NovaTec Immundiagnostica GmbH

Declaration of Conformity

We

NovaTec Immundiagnostica GmbH
Waldstraße 23 A6, 63128 Dietzenbach, Germany

herewith declare under our own responsibility, that the products listed below are in accordance with the requirements of the IVD Directive 98/79/EC of the European Parliament and Council of Oct. 27,1998 in regard to in vitro diagnostic medical devices (IVDs).

The accordance was shown by conformity assessment procedures in Annex III (2 – 5), resp. Annex IV.3.

Dietzenbach, 2017-10-23

Dr. Claudia Rezmer
Management Representative

The conformity of the above mentioned product is checked at least every 3 years
This is documented by rechecking and signing the general requirements.

Prod. No.		Name and Description
1.	ADVA0010	Adenovirus IgA
2.	ADVG0010	Adenovirus IgG
3.	ADVM0010	Adenovirus IgM
4.	ASCG0020	Ascaris lumbricoides IgG
5.	ASPG0680	Aspergillus fumigatus IgG
6.	ASPM0680	Aspergillus fumigatus IgM
7.	BOPA0030	Bordetella pertussis IgA
8.	BOPG0030	Bordetella pertussis IgG
9.	BOPM0030	Bordetella pertussis IgM
10.	BPTA0610	Bordetella pertussis toxin (PT) IgA
11.	BPTG0610	Bordetella pertussis toxin (PT) IgG
12.	BORG0040	Borrelia burgdorferi IgG
13.	BORM0040	Borrelia burgdorferi IgM
14.	BRUG0050	Brucella IgG
15.	BRUM0050	Brucella IgM
16.	CANA0060	Candida albicans IgA
17.	CANG0060	Candida albicans IgG
18.	CANM0060	Candida albicans IgM
19.	CHAG0560	Chagas (Trypanosoma cruzi) IgG
20.	TRYP0570	Chagas
21.	CHIG0590	Chikungunya IgG capture
22.	CHIM0590	Chikungunya IgM µ-capture
23.	CHLA0070	Chlamydia trachomatis IgA
24.	CHLG0070	Chlamydia trachomatis IgG
25.	CHLM0070	Chlamydia trachomatis IgM
26.	CHLA0510	Chlamydia pneumoniae IgA
27.	CHLG0510	Chlamydia pneumoniae IgG
28.	CHLM0510	Chlamydia pneumoniae IgM
29.	CMVG0110	Cytomegalovirus (CMV) IgG
30.	ACMV7110	Avidity Cytomegalovirus (CMV) IgG
31.	CMVM0110	Cytomegalovirus (CMV) IgM
32.	CORG0090	Corynebacterium diphtheriae toxin IgG
33.	CORG5009	Corynebacterium diphtheriae toxin 5S IgG
34.	PCORG009	Corynebacterium diphtheriae toxin 5S IgG plus
35.	COX1G0600	Coxiella burnetii (Q-Fever) Phase 1 IgG
36.	COX2G0600	Coxiella burnetii (Q-Fever) Phase 2 IgG
37.	COX2M0600	Coxiella burnetii (Q-Fever) Phase 2 IgM
38.	DENG0120	Dengue Virus IgG
39.	DENM0120	Dengue Virus IgM
40.	DVM0460	Dengue Virus IgM µ-capture
41.	ECHG0130	Echinococcus IgG
42.	ENTG0140	Entamoeba histolytica IgG
43.	EBVA0150	Epstein-Barr Virus (VCA) IgA
44.	EBVG0150	Epstein-Barr Virus (VCA) IgG
45.	AEBV7150	Avidity Epstein-Barr Virus (VCA) IgG
46.	EBVM0150	Epstein-Barr Virus (VCA) IgM
47.	EBVG0580	Epstein-Barr Virus (EBNA) IgG

The conformity of the above mentioned product is checked at least every 3 years
This is documented by rechecking and signing the general requirements.

48.	FIL0760	Filariasis
49.	GIA0160S	Giardia lamblia antigen
50.	HANG0670	Hantavirus IgG
51.	HANM0670	Hantavirus IgM
52.	HELA0220	Helicobacter pylori IgA
53.	PHELA022	Helicobacter pylori IgA plus
54.	HELG0220	Helicobacter pylori IgG
55.	PHELG022	Helicobacter pylori IgG plus
56.	HSVG0250	Herpes simplex Virus 1+2 (HSV) IgG
57.	HSVM0250	Herpes simplex Virus 1+2 (HSV) IgM
58.	HSV1G0500	Herpes simples Virus 1 (HSV 1) IgG
59.	HSV1M0500	Herpes simplex Virus 1 (HSV 1) IgM
60.	HSV2G0540	Herpes simplex Virus 2 (HSV 2) IgG
61.	HSV2M0540	Herpes simplex Virus 2 (HSV 2) IgM
62.	INFA0290	Influenza Virus A IgA
63.	INFG0290	Influenza Virus A IgG
64.	INFM0290	Influenza Virus A IgM
65.	INFA0300	Influenza Virus B IgA
66.	INFG0300	Influenza Virus B IgG
67.	INFM0300	Influenza Virus B IgM
68.	LEGG0650	Legionella pneumophila IgG
69.	LEGM0650	Legionella pneumophila IgM
70.	LEIG0310	Leishmania infantum IgG
71.	LEPG0660	Leptospira IgG
72.	LEPM0660	Leptospira IgM
73.	LEIG0310	Leishmania infantum IgG
74.	MAL0620	Malaria
75.	MEAG0330	Measles Virus IgG
76.	AEA7330	Avidity Measles Virus IgG
77.	MEAM0330	Measles Virus IgM
78.	MUMG0340	Mumps Virus IgG
79.	MUMM0340	Mumps Virus IgM
80.	MYCA0350	Mycoplasma pneumoniae IgA
81.	MYCG0350	Mycoplasma pneumoniae IgG
82.	MYCM0350	Mycoplasma pneumoniae IgM
83.	PAIA0360	Parainfluenza Virus 1,2,3 IgA
84.	PAIG0360	Parainfluenza Virus 1,2,3 IgG
85.	PARG0370	Parvovirus B19 IgG
86.	PARM0370	Parvovirus B19 IgM
87.	RSVA0380	Respiratory Syncytial Virus IgA
88.	RSVG0380	Respiratory syncytial Virus IgG
89.	RSVM0380	Respiratory syncytial Virus IgM
90.	RUBG0400	Rubella Virus IgG
91.	ARUB7400	Avidity Rubella Virus IgG
92.	RUBM0400	Rubella Virus IgM µ-capture
93.	SCHG0410	Schistosoma mansoni IgG
94.	SCHM0410	Schistosoma mansoni IgM
95.	STRO0690	Strongyloides
96.	TAEG0420	Taenia solium IgG
97.	TETG0430	Clostridium tetani toxin IgG
98.	TETG5043	Clostridium tetani toxin 5S IgG

