Muestras	200µl dil.+10µl
Diluyente de ensayo (DILAS)	50 µl
1ª incubación	45 min
Temperatura	+37°C
Lavado	5 ciclos con 20"de remojo o 6 ciclos sin remojo
Conjugado	100 µl
2ª incubación	45 min
Temperatura	+37°C
Lavado	5 ciclos con 20"de remojo o 6 ciclos sin remojo
TMB/H2O2	100 µl
3ª incubación	15 min
Temperatura	18-24°C
Acido Sulfúrico	100 µl
Lectura D.O.	450nm / 620-630nm

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 CP = Control Positivo M = Muestra

O. CONTROL DE CALIDAD INTERNO.

Se realiza un grupo de pruebas con los controles/calibrador cada vez que se usa el equipo para verificar si los valores DO450nm 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
Calibrador	M/Co > 1.1
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.
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.
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 responsable para tomar las medidas pertinentes.

P. CÁLCULO DEL VALOR DE CORTE.

Los resultados se calculan por medio de un valor de corte (cut-off) hallado con la siguiente fórmula:

$$\text{Valor de corte} = \text{CN medio DO450nm} + 0.350$$

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.

La interpretación de los resultados se realiza mediante la razón entre las DO a 450nm de las muestras y el Valor de corte (M/Co).

Los resultados se interpretan según 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 está infectado por HCV y la unidad de sangre se puede transfundir. 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. En este caso la unidad de sangre no debe ser transfundida. Un resultado positivo es indicativo de infección por HCV y por consiguiente el paciente debe ser tratado adecuadamente. La unidad de sangre debe ser descartada.

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. Antes de formular un diagnóstico de hepatitis viral, los resultados positivos deben comprobarse a través de un método alternativo, capaz de detectar anticuerpos IgG e IgM (prueba confirmatoria).
3. Según se demuestra en la Evaluación del Performance del producto, el ensayo es capaz de detectar los anticuerpos anti HCV core, en etapas más tempranas en comparación con otros equipos comerciales. Sin embargo, un resultado positivo, no confirmado con estos equipos comerciales, no debe necesariamente considerarse como falso positivo! Es necesario realizar una prueba de confirmación (suministrada, bajo solicitud del cliente, por Dia.pro srl. Codificada CCONF).
4. Como el ensayo es capaz de detectar además anticuerpos IgM, pueden presentarse resultados discrepantes (pérdida de reactividad IgM) con respecto a otros productos comerciales para la detección de anticuerpos anti-HCV. La positividad real de una muestra debe confirmarse probando la reactividad IgM, lo cual resulta muy importante para el diagnóstico de infección por HCV.
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 con un virus de la hepatitis debe ser evaluado y comunicado al paciente por un médico calificado.

A continuación, un ejemplo de los cálculos a realizar:

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: 0.550 - 0.530 DO450nm
 Valor medio: 0.540 DO450nm M/Co = 1.4
 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 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 ha sido calculado por medio del estándar de trabajo británico anti-HCV NIBSC, código 99/558-003-WI). La siguiente tabla muestra los valores medios de DO450nm de este estándar diluido en plasma negativo y examinado:

Dilución	Lote # 1	Lote # 2
Factor	M/Co	M/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
Plasma Negativo	0.3	0.3

Se evaluó además la muestra Accurun 1 –serie 3000– suministrado por Boston Biomedica Inc., Estados Unidos.

Los resultados son los siguientes:

CVAB.CE Lote ID	Accurun 1 Serie	M/Co
1201	3000	1.5
0602	3000	1.5
1202	3000	1.9

Por otra parte, un total de 7 muestras, positivas para HCVAb según Ortho HCV 3.0 SAVe, código 930820, lote # EXE065-1, fueron diluidas en plasma negativo a HCVAb con el fin de obtener diluciones limitantes y luego fueron probadas nuevamente en CVAB.CE, lote # 1202, y Ortho.

Las tablas siguientes reflejan los resultados obtenidos:

Muestra nº	Dilución Límite	CVAB.CE M/Co	Ortho 3.0 M/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. ESPECIFICIDAD Y SENSIBILIDAD DIAGNÓSTICAS.

La evaluación del procedimiento diagnóstico se realizó mediante un ensayo con más de 5000 muestras.

2.1 Especificidad Diagnóstica:

Se define como la probabilidad del ensayo de detectar negativos en ausencia del analito específico.

Además del primer estudio, donde se examinaron en total 5043 muestras de donantes de sangre no seleccionados, (incluyendo donantes por 1ª vez), 210 muestras de pacientes hospitalizados y 162 muestras que pudieran provocar interferencia (otras enfermedades infecciosas, positivas para anticuerpos de E. coli, pacientes con enfermedades hepáticas no virales, pacientes en diálisis, mujeres embarazadas, hemolizadas, lipémicas, etc.), la especificidad diagnóstica se evaluó recientemente examinando un total de 2876 muestras de donantes de sangre negativas en seis lotes distintos. Se observó un valor de especificidad de 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.

2.2 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 en un total de 359 muestras, el valor obtenido fue de 100%. Más de 50 muestras positivas fueron probadas de forma interna, en este caso el resultado fue también de 100%.

Se evaluaron además, muestras positivas producto de infecciones por diferentes genotipos de HCV, así como también se estudió gran parte de los paneles de seroconversión de Boston Biomedica Inc (PHV) y Zeptometrix, USA (HCV), disponibles.

Los resultados para algunos de ellos se describen a continuación:

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

Por último, el producto ha sido probado contra el panel EFS Ac HCV, lote n° 01/08.03.22C/01/A, suministrado por Etablissement Francais Du Sang (EFS), Francia, obteniéndose los siguientes resultados:

EFS Panel Ac HCV

Muestra	Lote # 1 M/Co	Lote # 2 M/Co	Lote# 3 M/Co	Resultados esperados
HCV 1	2.2	2.4	2.6	positivo
HCV 2	1.6	2.0	2.1	positivo
HCV 3	1.5	1.7	1.6	positivo
HCV 4	5.2	6.5	5.5	positivo
HCV 5	1.6	1.8	1.6	positivo
HCV 6	0.4	0.4	0.4	negativo

3. PRECISIÓN.

Ha sido calculada utilizando dos muestras, una negativa y una débil positiva, examinadas en 16 réplicas en tres corridas separadas.

Los resultados se muestran a continuación:

Lote # 1202

Muestra Negativa (N = 16)

Valores medios	1ª corrida	2ª corrida	3ª corrida	Valor Promedio
DO 450nm	0.094	0.099	0.096	0.096
Desviación estándar	0.008	0.007	0.008	0.007
CV %	8.7	6.6	7.9	7.7

Cal # 2 – 7K (N = 16)

Valores medios	1ª corrida	2ª corrida	3ª corrida	Valor Promedio
DO 450nm	0.396	0.403	0.418	0.406
Desviación estándar	0.023	0.029	0.027	0.026
CV %	5.9	7.1	6.4	6.5
M/Co	1.1	1.1	1.2	1.1

Lote # 0602

Muestra Negativa (N = 16)

Valores medios	1ª corrida	2ª corrida	3ª corrida	Valor Promedio
DO 450nm	0.097	0.096	0.094	0.096
Desviación estándar	0.009	0.010	0.008	0.009
CV %	8.9	10.1	8.4	9.1

Cal # 2 – 7K (N = 16)

Valores medios	1ª corrida	2ª corrida	3ª corrida	Valor Promedio
DO 450nm	0.400	0.395	0.393	0.396
Desviación estándar	0.021	0.025	0.026	0.024
CV %	5.4	6.2	6.6	6.1
M/Co	1.2	1.2	1.1	1.2

Lote # 0602/2

Muestra Negativa (N = 16)

Valores medios	1 ^{ra} corrida	2 ^{da} corrida	3 ^{ra} corrida	Valor Promedio
DO 450nm	0.087	0.091	0.088	0.089
Desviación estándar	0.009	0.007	0.008	0.008
CV %	10.0	8.2	8.6	8.9

Cal # 2 – 7K (N = 16)

Valores medios	1 ^{ra} corrida	2 ^{da} corrida	3 ^{ra} corrida	Valor Promedio
DO 450nm	0.386	0.390	0.391	0.389
Desviación estándar	0.023	0.021	0.023	0.022
CV %	6.0	5.3	5.8	5.7
M/Co	1.1	1.2	1.2	1.2

La variabilidad mostrada en las tablas no dió como resultado una clasificación errónea de las muestras.

S. LIMITACIONES.

Los falsos positivos repetibles, no confirmados por RIBA o similares técnicas de confirmación, fueron estimados como menos del 0.1% de la población normal.

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 aprobado por un organismo notificado para el mercado 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:
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0318

HCV IgM

**Enzyme ImmunoAssay (ELISA) for
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of IgM antibodies to
Hepatitis C Virus
in human serum and plasma**

- for “in vitro” diagnostic use only -



DIA.PRO

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HCV IgM

A. INTENDED USE

Enzyme ImmunoAssay (ELISA) for the quantitative/qualitative determination of IgM antibodies to Hepatitis C Virus in human plasma and sera. The kit is mainly intended for the follow-up of HCV chronic patients submitted to anti-viral pharmaceutical treatment. For "in vitro" diagnostic use only.

B. INTRODUCTION

Antiviral drugs, such as Interferon taken alone or in combination with Ribavirin, can be used for the treatment of persons with chronic viral hepatitis C.

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.

Active production of HCV antigens in the liver of chronic patients generates spikes of IgM antibodies production and release of liver specific enzymes, similar to what happen in HBV chronic patients. The presence of anti viral IgM is usually correlated to a phase of sufferance and cellular damage of the liver.

During the pharmaceutical treatment HCV IgM may represent a marker for the follow-up of the efficiency of the drug itself, monitoring the balance between its effectiveness and the side effects, that often may be heavy for the patient.