99.	PTETG043	Clostridium tetani toxin 5S IgG plus
100.	TICG0440	TBE/ FSME IgG
101.	TICM0440	TBE/ FSME IgM
102.	PTICG044	TBE/ FSME IgG plus
103.	TOCG0450	Toxocara canis IgG
104.	TOXA0460	Toxoplasma gondii IgA
105.	TOXG0460	Toxoplasma gondii IgG
106.	ATOX7460	Avidity Toxoplasma gondii IgG
107.	TOXM0460	Toxoplasma gondii IgM µ-capture
108.	TRIG0480	Trichinella spiralis IgG
109.	VZVA0490	Varicella-Zoster Virus (VZV) IgA
110.	VZVG0490	Varicella-Zoster Virus (VZV) IgG
111.	VZVM0490	Varicella-Zoster Virus (VZV) IgM
112.	ZVG0790	Zika Virus IgG capture
113.	ZVM0790	Zika Virus IgM µ-capture
114.	ATG1010	Anti-TG
115.	ATPO1020	Anti-TPO
116.	TSH1030	TSH
117.	RFM3010	Rheumatoid Factor IgM
118.	DNOV001	Cortisol
119.	DNOV002	Testosterone
120.	DNOV003	17 beta-Estradiol
121.	DNOV004	17-OH Progesterone
122.	DNOV005	DHEA-S
123.	DNOV006	Progesterone
124.	DNOV007	Free Estriol
125.	DNOV008	Androstenedione
126.	DNOV009	Free Testosterone
127.	DNOV010	Urinary Cortisol
128.	DNOV011	Total Estriol
129.	DNOV012	Aldosterone
130.	DSNOV20	Cortisol Saliva
131.	DSNOV21	Testosterone Saliva
132.	DSNOV22	17 beta-Estradiol Saliva
133.	DSNOV24	DHEA-S Saliva
134.	DSNOV25	Progesterone Saliva
135.	DSNOV26	Estriol Saliva
136.	DSNOV27	Androstenedione Saliva
137.	DNOV030	LH
138.	DNOV031	FSH
139.	DNOV032	Prolactin
140.	DNOV033	AFP
141.	DNOV034	beta-HCG
142.	DNOV051	Free T3
143.	DNOV052	Free T4
144.	DNOV053	Total T3
145.	DNOV054	Total T4
146.	DNOV057	Thyroglobulin
147.	DNOV060	CEA
148.	DNOV061	CA 125
149.	DNOV062	CA 15-3

150.	DNOV063	CA 19-9
151.	DNOV093	CIC-C1q
152.	DNOV094	CIC-C3d
153.	DNOV096	CH-50
154.	DNOV100	Ferritin
155.	DNOV101	HGH
156.	DNOV102	IgE
157.	DNOV111	Insulin
158.	DNOV112	C-Peptide

NovaTec Immundiagnostica GmbH

NovaLisa®

Ascaris lumbricoides IgG

ELISA

CE

Only for in-vitro diagnostic use

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
Ascaris lumbricoides	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 pulmonal symptoms like cough and dyspnoea.	Ingestion of infectious Ascaridae eggs (classical way of infestation is the consumption of insufficient washed salad)

The presence of pathogen or infection 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.
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 anlibodies 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

- **Ascaris lumbricoides Coated Microplate (IgG):** 12 break-apart 8-well snap-off strips coated with Ascaris lumbricoides 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.
- **Protein A 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; < 5 % NMP.
- **Ascaris lumbricoides IgG Positive Control:** 1 vial containing 2 ml control (human serum or plasma); coloured yellow; ready to use; red cap.
- **Ascaris lumbricoides IgG Cut-off Control:** 1 vial containing 3 ml control (human serum or plasma); coloured yellow; ready to use; green cap.
- **Ascaris lumbricoides 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 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. For CSF please use the instruction for use ABVL0001. If the assay is performed within 5 days after sample collection, the samples should be kept at 2...8 °C; otherwise they should be aliquoted and stored deep-frozen (-70...-20 °C). If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing.
Heat inactivation of samples is not recommended.

7.1. Sample Dilution

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

8. ASSAY PROCEDURE

8.1. Test Preparation

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

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

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

Adjust the incubator to 37 ± 1 °C.

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

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

8.2. Measurement

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

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

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

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

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

9. RESULTS

9.1. Run Validation Criteria

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

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

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

9.2. Calculation of Results

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

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

Cut-off = 0.43

9.2.1. Results in Units [NTU]

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

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

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 100.0 % (95% confidence interval: 47.82% - 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

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

- 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.: ASCG0020 Ascaris lumbricoides IgG ELISA (96 Determinations)

NovaTec
Immundiagnostica GmbH

NovaLisa®

Bordetella pertussis IgM

ELISA

CE

Only for in-vitro diagnostic use

Product Number: BOPM0030 (96 Determinations)

ENGLISH

1. INTRODUCTION

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

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

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

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

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

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

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

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

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

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

The presence of pathogen or infection 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.
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

- **Bordetella pertussis Coated Microplate (IgM):** 12 break-apart 8-well snap-off strips coated with Bordetella pertussis 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.
- **Bordetella pertussis 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.
- **Bordetella pertussis IgM Positive Control:** 1 vial containing 2 ml control (human serum or plasma); coloured yellow; ready to use; red cap.
- **Bordetella pertussis IgM Cut-off Control:** 1 vial containing 3 ml control (human serum or plasma); coloured yellow; ready to use; green cap.
- **Bordetella pertussis 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 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. For CSF please use the instruction for use ABVL0001. If the assay is performed within 5 days after sample collection, the samples should be kept at 2...8 °C, otherwise they should be aliquoted and stored deep-frozen (-70...-20 °C). If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing.
Heat inactivation of samples is not recommended.

7.1. Sample Dilution

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

8. ASSAY PROCEDURE

8.1. Test Preparation

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

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

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

Adjust the incubator to 37 ± 1 °C.

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

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

8.2. Measurement

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

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

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

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

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

9. RESULTS

9.1. Run Validation Criteria

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

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

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

9.2. Calculation of Results

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

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

9.2.1. Results in Units [NTU]

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

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

9.3. Interpretation of Results

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

9.3.1. Antibody Isotypes and State of Infection

Serology	Significance
IgM	Characteristic of the primary antibody response High IgM titer with low IgG titer: → suggests a current or very recent infection Rare: → persisting IgM
IgG	Characteristic of the secondary antibody response May persist for several years High IgG titer with low IgM titer: → may indicate a past infection
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% - 100.0%).

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 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

- 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.: BOPM0030 Bordetella pertussis IgM ELISA (96 Determinations)

NovaTec
Immundiagnostica GmbH

NovaLisa®

Bordetella pertussis IgG

ELISA

CE

Only for in-vitro diagnostic use

Product Number: BOPG0030 (96 Determinations)

ENGLISH

1. INTRODUCTION

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

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

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

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

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

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

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

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

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

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

The presence of pathogen or infection 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.
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

- **Bordetella pertussis Coated Microplate (IgG):** 12 break-apart 8-well snap-off strips coated with Bordetella pertussis 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.
- **Bordetella pertussis 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.
- **Bordetella pertussis IgG Positive Control:** 1 vial containing 2 ml control (human serum or plasma); coloured yellow; ready to use; red cap.
- **Bordetella pertussis IgG Cut-off Control:** 1 vial containing 3 ml control (human serum or plasma); coloured yellow; ready to use; green cap.
- **Bordetella pertussis 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 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. For CSF please use the instruction for use ABVL0001. If the assay is performed within 5 days after sample collection, the samples should be kept at 2...8 °C; otherwise they should be aliquoted and stored deep-frozen (-70...-20 °C). If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing.
Heat inactivation of samples is not recommended.

7.1. Sample Dilution

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

8. ASSAY PROCEDURE

8.1. Test Preparation

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

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

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

Adjust the incubator to 37 ± 1 °C.

1. Dispense 100 µl standards/controls and diluted samples into their respective wells. Leave well A1 for the Substrate Blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 1 hour ± 5 min at 37 ± 1 °C.**
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µl of Washing Buffer. Avoid overflows from the reaction wells The interval between washing and aspiration should be > 5 sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!
   Note: Washing is important! Insufficient washing results in poor precision and false results.
5. Dispense 100 µl Conjugate into all wells except for the Substrate Blank well A1.
6. **Incubate for 30 min at room temperature (20...25 °C).** Do not expose to direct sunlight.
7. Repeat step 4.
8. Dispense 100 µl TMB Substrate Solution into all wells.
9. **Incubate for exactly 15 min at room temperature (20...25 °C) in the dark.** A blue colour occurs due to an enzymatic reaction.
10. Dispense 100 µl Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution, thereby a colour change from blue to yellow occurs.
11. Measure the absorbance at 450/620 nm within 30 min after addition of the Stop Solution.