C. PRINCIPLE OF THE TEST

Microplates are coated with HCV immunodominant synthetic antigens (core peptide, recombinant NS3, NS4 and NS5 peptides).

In the 1st incubation, the solid phase is treated with diluted samples and anti HCV IgM are captured, if present, by the antigens. After washing out all the other components of the sample, in the 2nd incubation bound anti-HCV IgM are detected by the addition of anti hlgM 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-HCV IgM antibodies present in the sample.

The presence of IgM in the sample may therefore be quantitated by means of a calibration curve able to determine the content of the antibody in arbU/ml.

Neutralization of IgG anti-HCV, 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 HCV-specific synthetic antigens (core, NS4 and NS5 peptides and recombinant NS3). Plates are sealed into a bag with desiccant.

2. Calibration Curve: CAL N°...

6x2.0 ml/vial. Ready to use and color coded standard curve calibrated on an Internal Gold Standard (in absence of a defined international one) or IGS, ranging:

CAL 1 = 0 arbU/ml	CAL 2 = 10 arbU/ml
CAL 3 = 25 arbU/ml	CAL 4 = 50 arbU/ml
CAL 5 = 100 arbU/ml	CAL 6 = 250 arbU/ml.

It contains chemical inactivated HCV IgM positive human plasma, 100 mM Tris buffer pH 7.4+/-0.1, 0.2% Tween 20, 0.09% sodium azide and 0.1% Kathon GC 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 0.05% Kathon GC.

4. 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.

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 H₂O₂).

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 H₂SO₄ 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, 0.2% Tween 20, 0.09% Na-azide and 0.1% Kathon GC as preservatives. To be used to dilute the sample.

8. Neutralizing Reagent: SOLN NEUT

1x8ml/vial. It contains goat anti hlgG, 2% casein, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.09% Na-azide and 0.1% Kathon GC as preservatives.

9. Plate sealing foils n°2

10. 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-borne microbial agents, when opening kit vials and microplates and when performing the test. Protect the Chromogen/Substrate (or 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 also 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/calibrators 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/calibrators, 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 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 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.

Calibration Curve

Ready to use components. 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, possibly 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/calibrators 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/calibrators 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 have to be carried out according to the instructions of the manufacturer.
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 (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; 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 workstation**, 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/Substrate 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.

Two methods of analysis are possible, as described below:

M.1 QUANTITATIVE ASSAY

1. Place the required number of strips in the plastic holder and carefully identify the wells for calibrators 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+B1 wells empty for blanking purposes.
4. Dispense 50 µl Neutralizing Reagent in all the wells, except A1+B1 wells used for blanking operations and the wells used for the Calibration Curve.
5. In the identified positions pipette 100 µl of the Calibrators in duplicate followed by 100 µl of diluted samples. Check that Calibrators and samples have been correctly added.
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. 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 plastic inner surface of the well with the tip filled with the Enzyme Conjugate. Contamination might occur.

- When the second incubation is finished, wash the microwells as previously described (section I.3)
- 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.

- Incubate the microplate protected from light at **room temperature (18-24°C) for 20 minutes**. Wells dispensed with positive samples and with positive calibrators will turn from clear to blue.
- Pipette 100 µl Sulphuric Acid into all the wells using the same pipetting sequence as in step 10 to block the enzymatic reaction. Addition of the stop solution will turn the positive calibrators and the positive samples from blue to yellow.
- 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, strongly recommended), blanking the instrument on A1 or B1 or both.

M.2 QUALITATIVE ASSAY

- Place the required number of strips in the plastic holder and carefully identify the wells for calibrators and samples.
- Dilute samples **1:101** dispensing 1 ml Sample Diluent into a disposable tube and then 10 µl sample; mix on vortex before use. Do not dilute the Calibrators as they are ready-to-use.
- Leave the A1 well empty for blanking purposes.
- Dispense 50 µl Neutralizing Reagent in all the wells, except A1 well used for blanking operations and the wells used for the Calibrators.
- Then pipette 100 µl of Calibrator 0 arbU/ml in duplicate, 100 µl of Calibrator 10 arbU/ml in duplicate and finally 100 µl of diluted samples. Check that Calibrators and samples have been correctly added.
- 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.

- When the first incubation is finished, wash the microwells as previously described (section I.3)
- 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 plastic inner surface of the well with the tip filled with the Enzyme Conjugate. Contamination might occur.

- When the second incubation is finished, wash the microwells as previously described (section I.3)
- Pipette then 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.

- Incubate the microplate protected from light at **room temperature (18-24°C) for 20 minutes**. Wells dispensed with positive samples and with positive calibrators will turn from clear to blue.
- Pipette 100 µl Sulphuric Acid into all the wells using the same pipetting sequence as in step 10 to block the enzymatic reaction. Addition of the stop solution will turn the positive calibrators and the positive samples from blue to yellow.
- 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, strongly recommended), blanking the instrument on A1.

General Important notes:

- 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.
- 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 TMB chromogen can occur leading to high background.

N. ASSAY SCHEME

Method	Operations
Neutralizing Reagent	50 µl
Calibrators (no SOLN NEUT !)	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 µl
Reading OD	450nm

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	CAL6	S7									
F	CAL2	CAL6	S8									
G	CAL3	S1	S9									
H	CAL3	S2	S10									

Legenda: BLK = Blank // CAL = Calibrators // 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	S4										
B	CAL1	S5										
C	CAL1	S6										
D	CAL2	S7										
E	CAL2	S8										
F	S1	S9										
G	S2	S10										
H	S3	S11										

Legenda: BLK = Blank // CAL = Calibrators // CS = Control Serum // S = Sample

O. INTERNAL QUALITY CONTROL

A validation check is carried out 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 arbU/ml	< 0.200 OD450nm after blanking
Calibrator 10 arbU/ml	OD450nm > OD450nm CAL 0 arbU/ml + 0.100
Calibrator 250 arbU/ml	3.500 > OD450nm > 2.000

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 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 arbU/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 Cal 0 arbU/ml); 4. that no contamination of the Cal 0 arbU/ml, 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 10 arbU/ml < 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 250 arbU/ml < 2.000 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.
Calibrator 250 arbU/ml > 3.500 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; 4. that no contamination of the Cal 250 arbU/ml, 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.

If any of the above problems has occurred, report the problem to the supervisor for further actions.

P. RESULTS

If the test turns out to be valid, interpretation of results is carried out in the **quantitative assay** from the mean OD450nm value of the Calibration Curve elaborated with an appropriate curve fitting system (suggested : 4 parameters).

In the **qualitative assay** interpretation of results is done on the mean OD450nm value of the Calibrator 10 arbU/ml (or CAL 2) by means of the following formulation:

$$\text{Mean OD450nm CAL 2} = \text{cut-off (Co)}$$

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 generate the correct interpretation of results.

Q. INTERPRETATION OF RESULTS

Q.1 QUANTITATIVE ASSAY

Concentrations in arbU/ml are obtained elaborating OD450nm of samples on the fitted calibration curve.

The concentration of IgM is from Literature correlated proportionally with the liver damage produced by antibodies to HCV upon virus replication in hepatocytes.

A decrease in IgM concentration upon pharmacological treatment is usually clinically acknowledged as a sign of recovery and therapeutic efficacy.

Q.2 QUALITATIVE ASSAY

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
< 1.0	Negative
> 1.0	Positive

A negative result indicates that the patient has not developed IgM antibodies to HCV.

A positive result is indicative of an ongoing HCV active infection.

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.
4. The results of this ELISA assay should be anyway implemented with other diagnostic and clinical tests.

An example of calculation is reported below.

The following data must not be used instead of real figures obtained by the user.

CAL 1: 0.060 – 0.080 OD450nm
Mean Value: 0.070 OD450nm
Lower than 0.200 – Accepted

CAL 2: 0.200 – 0.220 OD450nm
Mean Value: 0.210 OD450nm
Higher than CAL1+0.100 = accepted
Cut-Off or Co = 0.210

Sample 1: 0.080 OD450nm
 Sample 2: 1.800 OD450nm
 Sample 1: S/Co < 1.0 = negative
 Sample 2: S/Co > 1.0 = positive

R. PERFORMANCE CHARACTERISTICS

Evaluation of Performances has been conducted on selected panels carried out in a clinical external center and internally.

1. Limit of detection

No international standard for HCV IgM 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 chronic HCV 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 study conducted in an external clinical center, with excellent experience in the diagnosis of infectious diseases and HCV.

The Diagnostic Sensitivity was studied on about 200 samples, pre-tested positive with an analytical system developed in house by the clinical laboratory where the study was conducted. Positive samples were collected from patients with a clinical history of HCV infection (acute and chronic).

In addition some Seroconversion Panels, purchased from Boston Biomedica Inc., USA, were examined.

The diagnostic specificity was determined on panels of more than 300 negative samples from normal individuals and blood donors, classified negative for anti HCV antibodies with the reference kit in use in the laboratory, including potentially interfering specimens.

A panel of potentially interfering samples (RF+, hemolised, lipemic, etc.) was also examined. No interference was observed on the samples examined.

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:

It has been calculated on two samples examined in replicates in different runs. Results are reported below summarized in a table:

Average values N = 48	Calibrator 2 10 arbU/ml	Calibrator5 100 arbU/ml
OD450nm	0.241	1.632
Std.Deviation	0.027	0.113
CV %	11.3	6.9

S. LIMITATIONS

False positivity has been assessed on 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.

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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



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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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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.

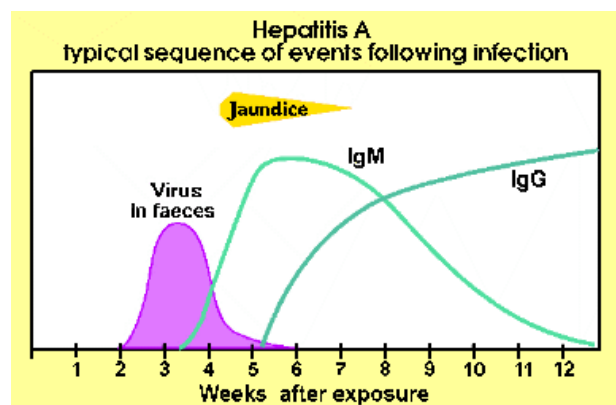
Hepatitis A cannot be differentiated from other types of viral hepatitis on the basis of clinical or epidemiologic features alone. Serologic testing to detect immunoglobulin M (IgM) antibody to the capsid proteins of HAV (IgM anti-HAV) is required to confirm a diagnosis of acute HAV infection. In most 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 H₂O₂.

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: **H₂SO₄ 0.3 M**

1x15ml/vial. Contains 0.3 M H₂SO₄ 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 0.2-0.8µ 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 $\pm 2\%$.
2. The ELISA incubator has to be set at +37°C (tolerance of $\pm 0.5^\circ\text{C}$) 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 ≤ 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+H₂O₂) 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 μ l of Negative Control in triplicate, 100 μ l of Calibrator in duplicate, 100 μ l 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 μ l 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 μ l TMB/H₂O₂ 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 μ l 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	50 ul
Controls/Calibrator(*)	100 ul
Samples	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/H ₂ O ₂ 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)	1. that the washing procedure and the washer settings are as validated in the

< 0.750 OD450nm after blanking coefficient of variation > 30%	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 ≥ 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:

$$\text{Cut-Off} = (\text{NC} + \text{PC}) / 3$$

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 of 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/S < 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

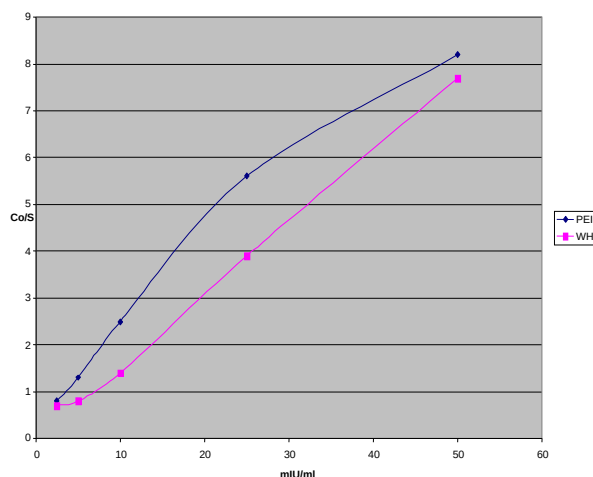
1. Limit of detection

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 mIU/ml.

Results of Quality Control are given in the following table:

WHO mIU/ml	OD450 nm	Co/S	PEI mIU/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





Dia.Pro
Diagnostic
Bio**Probes**

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 -



DIA.PRO

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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.

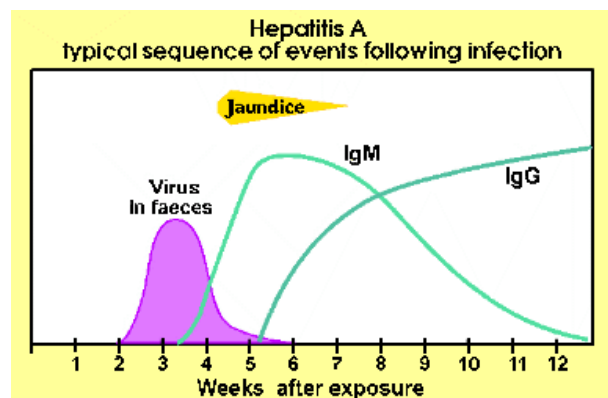
Hepatitis A cannot be differentiated from other types of viral hepatitis on the basis of clinical or epidemiologic features alone. Serologic testing to detect immunoglobulin M (IgM) antibody to the capsid proteins of HAV (IgM anti-HAV) is required to confirm a diagnosis of acute HAV infection. In most 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 H₂O₂.

Note: To be stored protected from light as sensitive to strong illumination.

10. Sulphuric Acid: H₂SO₄ 0.3 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/Substrate (TMB & H₂O₂) 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 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. 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 0.2-0.8µ 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 µl 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 $\pm 2\%$.

- The ELISA incubator has to be set at $+37^{\circ}\text{C}$ (tolerance of $\pm 0.5^{\circ}\text{C}$) 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.
- 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.
- Incubation times have a tolerance of $\pm 5\%$.
- 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.
- 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.
- 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

- Check the expiration date of the kit printed on the external label (primary container). Do not use the device if expired.
- 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.
- Dilute all the content of the 20x concentrated Wash Solution as described above.

- Dissolve the Calibrator as described above and gently mix.
- Allow all the other components to reach room temperature (about 1 hr) and then mix gently on vortex all liquid reagents.
- Set the ELISA incubator at $+37^{\circ}\text{C} \pm 0.1^{\circ}\text{C}$ 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.
- Check that the ELISA reader is turned on or ensure it will be turned on at least 20 minutes before reading.
- If using an automated work station, turn on, check settings and be sure to use the right assay protocol.
- Check that the micropipettes are set to the required volume.
- 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.

- Dilute samples 1:101 by dispensing first 10 μl sample and then 1 ml Sample Diluent into a dilution tube; mix gently on vortex.
- Place the required number of Microwells in the microwell holder. Leave the 1st well empty for the operation of blanking.
- 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 !
- 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.
- Incubate the microplate for **60 min at $+37^{\circ}\text{C}$** .

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.

- About 5-10 minutes before use, prepare the HAV Antigen/Antibody immunocomplex as described previously.
- Wash the microplate with an automatic washer as reported previously (section I.3).
- 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.

- Incubate the microplate for **60 min at $+37^{\circ}\text{C}$** .
- Wash microwells as in step 7.
- Pipette 100 μl Chromogen/Substrate mixture into each well, the blank well included. Then incubate the microplate at **room temperature ($18-24^{\circ}\text{C}$) for 20 minutes**.

Important note: Do not expose to strong direct illumination. High background might be generated.

- 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.
- 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:

- 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.
- 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 (*)	100 ul samples diluted 1:101
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/H ₂ O ₂ 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 of 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

REFERENCES

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2. Duermeier W., Van der Veen J., Koster B. "ELISA in Hepatitis A". Lancet. I.: 823-824, 1978
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



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.



Dia.Pro
Diagnostic
Bio**Probes**

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

	ZAO "Vector-Best"	Rev. 01
	EC Declaration of conformity	Page 1 of 4

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,
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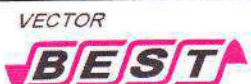
Date: 2013/04/12



Murat Khusainov
General Director ZAO «Vector-Best»

	ZAO "Vector-Best"	Rev. 01
	EC Declaration of conformity	Page 2 of 4

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

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		antigens	
24.	Ascarid-IgG-EIA-BEST	ELISA kit for determination of IgG to Ascaris lumbricoides	D-3452
25.	Lambliia-antibodies-EIA-BEST	ELISA kit for determination of IgG, IgM and IgA to Lambliia antibodies	D-3552
26.	Lambliia-IgM-EIA-BEST	ELISA kit for determination of IgM to Lambliia antibodies	D-3554
27.	Lambliia-antigen-EIA-BEST	ELISA kit for determination of Lambliia 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

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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



**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



Attachment of the certificate

No. D1213100019

date 2020-06-02

Page 1 of 1

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



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Head of Certification Body

ВЕКТОР



Набор реагентов
для иммуноферментного выявления
иммуноглобулинов классов А, М, G
к антигенам лямблий
в сыворотке (плазме) крови

ИНСТРУКЦИЯ ПО ПРИМЕНЕНИЮ

Утверждена 21.07.2011
приказом Росздравнадзора № 4449-Пр/11

Лямблия-антитела – ИФА – БЕСТ

НАБОР РЕАГЕНТОВ
D-3552

1. НАЗНАЧЕНИЕ

1.1. Набор реагентов Лямблия-антитела-ИФА-БЕСТ предназначен для иммуноферментного выявления иммуноглобулинов классов А (IgA), М (IgM), G (IgG) к антигенам лямблий в сыворотке (плазме) крови.

1.2. Лямблиоз – часто встречающееся паразитарное заболевание тонкого кишечника человека, вызываемое *Ciardia lamblia* – представителем семейства *Protozoe*. Лямблии существуют в двух отдельных формах – цистах (статическая форма) и трофозитах (пролиферативная форма).

1.3. Заражение человека происходит оральным путем, при попадании цист лямблий в желудочно-кишечный тракт. Источник инвазии – некипяченая питьевая вода, вода водоемов, немытые фрукты и овощи, грязные руки и контакт с домашними животными.

Традиционно диагностика лямблиоза проводится микроскопическим методом по обнаружению цист или трофозитов в образцах фекалий или дуоденальном содержимом.

Дополнительным методом диагностики лямблиоза является иммуноферментный анализ (ИФА), основанный на обнаружении в крови инвазированного антител, специфичных к антигенам лямблий.

Определение иммуноглобулинов различных классов к антигенам лямблий целесообразно дополнительно включать в комплексное

обследование детей с аллергическими симптомами, дерматитами, с гастродуоденитами, а также часто болеющих детей. Это способствует более надежному выявлению лямблиозной инвазии, позволяет своевременно провести специфическое лечение и в последующем осуществить контроль его эффективности.

1.4. Набор рассчитан на проведение 96 анализов образцов сыворотки (плазмы) крови, включая контроли, или 12 независимых постановок ИФА по 8 определений, включая контроли.

2. ХАРАКТЕРИСТИКА НАБОРА

2.1. Принцип метода

Метод определения суммарных антител к антигенам лямблий представляет собой твердофазный иммуноферментный анализ. Специфическими компонентами набора реагентов являются антигены лямблий, иммобилизованные в лунках планшетов, конъюгат моноклональных антител к IgA, IgM, IgG человека с пероксидазой хрена, положительный и отрицательный контрольные образцы.