8.2. Measurement

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

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

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

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

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

9. RESULTS

9.1. Run Validation Criteria

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

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

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

9.2. Calculation of Results

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

Example: Absorbance value Cut-off Control 0,44 + absorbance value Cut-off control 0,42 = 0,86 / 2 = 0,43
Cut-off = 0,43

9.2.1. Results in Units [NTU]

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

Example: $\frac{1.591 \times 10}{0,43} = 37$ NTU (Units)

9.3. Interpretation of Results

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

9.3.1. Antibody Isotypes and State of Infection

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

- 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.: BOPG0030 Bordetella pertussis IgG ELISA (96 Determinations)

NovaTec Immundiagnostica GmbH

NovaLisa®

Corynebacterium diphtheriae toxin IgG

ELISA

CE

Only for in-vitro diagnostic use

Product Number: CORG0090 (96 Determinations)

ENGLISH

1. INTRODUCTION

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

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

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

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

The presence of pathogen or infection 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.
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

- **Corynebacterium diphtheriae toxin Coated Microplate (IgG):** 12 break apart 8-well snap-off strips coated with Corynebacterium diphtheriae 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.
- **Corynebacterium diphtheriae 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.
- **Corynebacterium diphtheriae toxin IgG Standards:** 4 vials, each containing 2 ml standard (human serum or plasma); coloured yellow; ready to use.

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

7.1. Sample Dilution

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

8. ASSAY PROCEDURE

8.1. Test Preparation

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

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

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

Adjust the incubator to 37 ± 1 °C.

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

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

8.2. Measurement

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

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

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

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

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

9. RESULTS

9.1. Run Validation Criteria

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

- **Substrate blank:** **Absorbance value < 0.100**
- **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.

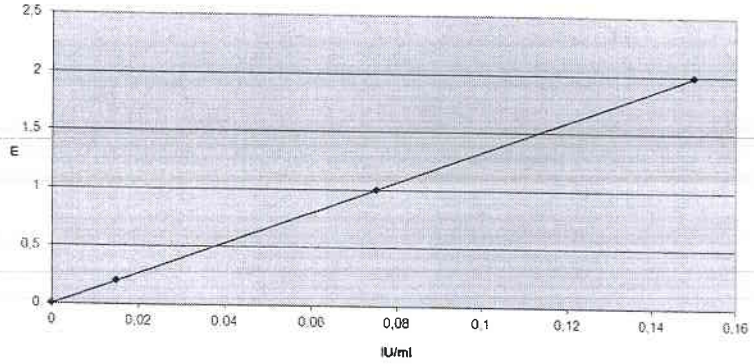
9.2. Calculation of Results

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 100.0% (95% confidence interval: 89.42% - 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: 95.44% - 100.0%).

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

- 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.: CORG0090 Corynebacterium diphtheriae toxin IgG ELISA (96 Determinations)

NovaTec Immundiagnostica GmbH

NovaLisa®

Echinococcus IgG

ELISA

CE

Only for in-vitro diagnostic use

Product Number: ECHG0130 (96 Determinations)

ENGLISH

1. INTRODUCTION

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

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

The presence of pathogen or infection 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.
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

- **Echinococcus Coated Microplate (IgG):** 12 break-apart 8-well snap-off strips coated with Echinococcus 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.
- **Echinococcus 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.
- **Echinococcus IgG Positive Control:** 1 vial containing 2 ml control (human serum or plasma); coloured yellow; ready to use; red cap.
- **Echinococcus IgG Cut-off Control:** 1 vial containing 3 ml control (human serum or plasma); coloured yellow; ready to use; green cap.
- **Echinococcus 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 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. For CSF please use the instruction for use ABVL0001. If the assay is performed within 5 days after sample collection, the samples should be kept at 2...8 °C; otherwise they should be aliquoted and stored deep-frozen (-70...-20 °C). If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing.
Heat inactivation of samples is not recommended.

7.1. Sample Dilution

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

8. ASSAY PROCEDURE

8.1. Test Preparation

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

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

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

Adjust the incubator to 37 ± 1 °C.

1. Dispense 100 µl standards/controls and diluted samples into their respective wells. Leave well A1 for the Substrate Blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 1 hour ± 5 min at 37 ± 1 °C.**
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µl of Washing Buffer. Avoid overflows from the reaction wells. The interval between washing and aspiration should be > 5 sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!
   Note: Washing is important! Insufficient washing results in poor precision and false results.
5. Dispense 100 µl Conjugate into all wells except for the Substrate Blank well A1.
6. **Incubate for 30 min at room temperature (20...25 °C).** Do not expose to direct sunlight.
7. Repeat step 4.
8. Dispense 100 µl TMB Substrate Solution into all wells.
9. **Incubate for exactly 15 min at room temperature (20...25 °C) in the dark.** A blue colour occurs due to an enzymatic reaction.
10. Dispense 100 µl Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution, thereby a colour change from blue to yellow occurs.
11. Measure the absorbance at 450/620 nm within 30 min after addition of the Stop Solution.

8.2. Measurement

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

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

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

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

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

9. RESULTS

9.1. Run Validation Criteria

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

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

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

9.2. Calculation of Results

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

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

9.2.1. Results in Units [NTU]

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

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

9.3. Interpretation of Results

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

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 99.41% (95% confidence interval: 96.77% - 99.99%).

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

- 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.: ECHG0130 Echinococcus IgG ELISA (96 Determinations)

Nova Tec
Immunodiagnostica GmbH

Giardia lamblia

ELISA

Предлагаемый к использование комплект Giardia in vitro diagnosticum предназначен для обнаружения Giardia lamblia антигена в фекальных образцах. Giardia lamblia является простейший, которые заражают по большей части тонкую кишку после внедрения кист Giardia. Заражаются люди через фекальную зараженную воду или пищу. В Соединенных Штатах, это - наиболее распространенный инфекционный агент в переносимых по воде взрывах поноса. В настоящем времени в мире, Giardia lamblia является - одного из первых энтеро патогенов, заражающих детей до 10 лет с показателями распространения 15 - 20 %. Приобретение Lambliasis происходит главным образом в группах где не соблюдается личная гигиена и передается от человека к человеку. Такие способы инфицирования наблюдаются детских садах , у сексуально- активных гомосексуалистах (вплоть до 19 %) и в учреждениях для пристарелых. Много инфицированные дети симптоматические распространяют болезнь в пределах их домов и окружениях. Giardiasis охарактеризован как острый или хронический понос. Период инкубации - 3 в 42 дней. Клиническое проявление симптоматической острой инфекции - внезапное начало водянистого поноса, брюшных судорог и метеоризм. Пациент выражает чувства недомогания, тошнота и анорексия, менее часто тошнота и лихорадка происходит; кровь, гной и слизь обычно отсутствуют

Диагноз Lambliasis в прошлом был сделан посредством обследования стула на трофозоидов или кист микроскопически методом потускнения. Эти методы требуют опытных лаборантов. Кроме того исследование должно быть выполнено периодически поскольку может произойти нерегулярное выделение. Эквивалентный метод является новым тестом ELISA для обследования Giardia lamblia антигена в образце стула.

ПРИНЦИП

Микропланшеты покрыты Giardia lamblia - специфичными антителами При первой инкубации твёрдая фаза обрабатывается разведёнными образцами захватываются, если имеются в наличии, твёрдой фазой.После отмывания других компонентов образца, во 2-й инкубации связавшиеся Giardia lamblia обнаруживаются посредством добавления антигена, меченного пероксидазой (HRP).

Энзим, захваченный на твёрдой фазе, действуя на субстрат/ хромогенную смесь, порождает оптический сигнал, который пропорционален количеству Giardia lamblia антигена , наличествующих в образце.

КОМПОНЕНТЫ

Каждый набор содержит достаточно реагентов, чтобы выполнить 96 тестов.