На первой стадии анализа при взаимодействии исследуемых образцов сывороток крови в лунках стрипов с иммобилизованными антигенами лямблий происходит связывание специфических антител и образование комплекса «антиген-антитело» на поверхности лунок. После удаления несвязавшихся компонентов сыворотки и добавления в лунки планшета конъюгата

моноклональных антител к IgA, IgM, IgG человека с пероксидазой хрена происходит включение ферментной метки в иммунный комплекс. После второй отмывки количество связавшегося конъюгата определяют цветной реакцией с использованием субстрата пероксидазы – перекиси водорода и хромогена – тетраметилбензидина. Реакцию останавливают добавлением стоп-реагента и измеряют оптическую плотность растворов в лунках при длине волны 450 нм, референс-волна в диапазоне 620–655 нм; допускается измерение при длине волны 450 нм. Интенсивность окрашивания пропорциональна концентрации суммарных антител к антигенам лямблий в анализируемом образце сыворотки.

2.2. Состав набора

В состав набора входят:

- планшет разборный (12 восьмилуночных стрипов) с иммобилизованными на внутренней поверхности лунок антигенами лямблий, готовый для использования – 1 шт.;
- положительный контрольный образец (K^+) на основе инактивированной сыворотки крови человека, содержащий IgA, IgM, IgG к антигенам лямблий, готовый для использования – 1 флакон (1,5 мл);
- отрицательный контрольный образец (K^-) на основе инактивированной сыворотки крови человека, не содержащий IgA, IgM, IgG к антигенам лямблий, готовый для использования – 1 флакон (2,5 мл);

- конъюгат моноклональных антител к IgA, IgM, IgG человека с пероксидазой хрена, концентрат – 1 флакон (1,5 мл);
- раствор для предварительного разведения сывороток (РПРС) – 1 флакон (10,0 мл);
- раствор для разведения сывороток (РРС) – 1 флакон (12,0 мл);
- 25-кратный концентрат фосфатно-солевого буферного раствора с твином (ФСБ-Т×25) – 1 флакон (28,0 мл);
- субстратный буферный раствор (СБР) – 1 флакон (13,0 мл);
- тетраметилбензидин, концентрат (ТМБ) – 1 флакон (1,0 мл);
- стоп-реагент (0,5 М серная кислота), готовый для использования – 1 флакон (12,0 мл).

Набор дополнительно комплектуется:

- пленками для заклеивания планшета – 2 шт.;
- ванночками для реагента – 2 шт.;
- наконечниками для пипеток на 5–200 мкл – 16 шт.

По согласованию с потребителем дополнительно поставляется:

- планшет для предварительного разведения исследуемых образцов – 1 шт.

3. АНАЛИТИЧЕСКИЕ И ДИАГНОСТИЧЕСКИЕ ХАРАКТЕРИСТИКИ

3.1. Перекрестные реакции при описторхозе, токсокарозе, эхинококкозе, трихинеллезе, аскаридозе не выявлены.

3.2. Специфическая активность

Чувствительность выявления иммуноглобулинов классов А, М, G к антигенам лямблий – соответствие результатов выявления набором IgA, IgM, IgG к антигенам лямблий требованиям стандартной панели предприятия (рег. № 05-2-134), аттестованной ОБТК АО «Вектор-Бест» – составляет 100%: средние арифметические значения оптической плотности для каждой пары лунок с положительными сыворотками больше либо равны величине диагностического значения оптической плотности.

Специфичность выявления иммуноглобулинов классов А, М, G к антигенам лямблий – соответствие результатов выявления набором IgA, IgM, IgG к антигенам лямблий требованиям стандартной панели предприятия (рег. № 05-2-134), аттестованной ОБТК АО «Вектор-Бест» – составляет 100%: средние арифметические значения оптической плотности для каждой пары лунок с отрицательными сыворотками меньше либо равны величине диагностического значения оптической плотности, умноженного на 0,85.

3.3. Титр стандартного образца предприятия СОП+ (рег. № 05-2-17), аттестованного ОБТК АО «Вектор-Бест», должен быть не менее 1:800.

4. МЕРЫ ПРЕДОСТОРОЖНОСТИ

4.1. Потенциальный риск применения набора – класс 1 (ГОСТ Р 51609-2000).

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 набором следует соблюдать «Правила устройства, техники безопасности, производственной санитарии, противоэпидемического режима и личной гигиены при работе в лабораториях (отделениях, отделах) санитарно-эпидемиологических учреждений системы Министерства здравоохранения СССР» (Москва, 1981 г.).

4.4. При работе с набором следует надевать одноразовые резиновые или пластиковые перчатки, так как в состав набора входят компоненты крови человека, которые следует рассматривать как потенциально инфицированные, способные длительное время сохранять и передавать ВИЧ, вирус гепатита или любой другой возбудитель вирусных 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 , дioxлор, хлорамин), приводит к серьезному искажению результатов.

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 при длине волны 450 нм;
- термостат, поддерживающий температуру $37 \pm 1^\circ\text{C}$;
- холодильник бытовой;
- пипетки полуавтоматические одноканальные с переменным объемом со сменными наконечниками, позволяющие отбирать объемы жидкости от 5 до 5000 мкл, аттестованные по значению средней дозы и сходимости результатов пипетирования;
- пипетки полуавтоматические многоканальные со сменными наконечниками, позволяющие отбирать

объемы жидкостей от 5 мкл до 200 мкл, аттестованные по значению средней дозы и сходимости результатов пипетирования;

- промывочное устройство для планшетов;
- перчатки резиновые или пластиковые;
- бумага фильтровальная лабораторная;
- флаконы вместимостью 15 мл;
- цилиндр мерный 2-го класса точности вместимостью 1000 мл;
- вода дистиллированная;
- дезинфицирующий раствор.

6. АНАЛИЗИРУЕМЫЕ ОБРАЗЦЫ

6.1. Для проведения анализа не следует использовать гемолизированную, мутную сыворотку крови, а также сыворотку, содержащую азид натрия.

6.2. Образцы сыворотки (плазмы) крови можно хранить при температуре от 2 до 8°C не более 5 суток или при температуре минус 20°C (и ниже) не более 3 мес. Допускается однократное замораживание-размораживание образцов сыворотки крови. После размораживания образцы тщательно перемешать.

6.3. Образцы сыворотки (плазмы) крови, содержащие осадок, необходимо очистить центрифугированием при 5000–10000 об/мин в течение 5 мин при температуре от 18 до 25°C.

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°C в течение времени не менее 60 мин.

7.2. Подготовка планшета

Вскрыть пакет с планшетом выше замка. Оставить на рамке необходимое для проведения анализа количество стрипов. Оставшиеся неиспользованными стрипы немедленно поместить вновь в пакет, удалить из него воздух и плотно закрыть замок.

Неиспользованные стрипы можно хранить при температуре от 2 до 8°C в течение всего срока годности набора.

7.3. Приготовление рабочего буферного раствора

Внести в мерный цилиндр необходимое количество ФСБ-Т×25 и добавить соответствующее количество дистиллированной воды. При выпадении осадка солей в концентрате необходимо прогреть его при температуре от 30 до 40°C до полного растворения осадка.

В таблице приведен расход реагентов в зависимости от количества используемых стрипов.

Приготовленный рабочий буферный раствор можно хранить при температуре от 2 до 8°C не более 5 суток.

7.4. Подготовка положительного (K^+) и отрицательного (K^-) контрольных образцов

Контрольные образцы готовы к использованию. Перед использованием встряхнуть.

Контрольные образцы после первого вскрытия флаконов можно хранить при температуре от 2 до 8°C в течение всего срока годности набора.

7.5. Предварительное разведение исследуемых образцов

В лунки планшета для предварительного разведения образцов внести по 90 мкл раствора для предварительного разведения сывороток (РПРС) и добавить по 10 мкл цельного образца сыворотки (плазмы) крови, тщательно перемешать. При этом темно-красный цвет должен измениться на желтый. Если изменения цвета не произошло, ИФА данного образца может дать неправильный результат. При разведении плазмы цвет раствора в лунке меняется незначительно.

Предварительно разведенные исследуемые образцы можно хранить при температуре от 18 до 25°C не более 3 ч.

7.6. Приготовление рабочего раствора конъюгата

Готовится в отдельном чистом флаконе или в пластиковой ванночке для реагента из концен-

Таблица расхода компонентов набора реагентов

Кол-во одновременно используемых стрипов	Рабочий раствор конъюгата		Рабочий раствор тетраметилбен- зидина		Рабочий буфер- ный раствор	
	Конъюгат, концентрат, мл	РБР, мл	ТМБ, концентрат, мл	СБР, мл	ФСБ-Т, концентрат, мл	Дистил. вода, мл
1	0,1	1,0	0,05	1,0	2,0	до 50
2	0,2	2,0	0,10	2,0	4,0	до 100
3	0,3	3,0	0,15	3,0	6,0	до 150
4	0,4	4,0	0,20	4,0	8,0	до 200
5	0,5	5,0	0,25	5,0	10,0	до 250
6	0,6	6,0	0,30	6,0	12,0	до 300
7	0,7	7,0	0,35	7,0	14,0	до 350
8	0,8	8,0	0,40	8,0	16,0	до 400
9	0,9	9,0	0,45	9,0	18,0	до 450
10	1,0	10,0	0,50	10,0	20,0	до 500
11	1,1	11,0	0,55	11,0	22,0	до 550
12	1,2	12,0	0,60	12,0	24,0	до 600

трата конъюгата и рабочего буферного раствора не более, чем за 5–10 мин до окончания первой инкубации.

Рабочий раствор конъюгата можно хранить при температуре от 18 до 25°C не более 3 ч.

В таблице приведен расход реагентов в зависимости от количества используемых стрипов.