Микролунки, покрытые антителом:

8x12 микролунковые полоски, покрытые Giardia lamblia - специфическими аффинными антителами в мешочке с десиккатом. Дайте микропланшете достичь комнатной температуры до открытия; повторно запечатайте неиспользованные полоски в мешочке с десиккантом и храните при 4°C.

Растворитель образца

1 x 100 мл растворитель образца (100 ml): буферное раствор NaCl для разбавления образца;

Промывочный буферный концентрат:

1x100мл / бутылка. Буфер для промывки; pH 7.2

Положительный контроль:

1x 1.8 мл/флакон. Giardia lamblia антиген готовый к использованию.

Энзимный коньюгат:

1x10 мл/флакон. HRP энзимный коньюгат Giardia lamblia .

Субстрат/ Хромоген:

1x10мл/флакон. Перикись/ TMB, готовый к использованию

Примечание: Хранить в защищённом от света месте.

Останавливающий раствор:

1x6 мл/флакон. Содержит 1Н раствора H_2SO_4.

Инструкция

Требующиеся, но не поставляемые материалы

1. Микропипетки и сменные наконечники
2. Вода EIA степени чистоты
3. Таймер с 60-ти минутным диапазоном
4. Абсорбентная бумага.
5. Микропланшетный термостатический инкубатор, установленный на +37°C.
6. Считыватель микролунок с фильтром на 450нм и с фильтрами на 620-630нм.
7. Промыватель микропланшет.

Предупреждения и меры предосторожности

Набор содержит инактивированный антиген Giardia lamblia. Тем не менее, положительный , а также отрицательный контроли и образцы должны считаться потенциально заразными и необходимо принимать безопасные меры предосторожности.

Перекись мочевины может вызвать прижигание. Обращайтесь осторожно!

Стоповое реактив содержит 1Н серная кислота. Избегайте контакту с кожей и одеждой!

Все реагенты и материалы, входящие в контакт с потенциальными инфекционными образцами должны обеззараживатся дезинфицирующими средствами или автокловирование при 121 C в течение одного часа.

Инструкции хранения реагентов

Все реагенты должны быть сохранены при 2 - 8 C и могут быть израсходованы на до даты напечатанной на этикетках. Микробное заражение должно быть предупрежденно. Гарантия качества не может быть дана после истечение срока набора. Готовый буфер годен в течение 4 недель при 2 - 8 C. Вытаскиваете реагенты из холодильника, чтобы набрали комнатную температуру перед использованием.

Бесцветный Substrate/Chromogen должен быть защищен от света.

Нестабильность или ухудшения реагентов определяется последующим критериям:

- мутность или синяя окраска Substrate/Chromogen до использования ,
- величина оптической плотности отрицательного контроля выше чем 0,2 ,
- величина оптической плотности Положительного контроля ниже, чем 0.8

Образец стула может быть использован свежий или замороженным. Свежие образцы, должны быть сохранены при 4 C и должны быть протестированы в пределах 24 часов. Хранения при 4 C образец разбавленный может быть продлено в течение других 5 дней при 2 - 8 C.

Образцы, которые не могут быть протестированы в течение этого периода должно быть сохранено при -20 C пока не исследованы.

Процедура Теста

Оставьте все реагенты на час при комнатной температуры перед использованием. Смешайте реагенты хорошо перед использованием.

Воспроизводимость

Зависит от точного пипетирования, соблюдение времени инкубации и температуры, промывания.

Избегайте прямого солнечного света в течение всех инкубаций

- **Подготовка Буфера**

1 часть концентрированного буфера разбавляется 9 частями дистиллированной воды.Буфер годен в течение 4 недель при 2 - 8 C.

- **Подготовка образцов,**

Растворите образец 1:11 - наберите около 100 мкг стула и разбавте в 1 мл растворителя образца. Смешивать очень тщательно . После этого оставить короткое время (max. 10 минут) перед использованием.

ПРОЦЕДУРА АНАЛИЗА

Пожалуйста читаете процедуру теста тщательно прежде, чем выполнять тест. Надежность результата зависит от строгого соблюдения теста . Пожалуйста читаете процедуру теста тщательно прежде, чем выполнять тест. Надежность результата зависит от строгого соблюдения теста . При выполнении Toxocara canis мы рекомендуем увеличивать промывку от трех до пяти раз по 300ul на 350мкл.

Пожалуйста распределите лунки в такой последовательности:
1 лунка (напр.. A1) для бланка ,
1 лунка (напр.. B1) для отрицательного контроля (*разбавитель образцов*)
1лунка (напр.. E 1) для положительного контроля.

Накапайте пипеткой 100 µл контролей и затем 100 µл образцов.Накапайте пипеткой 100 µл Giardia lamblia коньюгата во все лунки исключая бланк.Инкубируйте микропланшету ***при комнатной температуре в течение 60 минут.***

1. Когда инкубация завершена, удалите фольгу, отсасывать содержимое лунки и мойте каждый хорошо пять раз по 300ul моющего раствора. Избегайте переполнений . Промывка между каждым циклом должна быть >5sec. В конечном счете тщательно удалите, оставшуюся жидкость встряхиванием на фильтровальнной бумаге!

Примечание: Недостаточная промывка уменьшает точность величины оптической плотности.

Когда инкубация завершена, удалите фольгу, отсасывать содержимое лунки и мойте каждый хорошо пять раз по 300ul моющего раствора. Избегайте переполнений . Промывка между каждым циклом должна быть >5sec. В конечном счете тщательно удалите, оставшуюся жидкость встряхиванием на фильтровальнной бумаге!

2.Накапайте пипеткой 100 µл ТМБ субстрата в каждой лунки.Инкубируйте микропланшету при комнатной ***температуре в течение 15 минут.Избегайте прямого света .***

5. Накапайте пипепткой **50 µл останавливающего** раствора в каждую лунку.

Синий цвет в течение инкубации переходит в желтый.

Примечание: Очень положительные образцы могут быть причиной заражение субстрата! Это влияет на оптическую плотность. Разбавляете образец с физиологическим раствором , например 1+1 рекомендуется. Затем разбавьте образец 1+1ОО с буфером разбавления и умножайте результат на 2.

Измерьте оптическую плотность образца на 450/620 nm в пределах 30 мин.

РЕЗУЛЬТАТЫ

- Негатив контроль: величина оптической плотности должна быть ниже чем 0.200.
- Положительный контроль : величина оптической плотности должна быть не менее 0.800.

Вычисление результатов отсечки является средней величиной оптической плотности кутова .

Пример :ОД негатив контроля + 0,15 = кутов

Интерпретация результатов считается положительной если величина оптической плотности выше чем 10% над кутовом.

Серая зона - рекомендовано повторять тест снова через 2-4 недели со свежим образцом. Если результаты во втором тесте - снова в серой зоне образец должен быть считавшим негатив.

Образцы считаются негативом если величина оптической плотности ниже, чем 10% ниже отсечки.

- Диагностическая специфичность определена как вероятность негатива в присутствии специфического analyte. Это 100 %.
- Диагностическая чувствительность определена как вероятность позитива в присутствии специфического analyte. Это 99,6 %.

ОГРАНИЧЕНИЯ ПРОЦЕДУРЫ

Бактериальное или регулярное замораживание образца могут повлиять на величины оптической плотности. Диагноз инфекционной болезни не должен быть установливан на основе единственного результата теста. Точный диагноз должен приниматься во внимание клиническим данным, а также серологически.

МЕРЫ ПРЕДОСТОРОЖНОСТИ И ПРЕДУПРЕЖДЕНИЯ

- В соответствии с статьей 1 - 2B Европейских директивы параграфа 98/79/EC использование in vitro диагностические медицинские продукты предлагаемые изготовителем, должны обеспечивать соответствие, исполнение и безопасность продукта. Следовательно процедура теста, информация, меры предосторожности и предупреждения в инструкциях для использования должны быть строго сопровождены. Использование наборов с анализаторами и аналогичным оборудованием должны быть подтверждены. Любое изменение в проекте, композиции и процедуры теста, а также для любого использования в комбинации с другими продуктами не одобренное изготовителем не разрешено; сам пользователь ответственный за такие изменения. Изготовитель не ответственный за ложные результаты .
- Все компоненты использованного для производства этих реагентов протестированы на антитела HIV, - антитела HCV и HBsAg и могут быть неинфицированы. Тем не менее, все материалы должны считаться потенциально инфекционными.
- Реагенты других изготовителей не должны быть использованы вместе с реагентами этого комплекта
- не используйте реагенты после того, как истекает дата устанавливаемая на этикетке.
- Закрываете флаконы реагента плотно немедленно после использования, чтобы избегать испарения и микробного заражения.

ПРЕДУПРЕЖДЕНИЕ:

Серная кислота раздражает глаза и кожу. Берегите от детей. В контакте с глазами, прополоскаете тщательно с водой и обращайтесь к доктору!

NovaTec Immundiagnostica GmbH

NovaLisa®

Mumps Virus IgM

ELISA

CE

Only for in-vitro diagnostic use

Product Number: MUMM0340 (96 Determinations)

ENGLISH

1. INTRODUCTION

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

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

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 ± 5 min at 37 ± 1 °C.**
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µl of Washing Buffer. Avoid overflows from the reaction wells. The interval between washing and aspiration should be > 5 sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!

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

8.2. Measurement

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

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

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

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

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

9. RESULTS

9.1. Run Validation Criteria

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

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

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

9.2. Calculation of Results

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

Example: Absorbance value Cut-off Control 0,44 + absorbance value Cut-off control 0,42 = 0,86 / 2 = 0,43
Cut-off = 0,43

9.2.1. Results in Units [NTU]

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

Example: $\frac{1,591 \times 10}{0,43} = 37$ NTU (Units)

9.3. Interpretation of Results

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

9.3.1. Antibody Isotypes and State of Infection

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

10. SPECIFIC PERFORMANCE CHARACTERISTICS

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

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

10.1. Precision

Intraassay	n	Mean (E)	CV (%)
#1	24	0,600	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)

NovaTec Immundiagnostica GmbH

NovaLisa®

Mumps Virus IgG

ELISA

CE

Only for in-vitro diagnostic use

Product Number: MUMG0340 (96 Determinations)

ENGLISH

1. INTRODUCTION

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

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

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 ± 5 min at 37 ± 1 °C.**
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µl of Washing Buffer. Avoid overflows from the reaction wells. The interval between washing and aspiration should be > 5 sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!

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

8.2. Measurement

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

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

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

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

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

9. RESULTS

9.1. Run Validation Criteria

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

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

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

9.2. Calculation of Results

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

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

9.2.1. Results in Units [NTU]

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

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

9.3. Interpretation of Results

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

9.3.1. Antibody Isotypes and State of Infection

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

10. SPECIFIC PERFORMANCE CHARACTERISTICS

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

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

10.1. Precision

Intraassay	n	Mean (E)	CV (%)
#1	24	0.411	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)

NovaTec Immundiagnostica GmbH

NovaLisa®

Clostridium tetani toxin IgG

ELISA

CE

Only for in-vitro diagnostic use

Product Number: TETG0430 (96 Determinations)

ENGLISH

1. INTRODUCTION

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

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

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

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

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

The presence of pathogen or infection may be identified by

- Microscopy
- Serology: e.g. ELISA

2. INTENDED USE

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

3. PRINCIPLE OF THE ASSAY

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

4. MATERIALS

4.1. Reagents supplied

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

Standard A:	0.0	IU/ml; blue cap
Standard B:	0.1	IU/ml; green cap
Standard C:	0.5	IU/ml; yellow cap
Standard D:	1.0	IU/ml; red cap

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

For potential hazardous substances please check the safety data sheet.

4.2. Materials supplied

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

4.3. Materials and Equipment needed

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

5. STABILITY AND STORAGE

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

6. REAGENT PREPARATION

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

6.1. Coated Microplate

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

6.2. Washing Buffer (20x conc.)

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

6.3. TMB Substrate Solution

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

7. SAMPLE COLLECTION AND PREPARATION

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

7.1. Sample Dilution

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

8. ASSAY PROCEDURE

8.1. Test Preparation

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

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

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

Adjust the incubator to 37 ± 1 °C.

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

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

8.2. Measurement

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

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

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

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

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

9. RESULTS

9.1. Run Validation Criteria

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

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

Standard A < Standard B < Standard C < Standard D

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

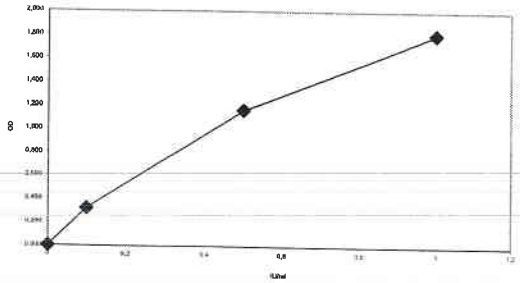
9.2. Calculation of Results

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

NovaTec Immundiagnostica GmbH

NovaLisa®

Brucella IgM

ELISA

CE

Only for in-vitro diagnostic use

Product Number: BRUM0050 (96 Determinations)

ENGLISH

1. INTRODUCTION

Brucella is a small Gram-negative bacterium (0.4-0.8 µm in diameter and 0.4-3.0 µm in length) which is non-flagellated, and non-spore-forming. They are named after the military doctor David Bruce, who on Malta in 1887 isolated the pathogens from the spleen of a soldier who died of undulating fever. Four species are pathogenic to human: Brucella abortus, Brucella melitensis, Brucella suis and Brucella canis. All four species are exciters of Brucellosis, a disease characterized by undulating fever. Depending on exciter the disease is also called Morbus Bang (B. abortus) or Malta fever (B. melitensis).

The pathogens are transmitted from animals, which are mainly affected. The infection is caused by contact with ill animals or their excrements as well as by non-pasteurized milk and milk products like fresh cheese from sheep or goat. Main entrances are skin wounds, conjunctives and digestive tract. The intact pathogens are transported by granulocytes into local lymph nodes, from where they spread haematogenous. All kind of organs can be infected. Symptoms depend up on the infected organ, where an inflammation takes place. Until now the pathogenic mechanism is not completely understood.

Brucellosis appears worldwide. In non-pasteurized milk and milk products Brucella is viably and infectious for weeks. Bovine brucellosis caused by Brucella abortus is still the most widespread form, although reported incidence and prevalence of the disease vary widely from country to country (from <0.01 to >200 per 100,000 population). Brucella melitensis is endemic in areas where keeping of sheeps and goats is frequent. It causes serious human infections. Working with these bacteria in laboratories necessitates highest carefulness because of high contagiosity. Risk groups include abattoir workers, meat inspectors, animal handlers, veterinarians, and laboratorians. Brucellosis is a nationally notifiable disease and reportable to the local health authority.

Species	Disease	Symptoms (e.g.)	Transmission route
B. abortus (cattle) B. melitensis (sheep, goats) B. suis (pigs) B. canis (dogs)	Brucella	Fever, chills (undulating fever), malaise, arthritis, hepatitis, endocarditis, hepatomegalie, osteomyelitis (OM)	Oral (non-pasteurized milk and milk products) Percutan (contact with ill animals or their excrements) In general no transmission from human to human

The presence of pathogen or infection may be identified by

- Histology
- Serology: e.g. ELISA

2. ~~INTENDED USE~~

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

3. PRINCIPLE OF THE ASSAY

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

4. MATERIALS

4.1. Reagents supplied

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

For potential hazardous substances please check the safety data sheet.