7.7. Приготовление рабочего раствора ТМБ

Готовится в отдельном чистом флаконе или в пластиковой ванночке для реагента из концентрата ТМБ и СБР не более, чем за 5-10 мин до окончания первой инкубации.

Рабочий раствор ТМБ можно хранить при температуре от 18 до 25°C не более 3 ч.

Необходимо исключить воздействие прямого света на раствор ТМБ.

В таблице приведен расход реагентов в зависимости от количества используемых стрипов.

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.

7.8. Стоп-реагент готов к использованию.

ПРОВЕДЕНИЕ ИФА

7.9. Внесение контрольных образцов

В лунку А-1 внести 100 мкл положительно-го контрольного образца (K^+). В лунки В-1 и С-1 внести по 100 мкл отрицательного контрольного образца (K^-).

7.10. Внесение исследуемых образцов сыворотки (плазмы)

В остальные лунки внести по 90 мкл раствора для разведения сывороток и по 10 мкл предварительно разведенных исследуемых образцов (п. 7.5.), тщательно перемешать.

Для повышения достоверности результатов исследуемые образцы рекомендуется анализировать в дублях, используя для каждого образца по две лунки.

Внесение образцов необходимо производить быстро, в течение времени не более 5–7 мин.

7.10.1. Определение титра исследуемых образцов

При определении титра антител тестирование исследуемых образцов произвести в семи последовательных двукратных разведениях в интервале от 1:100 до 1:12800. В соответствующие лунки горизонтального ряда А внести по 180 мкл РРС и по 20 мкл предварительно разведенных исследуемых образцов (п. 7.5.), тщательно перемешать. В соответствующие лунки рядов В–Н внести по 100 мкл РРС. Каждый образец титровать, перенося по 100 мкл раствора из лунок предыдущего ряда в лунки последующего от А до Н, тщательно перемешивая пипетированием. После разлития из каждой лунки последнего ряда Н удалить в дезинфицирующий раствор по 100 мкл раствора, оставляя в лунках по 100 мкл.

7.11. Планшет заклеить пленкой и инкубировать в течение 30 мин при температуре $37 \pm 1^\circ\text{C}$.

7.12. 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 цикла промывки.* По окончании промывки остатки влаги из лунок тщательно удалить, постукивая перевернутым планшетом по фильтровальной бумаге.

7.13. Внести во все лунки по 100 мкл рабочего раствора конъюгата.

Для внесения рабочего раствора конъюгата использовать ванночку для реагента и одноразовые наконечники.

Оставшийся после проведения ИФА рабочий раствор конъюгата из флакона или пластиковой ванночки удалить в сосуд с дезинфицирующим раствором.

7.14. Планшет заклеить пленкой и инкубировать в течение 30 мин при температуре $37 \pm 1^\circ\text{C}$.

7.15. По окончании второй инкубации удалить содержимое лунок и промыть планшет, как указано в п. 7.12.

7.16. Внести во все лунки по 100 мкл рабочего раствора ТМБ (см п. 7.7.).

Для внесения рабочего раствора ТМБ использовать ванночку для реагента и одноразовые наконечники.

Планшет инкубировать в защищенном от света месте в течение 25 мин при температуре от 18 до 25°C.

Оставшийся после проведения ИФА рабочий раствор ТМБ удалить из стеклянного флакона или пластиковой ванночки в сосуд с дезинфицирующим раствором.

7.17. Внести во все лунки с той же скоростью и в той же последовательности, как и раствор ТМБ, по 100 мкл стоп-реагента.

8. РЕГИСТРАЦИЯ РЕЗУЛЬТАТОВ

8.1. Измерить величину оптической плотности растворов в лунках на спектрофотометре вертикального сканирования в двухволновом режиме: при основной длине волны 450 нм и длине волны сравнения в диапазоне 620–655 нм; допускается измерение при длине волны 450 нм (выведение спектрофотометра на нулевой уровень («бланк»)) осуществлять по воздуху). Измерение проводить не позднее 5 мин после остановки реакции.

9. УЧЕТ РЕЗУЛЬТАТОВ РЕАКЦИИ

9.1. Рассчитать средние арифметические значения оптической плотности в лунках с отрицательным контрольным образцом ($ОП_{cp}K^-$).

9.2. Среднее значение ОП в лунках с K^- не должно превышать 0,30 ед. опт. пл.

Значение ОП в лунке с K^+ должно быть не менее, чем 0,80 ед. опт. пл.

9.3. Только при соблюдении положений п. 9.2. можно учитывать результаты, полученные для анализируемых образцов сыворотки (плазмы) крови.

9.4. На основании полученных данных вычислить диагностическое значение оптической плотности ($ОП_Д$).

$$ОП_Д = ОП_{ср. K^-} + 0,2$$

9.5. Результат анализа считается **положительным**, если $ОП_{обр.} \geq ОП_Д$.

Результат анализа считается **отрицательным**, если $ОП_{обр.} \leq 0,85 \times ОП_Д$.

где $ОП_{обр.}$ – оптическая плотность в лунке с анализируемым образцом сыворотки (плазмы) крови.

Результат анализа считается **сомнительным**, если $0,85 \times ОП_Д < ОП_{обр.} < ОП_Д$. Рекомендуется повторно провести иммуноферментный анализ данного образца.

9.6. За титр положительной сыворотки (плазмы) принимают ее наибольшее разведение, при котором оптическая плотность образца $ОП_{обр.} \geq ОП_Д$.

10. УСЛОВИЯ ХРАНЕНИЯ И ЭКСПЛУАТАЦИИ НАБОРА

10.1. Транспортирование набора должно проводиться при температуре от 2 до 8°C. Допускается транспортирование при температуре до 25°C не более 10 сут. Замораживание компонентов набора не допускается.

10.2. Набор реагентов должен храниться в упаковке предприятия-изготовителя при температуре от 2 до 8°C в течение всего срока годности (9 мес). Замораживание компонентов набора не допускается.

10.3. Дробное использование набора может быть реализовано в течение всего срока годности набора:

- неиспользованные стрипы после первого вскрытия пакета можно хранить в закрытом на замок пакете при температуре от 2 до 8°C в течение всего срока годности набора;
- 25-кратный концентрат фосфатно-солевого буферного раствора с твином, субстратный буферный раствор, раствор для разведения сывороток, раствор для предварительного разведения сывороток, концентрат конъюгата, концентрат тетраметилбензидина и стоп-реагент после вскрытия флаконов можно хранить в плотно закрытых флаконах при температуре от 2 до 8°C в течение всего срока годности набора;
- положительный и отрицательный контрольные образцы можно хранить при температуре от 2 до 8°C в течение всего срока годности набора;

- рабочий буферный раствор можно хранить при температуре от 2 до 8°C не более 5 суток;
- рабочий раствор конъюгата можно хранить при температуре от 18 до 25°C не более 3 ч;
- рабочий раствор тетраметилбензидина можно хранить при температуре от 18 до 25°C не более 3 ч.

10.4. Для получения надежных результатов необходимо строгое соблюдение инструкции по применению набора.

10.5. При постановке ИФА нельзя использовать компоненты из наборов разных серий или смешивать их при приготовлении растворов, кроме неспецифических компонентов (ФСБ-Т×25, СБР, РПРС, стоп-реагент), которые взаимозаменяемы во всех наборах АО «Вектор-Бест».

Нельзя использовать реагенты из наборов других фирм-производителей.

По вопросам, касающимся качества набора «Лямблия-антитела-ИФА-БЕСТ»,
обращаться в АО «Вектор-Бест» по адресу:
630559, Новосибирская обл.,
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.

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.

При динамическом наблюдении пациента для получения результатов, адекватно отражающих изменение концентрации иммуноглобулинов классов А, М и G 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 в соответствии п. 4.7 настоящей инструкции и согласно 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 субстрата;

- 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 рабочего раствора ТМБ;
- перед отбором ТМБ из флакона необходимо обрабатывать конус пипетки (внутреннюю и внешнюю поверхности) 70% этиловым спиртом, так как малейшее загрязнение пипеток конъюгатом может привести к контаминации всего содержимого флаконов с СБР и ТМБ;
- 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.

4. Количественная оценка результатов анализа

Результаты анализа можно оценить по коэффициенту позитивности (КП), рассчитывая отношение ОП в лунке с образцом пациента относительно ОП_Д. (п. 9.4.).

Для расчета коэффициента позитивности образцов, имеющих ОП₄₅₀ ≤ 3,5 о.е., использовать формулу:

$$КП_{обр.} = \frac{ОП_{450 \text{ обр.}}}{ОП_{Д}},$$

где 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 ОП₄₅₀ > 3,5 о.е., использовать формулу:

$$КП_{обр.} = 3,2 \times \frac{ОП_{450 \text{ обр.}}}{ОП_{Д}},$$

где ОП_{405 обр.} — ОП образца, полученная в двухволновом режиме 405 / 620–655 нм (или только с фильтром 405 нм).

Результат анализа **положительный**, если $KП_{обр.} \geq 1$, где $KП_{обр.}$ – коэффициент позитивности исследуемого образца.

Результат анализа **отрицательный**, если $KП_{обр.} \leq 0,85$.

Результат анализа **сомнительный**, если соответствующее ему значение $KП_{обр.}$ попадает в интервал от 0,85 до 1,0.

Расчет КП целесообразно проводить для оценки концентрации специфических антител в исследуемых образцах и при наблюдении за изменением концентрации суммарных антител к антигенам лямблий в динамике в парных образцах сывороток.

5. Диагностическая значимость полученных результатов

Лямблии обитают в проксимальном отделе тонкой кишки, поэтому для лямблиоза характерно развитие местных иммунологических реакций. Однако и в сыворотках крови инвазированных лямблиями людей выявляются антитела к антигенам лямблий, относящиеся к различным классам иммуноглобулинов. Показано, что попадание антигенов лямблий в периферическую кровь увеличивается при резорбции слизистой оболочки кишечника, проницаемость которой, как известно, возрастает при ее воспалении. В связи с этим выявление антител к антигенам лямблий в сыворотке крови может свидетельствовать о наличии патологического процесса.