4.2. Materials supplied

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

4.3. Materials and Equipment needed

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

5. STABILITY AND STORAGE

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

6. REAGENT PREPARATION

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

6.1. Coated Microplate

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

6.2. Washing Buffer (20x conc.)

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

6.3. TMB Substrate Solution

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

7. SAMPLE COLLECTION AND PREPARATION

Use human serum or plasma (citrate, heparin) samples with this assay. For CSF please use the instruction for use ABVL0601. If the assay is performed within 5 days after sample collection, the samples should be kept at 2...8 °C; otherwise they should be aliquoted and stored deep-frozen (-70...-20 °C). If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing.
Heat inactivation of samples is not recommended.

7.1. Sample Dilution

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

8. ASSAY PROCEDURE

8.1. Test Preparation

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

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

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

Adjust the incubator to 37 ± 1 °C.

1. Dispense 100 µl standards/controls and diluted samples into their respective wells. Leave well A1 for the Substrate Blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 1 hour ± 5 min at 37 ± 1 °C.**
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µl of Washing Buffer. Avoid overflows from the reaction wells. The interval between washing and aspiration should be > 5 sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!
   Note: Washing is important! Insufficient washing results in poor precision and false results.
5. Dispense 100 µl Conjugate into all wells except for the Substrate Blank well A1.
6. **Incubate for 30 min at room temperature (20...25 °C).** Do not expose to direct sunlight.
7. Repeat step 4.
8. Dispense 100 µl TMB Substrate Solution into all wells.
9. **Incubate for exactly 15 min at room temperature (20...25 °C) in the dark.** A blue colour occurs due to an enzymatic reaction.
10. Dispense 100 µl Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution, thereby a colour change from blue to yellow occurs.
11. Measure the absorbance at 450/620 nm within 30 min after addition of the Stop Solution.

8.2. Measurement

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

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

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

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

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

9. RESULTS

9.1. Run Validation Criteria

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

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

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

9.2. Calculation of Results

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

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

9.2.1. Results in Units [NTU]

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

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

9.3. Interpretation of Results

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

9.3.1. Antibody Isotypes and State of Infection

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

10. SPECIFIC PERFORMANCE CHARACTERISTICS

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

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

10.1. Precision

Intraassay	n	Mean (OD)	CV (%)
#1	24	0.550	5.82
#2	24	1.048	4.27
#3	24	0.998	4.18
Interassay	**n**	**Mean (NTU)**	**CV (%)**
#1	12	19.82	8.53
#2	12	14.08	13.38
#3	12	2.28	7.43

10.2. Diagnostic Specificity

The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte.
It is 100.0% (95% confidence interval: 97.49% - 100.0%).

10.3. Diagnostic Sensitivity

The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte.
It is 100.0% (95% confidence interval: 81.47% - 100.0%).

10.4. Interferences

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

10.5. Cross Reactivity

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

11. LIMITATIONS OF THE PROCEDURE

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

12. PRECAUTIONS AND WARNINGS

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

12.1. Disposal Considerations

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

13. ORDERING INFORMATION

Prod. No.: BRUM0050 Brucella IgM ELISA (96 Determinations)

NovaTec Immundiagnostica GmbH

NovaLisa®

Brucella IgG

ELISA

CE

Only for in-vitro diagnostic use

Product Number: BRUG0050 (96 Determinations)

ENGLISH

1. INTRODUCTION

Brucella is a small Gram-negative bacterium (0.4-0.8 µm in diameter and 0.4-3.0 µm in length) which is non-flagellated, and non-spore-forming. They are named after the military doctor David Bruce, who on Malta in 1887 isolated the pathogens from the spleen of a soldier who died of undulating fever. Four species are pathogenic to human: Brucella abortus, Brucella melitensis, Brucella suis and Brucella canis. All four species are exciters of Brucellosis, a disease characterized by undulating fever. Depending on exciter the disease is also called Morbus Bang (B. abortus) or Malta fever (B. melitensis).

The pathogens are transmitted from animals, which are mainly affected. The infection is caused by contact with ill animals or their excrements as well as by non-pasteurized milk and milk products like fresh cheese from sheep or goat. Main entrances are skin wounds, conjunctives and digestive tract. The intact pathogens are transported by granulocytes into local lymph nodes, from where they spread haematogenous. All kind of organs can be infected. Symptoms depend up on the infected organ, where an inflammation takes place. Until now the pathogenic mechanism is not completely understood.

Brucellosis appears worldwide. In non-pasteurized milk and milk products Brucella is viably and infectious for weeks. Bovine brucellosis caused by Brucella abortus is still the most widespread form, although reported incidence and prevalence of the disease vary widely from country to country (from <0.01 to >200 per 100,000 population). Brucella melitensis is endemic in areas where keeping of sheeps and goats is frequent. It causes serious human infections. Working with these bacteria in laboratories necessitates highest carefulness because of high contagiosity. Risk groups include abattoir workers, meat inspectors, animal handlers, veterinarians, and laboratorians. Brucellosis is a nationally notifiable disease and reportable to the local health authority.

Species	Disease	Symptoms (e.g.)	Transmission route
B. abortus (cattle) B. melitensis (sheep, goats) B. suis (pigs) B. canis (dogs)	Brucella	Fever, chills (undulating fever), malaise, arthritis, hepatitis, endocarditis, hepatomegalie, osteomyelitis (OM)	Oral (non-pasteurized milk and milk products) Percutan (contact with ill animals or their excrements) In general no transmission from human to human

The presence of pathogen or infection may be identified by

- Histology
- Serology: e.g. ELISA

2. INTENDED USE

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

3. PRINCIPLE OF THE ASSAY

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

4. MATERIALS

4.1. Reagents supplied

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

For potential hazardous substances please check the safety data sheet.

4.2. Materials supplied

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

4.3. Materials and Equipment needed

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

5. STABILITY AND STORAGE

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

6. REAGENT PREPARATION

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

6.1. Coated Microplate

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

6.2. Washing Buffer (20x conc.)

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

6.3. TMB Substrate Solution

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

7. SAMPLE COLLECTION AND PREPARATION

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

7.1. Sample Dilution

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

8. ASSAY PROCEDURE

8.1. Test Preparation

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

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

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

Adjust the incubator to 37 ± 1 °C.

1. Dispense 100 µl standards/controls and diluted samples into their respective wells. Leave well A1 for the Substrate Blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 1 hour ± 5 min at 37 ± 1 °C.**
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µl of Washing Buffer. Avoid overflows from the reaction wells. The interval between washing and aspiration should be > 5 sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!
   Note: Washing is important! Insufficient washing results in poor precision and false results.
5. Dispense 100 µl Conjugate into all wells except for the Substrate Blank well A1.
6. **Incubate for 30 min at room temperature (20...25 °C).** Do not expose to direct sunlight.
7. Repeat step 4.
8. Dispense 100 µl TMB Substrate Solution into all wells.
9. **Incubate for exactly 15 min at room temperature (20...25 °C) in the dark.** A blue colour occurs due to an enzymatic reaction.
10. Dispense 100 µl Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution, thereby a colour change from blue to yellow occurs.
11. Measure the absorbance at 450/620 nm within 30 min after addition of the Stop Solution.

8.2. Measurement

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

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

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

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

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

9. RESULTS

9.1. Run Validation Criteria

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

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

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

9.2. Calculation of Results

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

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

9.2.1. Results in Units [NTU]

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

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

9.3. Interpretation of Results

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

9.3.1. Antibody Isotypes and State of Infection

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

10. SPECIFIC PERFORMANCE CHARACTERISTICS

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

10.1. Precision

Intraassay	n	Mean (OD)	CV (%)
#1	24	0.577	4.14
#2	24	1.276	3.34
#3	24	1.200	2.75
Interassay	**n**	**Mean (NTU)**	**CV (%)**
#1	12	23.22	4.97
#2	12	20.13	6.05
#3	12	5.10	8.55

10.2. Diagnostic Specificity

The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte.
It is 98.78% (95% confidence interval: 93.39% - 99.97%)

10.3. Diagnostic Sensitivity

The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte.
It is 100.0% (95% confidence interval: 66.37% - 100.0%).