**6. Краткая схема проведения ИФА
для набора реагентов
«Лямблия-антитела-ИФА-БЕСТ»**

*Использовать только после тщательного
ознакомления с инструкцией!*

- Внести:** по 100 мкл K^+ и K^- ;
по 90 мкл РРС и по 10 мкл
предварительно разведенных
анализируемых образцов.
- Инкубировать:** 30 мин, 37°C.
- Промыть:** рабочим буферным раствором,
400 мкл, 5 раз.
- Внести:** по 100 мкл рабочего раствора
конъюгата.
- Инкубировать:** 30 мин, 37°C.
- Промыть:** рабочим буферным раствором,
400 мкл, 5 раз.
- Внести:** по 100 мкл рабочего раствора
тетраметилбензидина.
- Инкубировать:** 25 мин, 18–25°C, в темноте.
- Внести:** по 100 мкл стоп-реагента.
- Измерить:** ОП при 450 нм / референсная
длина волны 620–655 нм.

7. Графические символы

	Номер по каталогу		Медицинское изделие для диагностики <i>in vitro</i>
	Содержимого достаточно для проведения n-количества тестов		Не стерильно
	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) 363-35-56.

14.05.18.

ВЕКТОР



Набор реагентов
для иммуноферментного выявления
иммуноглобулинов класса G
к вирусу Крымской-Конго
геморрагической лихорадки

ИНСТРУКЦИЯ ПО ПРИМЕНЕНИЮ

Утверждена 04.05.2010
Приказом Росздравнадзора № 3682-Пр/10

ВекторКрым-КГЛ-IgG

НАБОР РЕАГЕНТОВ
D-5052

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 (K^+), инаktivированный – на основе инаktivированной сыворотки крови человека, содержащей IgG к вирусу К-КГЛ – прозрачная жидкость красного цвета – 1 флакон (1,5 мл);
- отрицательный контрольный образец (K^-), инаktivированный – на основе инаktivированной

- 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)°C – 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°C;
- холодильник бытовой, поддерживающий температуру от 2 до 8°C;
- 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°C не более

5 суток, а также хранившихся при минус 20°C 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°C .

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°C.

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 посуды (*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°C 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°C в течение всего срока годности набора.

7.2.2. Приготовление контрольных образцов (K⁺, K⁻)

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 кратные разведенные сыворотки можно хранить до 3 часов при температуре от 18 до 25°C.

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)°C до полного растворения осадка.

Приготовленный промывочный раствор можно хранить при температуре от 2 до 8°C 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 (K^-) и в одну лунку, например, С-1, – 100 мкл положительного контрольного образца (K^+).

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 $(37 \pm 1)^\circ\text{C}$.

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 $(37 \pm 1)^\circ\text{C}$.

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 тетраметилбензидаина (см. п. 7.2.6.).

Для внесения раствора тетраметилбензидаина 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°C .

7.3.7. Внести во все лунки с той же скоростью и в той же последовательности, как и раствор тетраметилбензидаина, по 100 мкл стоп-реагента.

В случае попадания на кожу раствора тетраметилбензидаина 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:

$$ОП_{крит.} = ОП_{ср.} (K^-) + 0,2$$

где: $ОП_{ср.} (K^-)$ – среднее арифметическое значение оптической плотности в лунках с отрицательным контрольным образцом.

9.2. Среднее значение оптической плотности в лунках с отрицательным контрольным образцом $ОП_{ср.} (K^-)$ 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. Рекомендуются 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 дубликate 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 K^- , в лунку С-1 внести 100 мкл K^+ . 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 получают последовательные 2-кратные разведения исследуемых образцов.

Планшет закрыть и инкубировать при температуре $(37 \pm 1)^\circ\text{C}$ 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 $\text{ОП}_{\text{ср. К}^-}$.

10. УСЛОВИЯ ХРАНЕНИЯ И ПРИМЕНЕНИЯ НАБОРА

10.1. Транспортирование набора – при температуре от 2 до 8°C . Допускается транспортирование при температуре до 25°C не более 10 сут. Замораживание компонентов набора не допускается.

10.2. Хранение набора в упаковке предприятия-изготовителя – при температуре от 2 до 8°C . 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 $ОП_{450} \leq 3,5$ о.е., использовать формулу:

$$КП_{обр.} = \frac{ОП_{450 \text{ обр.}}}{ОП_{крит.}},$$

где 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 $ОП_{450} > 3,5$ о.е., использовать формулу:

$$КП_{обр.} = 3,2 \times \frac{ОП_{405\text{ обр.}}}{ОП_{крит.}},$$

где $ОП_{405\text{ обр.}}$ – 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 K^+ , K^- ;
по 90 мкл РРС и по 10 мкл
предварительно разведенных
анализируемых образцов.
- Инкубировать:** 60 мин, 37°C.
- Промыть:** промывочным раствором,
400 мкл, 5 раз.
- Внести:** по 100 мкл конъюгата.
- Инкубировать:** 30 мин, 37°C.
- Промыть:** промывочным раствором,
400 мкл, 5 раз.
- Внести:** по 100 мкл раствора тетраме-
тилбензидина.
- Инкубировать:** 25 мин, 18–25°C, в темноте.
- Внести:** по 100 мкл стоп-реагента.
- Измерить:** ОП при 450 нм / референсная
длина волны 620–655 нм.

6. Графические символы

	Номер по каталогу		Медицинское изделие для диагностики <i>in vitro</i>
	Содержимого достаточно для проведения n-количества тестов		Не стерильно
	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



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

ВекторКрым-КГЛ-IgM

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 планшето́в 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 (K^+), 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 (K^-), 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 мышинных 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 тетраметилбензидаина (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°C;
- холодильник бытовой, поддерживающий температуру от 2 до 8°C;
- 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°C не более

5 суток, а также хранившихся при минус 20°C 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°C .

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°C.

– 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 посуды (*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 негативно-го 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°C 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°C в течение всего срока годности набора.

7.2.2. Приготовление контрольных образцов (K⁺, K⁻)

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 кратные разведенные сыворотки можно хранить до 3 часов при температуре от 18 до 25°C.

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)°C до полного растворения осадка.

Приготовленный промывочный раствор можно хранить при температуре от 2 до 8°C 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 (K^-) и в одну лунку, например, С-1, — 100 мкл положительного контрольного образца (K^+).

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 $(37 \pm 1)^\circ\text{C}$.

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 $(37 \pm 1)^\circ\text{C}$.

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°C .

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:

$$\text{ОП}_{\text{крит.}} = \text{ОП}_{\text{ср.}} (K^-) + 0,2,$$

где: ОП_{ср.} (K⁻) — 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.} (K⁻) 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.} ≥ ОП_{крит.};

Результат анализа считают **сомнительным**, если $0,8 \times \text{ОП}_{\text{крит.}} < \text{ОП}_{\text{обр.}} < \text{ОП}_{\text{крит.}}$;

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. Рекомендуются 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°C. Допускается транспортирование при температуре до 25°C не более 10 сут. Замораживание компонентов набора не допускается.

10.2. Хранение набора в упаковке предприятия-изготовителя – при температуре от 2 до 8°C. 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 K^+ , K^- ;
по 90 мкл РРС и по 10 мкл
предварительно разведенных
анализируемых образцов.

Инкубировать: 60 мин, 37°C.

Промыть: промывочным раствором,
400 мкл, 5 раз.

Внести: по 100 мкл конъюгата.

Инкубировать: 90 мин, 37°C.

Промыть: промывочным раствором,
400 мкл, 5 раз.

Внести: по 100 мкл раствора тетраме-
тилбензидина.

Инкубировать: 25 мин, 18–25°C, в темноте.

Внести: по 100 мкл стоп-реагента.

Измерить: ОП при 450 нм / референсная
длина волны 620–655 нм.

6. Графические символы

	Номер по каталогу		Медицинское изделие для диагностики <i>in vitro</i>
	Содержимого достаточно для проведения n-количества тестов		Не стерильно
	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.

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БЕКТОР



Набор реагентов
для иммуноферментного выявления
антигена вируса Крымской-Конго
геморрагической лихорадки

ИНСТРУКЦИЯ ПО ПРИМЕНЕНИЮ

Утверждена 04.05.2010
Приказом Росздравнадзора № 3681-Пр/10

ВектоКрым-КГЛ-антиген

НАБОР РЕАГЕНТОВ
D-5056

1. НАЗНАЧЕНИЕ

1.1. Набор предназначен для выявления вируса Крымской-Конго геморрагической лихорадки в клещах и других вирусосодержащих 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 $37 \pm 1^\circ\text{C}$;
- холодильник бытовой, поддерживающий температуру от 2 до 8°C ;
- 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°C .

- 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 выдерживать 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°C.

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°C.

– 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 посуды (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 негативно-го 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°C 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°C в течение всего срока годности набора.

7.2.2. Приготовление контрольных образцов (K⁺, K⁻)

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)°C до полного растворения осадка.

Приготовленный промывочный раствор можно хранить при температуре от 2 до 8°C 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 (K^-) и в одну лунку, например, С-1, – 100 мкл положительного контрольного образца (K^+).

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 $(37 \pm 1)^\circ\text{C}$.

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 $(37 \pm 1)^\circ\text{C}$.

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 тетраметилбензи-
дина использовать пластиковую ванночку и одно-
разовые наконечники, входящие в состав набора.*

Планшет выдержать в защищенном от све-
та месте в течение 25 мин при температуре от 18
до 25°C.

7.3.7. Внести во все лунки с той же скоростью
и в той же последовательности, как и раствор те-
траметилбензидина, по 100 мкл стоп-реагента.

*В случае попадания на кожу раствора
тетраметилбензидина или стоп-реагента не-
обходимо немедленно смыть их водой с мылом.*

8. РЕГИСТРАЦИЯ РЕЗУЛЬТАТОВ

Результаты ИФА регистрировать с помо-
щью спектрофотометра, измеряя оптическую
плотность (ОП) в двухволновом режиме: основ-
ной фильтр – 450 нм, референс-фильтр в диа-
пазоне 620–650 нм. Допускается регистрация
результатов только с фильтром 450 нм (выве-
дение спектрофотометра на нулевой уровень
(бланк) осуществлять по воздуху).

Время между остановкой реакции и изме-
рением оптической плотности не должно превы-
шать 5 мин.

9. УЧЕТ РЕЗУЛЬТАТОВ АНАЛИЗА

9.1. Рассчитать среднее арифметическое
значение оптической плотности в лунках с отри-
цательным контрольным образцом.

На основании полученных данных вычислить критическое значение оптической плотности ($ОП_{\text{крит.}}$) по формуле:

$$ОП_{\text{крит.}} = ОП_{\text{ср.}}(K^-) + 0,2$$

где: $ОП_{\text{ср.}}(K^-)$ – среднее арифметическое значение оптической плотности в лунках с отрицательным контрольным образцом.

9.2. Среднее значение оптической плотности в лунках с отрицательным контрольным образцом $ОП_{\text{ср.}}(K^-)$ 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 $ОП_{\text{обр.}} \geq ОП_{\text{крит.}}$;

Результат анализа считают **сомнительным**, если $0,8 \times ОП_{\text{крит.}} < ОП_{\text{обр.}} < ОП_{\text{крит.}}$;

Результат анализа считают **отрицательным**, если $ОП_{\text{обр.}} \leq 0,8 \times ОП_{\text{крит.}}$;

где $ОП_{\text{обр.}}$ – 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°C. Допускается транспортирование при температуре до 25°C не более 10 сут. Замораживание не допускается.

10.2. Хранение набора в упаковке предприятия-изготовителя – при температуре от 2 до 8°C. 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 K^+ , K^- ;
по 100 мкл анализируемых образцов.
- Инкубировать:** 60 мин, 37°C.
- Промыть:** промывочным раствором, 400 мкл, 5 раз.
- Внести:** по 100 мкл конъюгата.
- Инкубировать:** 60 мин, 37°C.
- Промыть:** промывочным раствором, 400 мкл, 5 раз.
- Внести:** по 100 мкл раствора тетраметилбензидина.
- Инкубировать:** 25 мин, 18–25°C, в темноте.
- Внести:** по 100 мкл стоп-реагента.
- Измерить:** ОП при 450 нм / референсная длина волны 620–655 нм.

5. Графические символы

	Номер по каталогу		Медицинское изделие для диагностики <i>in vitro</i>
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	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.

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БЕКТОР



Набор реагентов
для иммуноферментного выявления
иммуноглобулинов класса М
к вирусу клещевого энцефалита

Инструкция утверждена 15.09.16

ВектоВКЭ-IgM

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 (K^+) на основе инактивированной сыворотки крови человека, содержащий IgM к ВКЭ, готовый для использования – 1 флакон (1,5 мл);
- отрицательный контрольный образец (K^-) 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 $(37 \pm 1)^\circ\text{C}$;
- 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, гемолизированную 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°C не более 5 суток при условии отсутствия микробной контаминации или при температуре минус 20°C (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°C.

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°C 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°C 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°C до полного растворения осадка.

Приготовленный промывочный раствор можно хранить при температуре от 2 до 8°C не более 5 суток.

Таблица расхода компонентов набора реагентов

Кол-во используемых стрипов	Промывочный раствор		Смесь конъюгата 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°C 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°C 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°C в течение 3 часов.

Конъюгат после первого вскрытия флакона можно хранить при температуре от 2 до 8°C в течение всего срока годности.

Антиген после первого вскрытия флакона можно хранить при температуре от 2 до 8°C 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°C 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°C в течение всего срока годности.

ПРОВЕДЕНИЕ ИФА

7.9. Внесение образцов.

В лунки А-1, В-1 внести по 100 мкл отрицательного контрольного образца (K^-).

В лунку С-1 внести 100 мкл положительного контрольного образца (K^+).

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 $(37 \pm 1)^\circ\text{C}$.

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 $(37 \pm 1)^\circ\text{C}$.

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 тетраметилбензидаина.

Планшет выдержать в защищенном от света месте в течение 25 мин при температуре от 18 до 25°C .

Для внесения раствора тетраметилбензидаина 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 ($ОП_{\text{крит.}}$) по формуле:

$$ОП_{\text{крит.}} = ОП_{\text{ср.}} K^- + 0,2$$

где $ОП_{\text{ср.}} K^-$ – 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°C. Допускается транспортирование при температуре до 25°C не более 10 суток.

10.2. Хранение набора в упаковке предприятия-изготовителя должно осуществляться при температуре от 2 до 8°C 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°C 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°C в течение всего срока годности;

- промывочный раствор можно хранить при температуре от 2 до 8°C 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 дубли 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 K^+ и в **4 лунки** – по 100 мкл K^- .

Например, в лунки А-1 и А-2, В-1 и В-2 внести по 100 мкл K^- , в лунки С-1, С-2 – по 100 мкл K^+ .

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°C.

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°C.

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°C.

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 K^+ , K^- ;
по 90 мкл РРС и по 10 мкл
предварительно разведенных
анализируемых образцов.
- Инкубировать:** 60 мин, 37°C.
- Промыть:** промывочным раствором,
400 мкл, 5 раз.
- Внести:** по 100 мкл смеси конъюгата и
антигена ВКЭ.
- Инкубировать:** 60 мин, 37°C.
- Промыть:** промывочным раствором,
400 мкл, 5 раз.
- Внести:** по 100 мкл раствора тетраме-
тилбензидина.
- Инкубировать:** 25 мин, 18–25°C в темноте.
- Внести:** по 100 мкл стоп-реагента.
- Измерить:** ОП при 450 нм / референсная
длина волны 620–655 нм.

5. Графические символы

	Номер по каталогу		Медицинское изделие для диагностики <i>in vitro</i>
	Содержимого достаточно для проведения n-количества тестов		Не стерильно
	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



Набор реагентов
для иммуноферментного выявления
антигена вируса клещевого энцефалита

ИНСТРУКЦИЯ ПО ПРИМЕНЕНИЮ

Утверждена 05.12.2016

ВекторВКЭ-антиген

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 (K^+) – буферный раствор, содержащий антиген ВКЭ, готовый для использования – 1 флакон (1,5 мл);
- отрицательный контрольный образец (K^-) – 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 клиничко-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°C или в течение 3-х месяцев при температуре минус (18–60)°C. 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°C 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°C 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°C до полного растворения осадка.

Приготовленный промывочный раствор можно хранить при температуре от 2 до 8°C 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°C 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°C 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°C 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°C в течение всего срока годности набора.

ПРОВЕДЕНИЕ ИФА

7.8. В лунки А-1, В-1 внести по 100 мкл отрицательного контрольного образца (K^-).

В лунку С-1 внести 100 мкл положительного контрольного образца (K^+).

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°C.

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°C. Допускается транспортирование при температуре до 25°C не более 10 суток.

10.2. Хранение набора в упаковке предприятия-изготовителя должно осуществляться при

температуре от 2 до 8°C 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°C 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°C в течение всего срока годности;
- промывочный раствор можно хранить при температуре от 2 до 8°C 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°C, 400 об/мин.

Промыть: промывочным раствором, 400 мкл, 5 раз.

Внести: по 100 мкл конъюгата.

Инкубировать: 1 час, 24–26°C, 400 об/мин.

Промыть: промывочным раствором, 400 мкл, 5 раз.

Внести: по 100 мкл раствора тетраметилбензидина.

Инкубировать: 25 мин, 18–25°C, в темноте.

Внести: 100 мкл стоп-реагента.

Измерить: ОП при 450 нм / референсная длина волны 620–655 нм.

3. Графические символы

	Номер по каталогу		Медицинское изделие для диагностики <i>in vitro</i>
	Содержимого достаточно для проведения n-количества тестов		Не стерильно
	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.

АКЦИОНЕРНОЕ ОБЩЕСТВО
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ISO 9001 и ISO 13485

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332-67-49, 332-67-52

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Internet: www.vector-best.ru



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



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

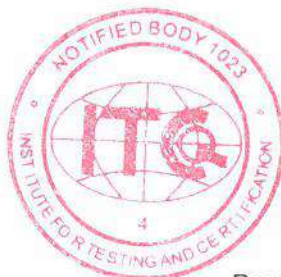
Name: **AmpliSens® CMV-FEP PCR kit**

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): -

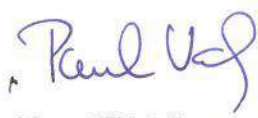
Model(s): variant FRT-100 F

Classification: List B

GMDN: 30798



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® EBV / CMV / HHV6-screen-FRT
PCR kit**

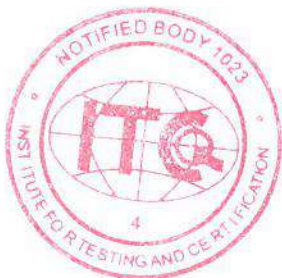
Trade name(s): -
Model(s): variant FRT-100 F
Classification: List B
GMDN: 61348

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



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® *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



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® *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
Revision: k



Mgr. Jiří Heš
Representative of the Notified Body No. 1023



Notified Body 1023
INSTITUTE FOR TESTING AND CERTIFICATION, Inc.,
trida 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® *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

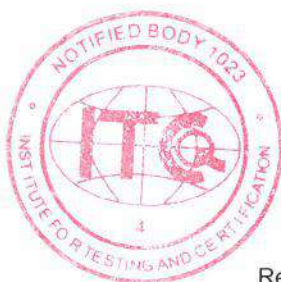
Name: **AmpliSens® *Mycoplasma pneumoniae* /
Chlamydomonas pneumoniae-FEP PCR kit**