10.4. Interferences

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

10.5. Cross Reactivity

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

11. LIMITATIONS OF THE PROCEDURE

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

12. PRECAUTIONS AND WARNINGS

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

12.1. Disposal Considerations

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

13. ORDERING INFORMATION

Prod. No.: BRUG0050 Brucella IgG ELISA (96 Determinations)

NovaTec Immundiagnostica GmbH

NovaLisa®

Hantavirus IgM

ELISA

CE

Only for in-vitro diagnostic use

Product Number: HANM0670 (96 Determinations)

ENGLISH

1. INTRODUCTION

Hantaviruses are negative sense RNA viruses in the Bunyaviridae family. Humans may be infected with Hantaviruses through urine, saliva or contact with rodent waste products. Some Hantaviruses may cause serious diseases in humans, such as hemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS).

Human infections of Hantaviruses have almost entirely been linked to human contact with rodent excrement, but recent human-to-human transmission has been reported with the Andes virus in South America.

Hantavirus has an incubation time of two to four weeks in humans before symptoms of infection occur. The symptoms of HFRS can be split into five phases:

- Febrile phase: Symptoms include fever, chills, sweaty palms, diarrhea, malaise, headaches, nausea, abdominal and back pain, respiratory problems such as the ones common in influenza virus infection, as well as gastro-intestinal problems. These symptoms normally occur for three to seven days and arise about two to three weeks after exposure.
- Hypotensive phase: This occurs when the blood platelet levels drop and symptoms can lead to tachycardia and hypoxemia. This phase can last for 2 days.
- Oliguric phase: This phase lasts for three to seven days and is characterized by the onset of renal failure and proteinuria occurs.
- Diuretic phase: This is characterized by diuresis of three to six liters per day, which can last for a couple of days up to weeks.
- Convalescent phase: This is normally when recovery occurs and symptoms begin to improve.

Regions especially affected by HFRS include China, the Korean Peninsula, Russia (Hantaan, Puumala and Seoul viruses), and northern and western Europe (Puumala and Dobrava virus).

Species	Disease	Symptoms (e.g.)	Transmission route
Puumala virus Dobrava virus	Hemorrhagic fever with renal syndrome (HFRS)	Initial: suddenly occurring symptoms like intense headache, back and abdominal pain, fever, chills, nausea, and blurred vison.	After exposure to aerosolized urine, droppings, or saliva of infected rodents or their nests (airborne transmission).
Hantaan virus Seoul virus		Late: low blood pressure, acute shock, vascular leakage, and acute kidney failure.	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]

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

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

9.3. Interpretation of Results

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

9.3.1. Antibody Isotypes and State of Infection

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

10. SPECIFIC PERFORMANCE CHARACTERISTICS

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

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

10.1. Precision

Intraassay	n	Mean (E)	Cv (%)
#1	24	0.649	4.11
#2	24	1.322	3.24
#3	24	1.064	4.29

Interassay	n	Mean (NTU)	Cv (%)
#1	12	23.49	12.94
#2	12	15.31	12.08
#3	12	0.73	12.86

10.2. Diagnostic Specificity

The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte. It is 99.03% (95% confidence interval: 94.71% - 99.98%).

10.3. Diagnostic Sensitivity

The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte. It is 96.3% (95% confidence interval: 89.56% - 99.23%).

10.4. Interferences

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

10.5. Cross Reactivity

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

11. LIMITATIONS OF THE PROCEDURE

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

12. PRECAUTIONS AND WARNINGS

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

12.1. Disposal Considerations

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

13. ORDERING INFORMATION

Prod. No.: HANM0670 Hantavirus IgM ELISA (96 Determinations)

NovaTec Immundiagnostica GmbH

NovaLisa®

Hantavirus IgG

ELISA

CE

Only for in-vitro diagnostic use

Product Number: HANG0670 (96 Determinations)

ENGLISH

1. INTRODUCTION

Hantaviruses are negative sense RNA viruses in the Bunyaviridae family. Humans may be infected with Hantaviruses through urine, saliva or contact with rodent waste products. Some Hantaviruses may lead to serious diseases in humans, such as hemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS).

Human infections of Hantaviruses have almost entirely been linked to human contact with rodent excrement, but recent human-to-human transmission has been reported with the Andes virus in South America.

Hantavirus has an incubation time of two to four weeks in humans before symptoms of infection occur. The symptoms of HFRS can be split into five phases:

- Febrile phase: Symptoms include fever, chills, sweaty palms, diarrhea, malaise, headaches, nausea, abdominal and back pain, respiratory problems such as the ones common in influenza virus infection, as well as gastro-intestinal problems. These symptoms normally occur for three to seven days and arise about two to three weeks after exposure.
- Hypotensive phase: This occurs when the blood platelet levels drop and symptoms can lead to tachycardia and hypoxemia. This phase can last for 2 days.
- Oliguric phase: This phase lasts for three to seven days and is characterized by the onset of renal failure and proteinuria occurs.
- Diuretic phase: This is characterized by diuresis of three to six liters per day, which can last for a couple of days up to weeks.
- Convalescent phase: This is normally when recovery occurs and symptoms begin to improve.

Regions especially affected by HFRS include China, the Korean Peninsula, Russia (Hantaan, Puumala and Seoul viruses), and northern and western Europe (Puumala and Dobrava virus).

Species	Disease	Symptoms (e.g.)	Transmission route
Puumala virus Dobrava virus Hantaan virus Seoul virus	Hemorrhagic fever with renal syndrome (HFRS)	Initial: suddenly occurring symptoms like intense headache, back and abdominal pain, fever, chills, nausea, and blurred vison. Late: low blood pressure, acute shock, vascular leakage, and acute kidney failure	After exposure to aerosolized urine, droppings, or saliva of infected rodents or their nests (airborne transmission). Also by direct contact with these materials to broken skin or onto mucous membranes.
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 ± 5 min at 37 ± 1 °C.**
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µl of Washing Buffer. Avoid overflows from the reaction wells. The interval between washing and aspiration should be > 5 sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!
   Note: Washing is important! Insufficient washing results in poor precision and false results.
5. Dispense 100 µl Conjugate into all wells except for the Substrate Blank well A1.
6. **Incubate for 30 min at room temperature (20...25 °C).** Do not expose to direct sunlight.
7. Repeat step 4.
8. Dispense 100 µl TMB Substrate Solution into all wells.
9. **Incubate for exactly 15 min at room temperature (20...25 °C) in the dark.** A blue colour occurs due to an enzymatic reaction.
10. Dispense 100 µl Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution, thereby a colour change from blue to yellow occurs.
11. Measure the absorbance at 450/620 nm within 30 min after addition of the Stop Solution.

8.2. Measurement

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

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

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

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

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

9. RESULTS

9.1. Run Validation Criteria

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

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

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

9.2. Calculation of Results

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

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

9.2.1. Results in Units [NTU]

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

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

9.3. Interpretation of Results

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

9.3.1. Antibody Isotypes and State of Infection

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

10. SPECIFIC PERFORMANCE CHARACTERISTICS

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

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

10.1. Precision

Intraassay	n	Mean (E)	Cv (%)
#1	24	0.450	3.61
#2	24	1.333	6.41
#3	24	1.264	4.78

Interassay	n	Mean (NTU)	Cv (%)
#1	12	27.44	5.34
#2	12	25.44	8.15
#3	12	1.09	12.09

10.2. Diagnostic Specificity

The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte. It is 96.59% (95% confidence interval: 90.36% - 99.29%).

10.3. Diagnostic Sensitivity

The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte. It is 99.16% (95% confidence interval: 95.41% - 99.98%).