Trade name(s): -

Model(s): variant FEP (0.2 ml tubes)

Classification: List B

GMDN: 58957



Date: 2022-04-28
Revision: k

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® *Mycoplasma pneumoniae* /
Chlamydomonas 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:

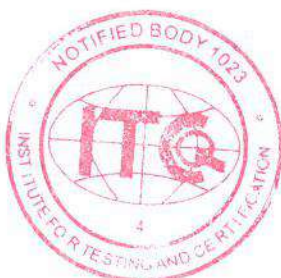
List B

GMDN:

61144

Facility(ies):

Federal Budget Institute of Science “Central Research Institute for Epidemiology”
3a Novogireevskaya Street, Moscow 111123, Russia



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

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



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

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 25 th January 2022



Date: 2022-04-28
Revision: k

Mgr. Jiří Heš
Representative of the Notified Body No. 1023

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



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 <i>in Vitro</i> 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. třída Tomáše Bati 299 Louky, 763 02 Zlín, 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:	Zlín, Czech Republic, 2022-04-28

Signed

Full name: Vasily G. Akimkin
Title: Director



Valid from 2022-04-28

Valid until 2025-05-26

NoNo	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 / HHV6-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



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: ecolix@ecolix.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 <i>in Vitro</i> Diagnostic Devices
Conformity Assessment Route:	Annex III (IVDD)

Signed _____

Full name: Vasiliy G. Akimkin
Title: Director



Valid from 25.05.2022

№№	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® Ascariidosis-FRT PCR kit	H-1971-1-CE
6.	AmpliSens® <i>Bacillus anthracis</i> -FRT PCR kit	R-B41(RG)-CE
7.	AmpliSens® <i>Bordetella</i> multi-FRT PCR kit	R-B84-100-F(RG,iQ,Dt)-CE
8.	AmpliSens® <i>Borrelia burgdorferi sensu lato</i> -FRT PCR kit	R-B37(RG)-CE
9.	AmpliSens® <i>Borrelia miyamotoi</i> -FRT PCR kit	H-2791-1-CE H-2792-1-4-CE
10.	AmpliSens® BRCA1-FRT PCR kit	S-3901-1-CE
11.	AmpliSens® <i>Brucella</i> spp.-FRT PCR kit	R-B10(RG)-CE
12.	AmpliSens® <i>C.albicans</i> / <i>C.glabrata</i> / <i>C.krusei</i> -MULTIPRIME-FRT PCR kit	R-F3-F(RG,iQ)-CE
13.	AmpliSens® <i>Candida albicans</i> -FEP PCR kit	F1-100-R0,2-FEP-CE
14.	AmpliSens® <i>Candida albicans</i> -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® <i>Corynebacterium diphtheriae</i> / <i>tox-genes</i> -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® <i>Coxiella burnetii</i> -FRT PCR kit	R-B85-50-F(RG,iQ,Mx,Dt)-CE
20.	AmpliSens® <i>Cryptococcus neoformans</i> -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 / <i>Candida</i> -FRT PCR kit	R-F5-100-FT(RG,CFX)-CE
35.	AmpliSens® Florocenosis / <i>Mycoplasma</i> -FRT PCR kit	R-B75-100-FT(RG,iQ,Mx)-CE
36.	AmpliSens® <i>Giardia lamblia</i> -FRT PCR kit	H-2821-1-CE H-2822-1-4-CE

№№	Description	Product Code (for reference only)
37.	AmpliSens® <i>Gardnerella vaginalis</i> -FEP PCR kit	B7-100-R0,2-FEP-CE
38.	AmpliSens® <i>Gardnerella vaginalis</i> -FRT PCR kit	R-B7-F(RG,iQ)-CE
39.	AmpliSens® Genoscreen-IL28B-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® <i>Helicobacter pylori</i> -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® HHV6-screen-titre-FRT PCR kit	R-V10-T(RG,iQ,Mx)-CE
45.	AmpliSens® HHV7-screen/monitor-FRT PCR kit	H-2431-1-1-CE
46.	AmpliSens® HHV8-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

NoNo	Description	Product Code (for reference only)
68.	AmpliSens® <i>Legionella pneumophila</i> -FEP PCR kit	B50-R0,2-FEP-CE
69.	AmpliSens® <i>Legionella pneumophila</i> -FRT PCR kit	R-B50(RG)-CE
70.	AmpliSens® <i>Leptospira</i> -FRT PCR kit	R-B49(RG,iQ)-CE
71.	AmpliSens® Leucosis Quantum <i>M-bcr</i> -FRT PCR kit	TR-O1(RG,iQ,Mx,A)-CE
72.	AmpliSens® <i>Listeria monocytogenes</i> -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® <i>MRSA</i> -screen-titre-FRT PCR kit	R-B78-100-FT(RG,iQ)-CE
79.	AmpliSens® <i>MTC</i> -diff-FRT PCR kit	R-B80(RG,iQ,Dt,SC)-CE
80.	AmpliSens® <i>MTC</i> -MDR-FRT PCR kit	H-3611-1-CE H-3612-1-4-CE
81.	AmpliSens® <i>MTC</i> -FEP PCR kit	B57-FEP-CE
82.	AmpliSens® <i>MTC</i> -FRT PCR kit	R-B57(RG,iQ,SC,Dt)-CE
83.	AmpliSens® <i>MTHFR</i> -SNP-FRT PCR kit	S-3721-1-CE S-3722-1-4-CE
84.	AmpliSens® <i>Mycoplasma genitalium</i> -FEP PCR kit	B4-100-R0,2-FEP-CE
85.	AmpliSens® <i>Mycoplasma genitalium</i> -FRT PCR kit	R-B4(RG)-CE R-B4-F(RG,iQ)-CE
86.	AmpliSens® <i>Mycoplasma hominis</i> -FEP PCR kit	B3-100-R0,2-FEP-CE
87.	AmpliSens® <i>Mycoplasma hominis</i> -FRT PCR kit	R-B3(RG)-CE R-B3-F(RG,iQ)-CE
88.	AmpliSens® <i>M.genitalium</i> -ML/FQ-Resist-FRT PCR kit	H-3971-1-CE
89.	AmpliSens® <i>N.meningitidis</i> / <i>H.influenzae</i> / <i>S.pneumonia</i> -FRT PCR kit	R-B25(RG,iQ)-CE
90.	AmpliSens® <i>Neisseria gonorrhoeae</i> -screen-FEP PCR kit	B51-100-R0,2-FEP-CE
91.	AmpliSens® <i>Neisseria gonorrhoeae</i> -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® <i>Norovirus</i> GI / GII-FRT PCR kit	H-2751-1-3-CE
94.	AmpliSens® <i>Parvovirus</i> B19-FRT PCR kit	R-V49(RG,iQ,Mx)-CE
95.	AmpliSens <i>Plasmodium</i> spp. / <i>P.falciparum</i> / <i>P.vivax</i> -FRT PCR kit	H-3981-1-CE H-3982-1-4-CE
96.	AmpliSens® <i>Pneumocystis jirovecii</i> (<i>carinii</i>)-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® <i>Poliovirus</i> -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

No№	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® <i>Rickettsia conorii</i> -FRT PCR kit	H-2741-1-CE H-2742-1-4-CE
105.	AmpliSens® <i>Rickettsia</i> 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® <i>Shigella</i> spp. and <i>EIEC</i> / <i>Salmonella</i> spp. / <i>Campylobacter</i> spp.-FRT PCR kit	R-B44(RG,iQ)-CE
110.	AmpliSens® <i>Streptococcus agalactiae</i> -screen-titre-FRT PCR kit	R-B77-100-FT(RG,iQ)-CE
111.	AmpliSens® <i>Streptococcus pyogenes</i> -screen/monitor-FRT PCR kit	H-2171-1-1-CE H-2172-1-14-CE
112.	AmpliSens® <i>T.vaginalis</i> / <i>N.gonorrhoeae</i> -MULTIPRIME-FRT PCR kit	R-B65-F(RG,iQ)-CE
113.	AmpliSens® TBE-FRT PCR kit	R-V52(RG)-CE
114.	AmpliSens® TBEV, <i>B.burgdorferi</i> sl, <i>A.phagocytophilum</i> , <i>E.chaffeensis</i> / <i>E.muris</i> -FRT PCR kit	R-V59(RG,iQ,Mx,Dt)-CE
115.	AmpliSens® <i>Treponema pallidum</i> -FRT PCR kit	R-B20-F(RG,iQ)-CE
116.	AmpliSens® <i>Trichomonas vaginalis</i> -EPH PCR kit	B6-100-R0,2-CE
117.	AmpliSens® <i>Trichomonas vaginalis</i> -FEP PCR kit	B6-100-R0,2-FEP-CE
118.	AmpliSens® <i>Trichomonas vaginalis</i> -FRT PCR kit	R-B6-F(RG,iQ)-CE
119.	AmpliSens® <i>U.parvum</i> / <i>U.urealyticum</i> -FEP PCR kit	B19-100-R0,2-FEP-CE
120.	AmpliSens® <i>U.parvum</i> / <i>U.urealyticum</i> -FRT PCR kit	R-B19(RG)-CE R-B19-F(RG,iQ)-CE
121.	AmpliSens® <i>Ureaplasma</i> spp.-FRT PCR kit	R-B2(RG)-CE R-B2-F(RG,iQ)-CE
122.	AmpliSens® <i>Ureaplasma</i> spp.-screen-titre-FRT PCR kit	R-B2-100-FT(RG,iQ,Mx)-CE
123.	AmpliSens® <i>Vibrio cholerae</i> -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® <i>Yersinia enterocolitica</i> / <i>Y.pseudotuberculosis</i> -FRT PCR kit	R-B64(RG,iQ)-CE
128.	AmpliSens® <i>Yersinia pestis</i> -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



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Date of the first certification awarding: 20. 05. 2015


Ing. Pavel Vaněk
Head of Certification Body