10.4. Interferences

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

10.5. Cross Reactivity

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

11. LIMITATIONS OF THE PROCEDURE

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

12. PRECAUTIONS AND WARNINGS

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

12.1. Disposal Considerations

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

13. ORDERING INFORMATION

Prod. No.: HANG0670 Hantavirus IgG ELISA (96 Determinations)

NovaTec Immundiagnostica GmbH

NovaLisa®

Legionella pneumophila IgM

ELISA

CE

Only for in-vitro diagnostic use

Product Number: LEGM0650 (96 Determinations)

ENGLISH

1. INTRODUCTION

Legionellae are aerobic gram-negative facultative intracellular parasites of certain protozoa. They are found in freshwater environments worldwide and can cause respiratory disease (legionellosis) in humans.

Legionella was first identified after an outbreak of pneumonia involving delegates of the 1976 American Legion Convention at a Philadelphia hotel.

The genus Legionella currently has at least 50 species comprising 70 distinct serogroups. One species of Legionella, L. pneumophila, is the aetiological agent of approximately 90 % of legionellosis cases, and serogroup 1 (Sg1) accounts for about 84 % of these cases.

L. pneumophila multiplies itself at temperatures between 25 and 42 °C, with an optimal growth temperature of 35 °C. Legionella thrives in warm, stagnant water in the environment and in artificial systems such as cooling towers, evaporative condensers, hot and cold water systems and spa pools that mimic the natural environment in which the organism thrives. These systems also provide the means by which aerosols/droplets are generated and the organism dispersed into the atmosphere.

Legionellosis can be acquired by the inhalation of aerosols containing Legionella bacteria or by micro-aspiration of ingested water contaminated with Legionella. Person-to-person transmission is not thought to be a risk.

The likelihood of contracting Legionnaires' disease depends on the level of contamination in the water source, the susceptibility of the person exposed, and the intensity of exposure. Legionnaires' disease is characterized as an "opportunistic" disease that attacks individuals who have an underlying illness or a weakened immune system. Predisposing risks include increasing age, being male, heavy smoking, alcohol abuse, chronic lung disease, immunosuppressive therapy, cancer chemotherapy, organ or bone marrow transplant, and corticosteroid therapy.

Legionellosis can appear in two distinct clinical presentations: Legionella pneumonia (Legionnaires' disease) with an incubation period of approx. 2-10 days (may extend up to 16-20 days) and Pontiac fever (incubation period: normally 12-48 hours).

Legionella pneumonia (Legionnaires' disease) is a serious form of pneumonia that carries with it a case-fatality ratio of 10-15 %. Legionnaires' disease patients initially present with cough, fever and nonspecific symptoms including malaise, myalgia and headache. Some patients develop shaking chills, chest pain, diarrhea, delirium or other neurologic symptoms. Extra pulmonary involvement is rare.

Pontiac fever is a milder form of the disease without manifestations of pneumonia and presents as an influenza-like illness. Symptoms may include headache, chills, muscle aches, a dry cough and fever. It is usually self-limiting and typically does not require treatment. The attack rate is much higher than for Legionnaires' disease (up to 95 % of those exposed).

Species	Disease	Symptoms (e.g.)	Transmission route
Legionella pneumophila	Legionella pneumonia (Legionnaires' disease)	Cough, fever and nonspecific symptoms (malaise, myalgia, headache). Some patients develop shaking chills, chest pain, diarrhea, delirium or other neurologic symptoms.	Inhalation of aerosols containing Legionella bacteria or micro-aspiration of ingested water contaminated with Legionella
	Pontiac fever	Influenza-like illness (headache, chills, muscle aches, a dry cough and fever) without manifestations of pneumonia	

The presence of pathogen or infection 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.
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

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

8.2. Measurement

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

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

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

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

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

9. RESULTS

9.1. Run Validation Criteria

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

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

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

9.2. Calculation of Results

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

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

9.2.1. Results in Units [NTU]

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

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

9.3. Interpretation of Results

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

9.3.1. Antibody Isotypes and State of Infection

Serology	Significance
IgM	Characteristic of the primary antibody response High IgM titer with low IgG liter: → 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 100.0% (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 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 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.: LEGM0650 Legionella pneumophila IgM ELISA (96 Determinations)

NovaTec Immundiagnostica GmbH

NovaLisa®

Legionella pneumophila IgG

ELISA

CE

Only for in-vitro diagnostic use

Product Number: LEGG0650 (96 Determinations)

ENGLISH

1. INTRODUCTION

Legionellae are aerobic gram-negative facultative intracellular parasites of certain protozoa. They are found in freshwater environments worldwide and can cause respiratory disease (legionellosis) in humans.
Legionella was first identified after an outbreak of pneumonia involving delegates of the 1976 American Legion Convention at a Philadelphia hotel.
The genus Legionella currently has at least 50 species comprising 70 distinct serogroups. One species of Legionella, L. pneumophila, is the aetiological agent of approximately 90 % of legionellosis cases, and serogroup 1 (Sg1) accounts for about 84 % of these cases.
L. pneumophila multiplies itself at temperatures between 25 and 42 °C, with an optimal growth temperature of 35 °C. Legionella thrives in warm, stagnant water in the environment and in artificial systems such as cooling towers, evaporative condensers, hot and cold water systems and spa pools that mimic the natural environment in which the organism thrives. These systems also provide the means by which aerosols/droplets are generated and the organism dispersed into the atmosphere.
Legionellosis can be acquired by the inhalation of aerosols containing Legionella bacteria or by micro-aspiration of ingested water contaminated with Legionella. Person-to-person transmission is not thought to be a risk.
The likelihood of contracting Legionnaires' disease depends on the level of contamination in the water source, the susceptibility of the person exposed, and the intensity of exposure. Legionnaires' disease is characterized as an "opportunistic" disease that attacks individuals who have an underlying illness or a weakened immune system. Predisposing risks include increasing age, being male, heavy smoking, alcohol abuse, chronic lung disease, immunosuppressive therapy, cancer chemotherapy, organ or bone marrow transplant, and corticosteroid therapy.
Legionellosis can appear in two distinct clinical presentations: Legionella pneumonia (Legionnaires' disease) with an incubation period of approx. 2-10 days (may extend up to 16-20 days) and Pontiac fever (incubation period: normally 12-48 hours).
Legionella pneumonia (Legionnaires' disease) is a serious form of pneumonia that carries with it a case-fatality ratio of 10-15 %. Legionnaires' disease patients initially present with cough, fever and nonspecific symptoms including malaise, myalgia and headache. Some patients develop shaking chills, chest pain, diarrhea, delirium or other neurologic symptoms. Extra pulmonary involvement is rare.
Pontiac fever is a milder form of the disease without manifestations of pneumonia and presents as an influenza-like illness. Symptoms may include headache, chills, muscle aches, a dry cough and fever. It is usually self-limiting and typically does not require treatment. The attack rate is much higher than for Legionnaires' disease (up to 95 % of those exposed).

Species	Disease	Symptoms (e.g.)	Transmission route
Legionella pneumophila	Legionella pneumonia (Legionnaires' disease)	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	

The presence of pathogen or infection 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.
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

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

8.2. Measurement

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

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

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

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

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

9. RESULTS

9.1. Run Validation Criteria

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

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

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

9.2. Calculation of Results

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

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

9.2.1. Results in Units [NTU]

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

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

9.3. Interpretation of Results

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

9.3.1. Antibody Isotypes and State of Infection

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

10. SPECIFIC PERFORMANCE CHARACTERISTICS

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

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

10.1. Precision

Intraassay	n	Mean (E)	CV (%)
#1	24	0.275	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 100.0% (95% confidence interval: 88.78% - 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 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

- 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.: LEGG0650 Legionella pneumophila IgG ELISA (96 Determinations)

EUROIMMUN Medizinische Labordiagnostika AG

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 -

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