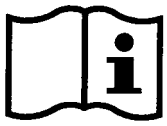
Product information



Userś Manual

Trichinella spiralis ELISA

Enzyme immunoassay for the determination of antibodies against Trichinella spiralis in human serum or plasma

REF **IB79830**

Σ **96**

IVD **For *in-vitro* diagnostic use only.**

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1. INTRODUCTION

Trichinosis (also called trichinellosis) is caused by nematodes (roundworms) of the genus Trichinella. In addition to the classical agent Trichinella spiralis, which is found worldwide in many carnivorous and omnivorous animals, four other species (T. pseudospiralis, T. nativa, T. nelsoni, and T. britovi) are recognized. Trichinosis is acquired by ingesting meat containing cysts of Trichinella. After exposure to gastric acid and pepsin, the larvae are released from the cysts and invade the small bowel mucosa where they develop into adult worms (female 2.2 mm in length, males 1.2 mm). After 1 week, the females release larvae that migrate to the striated muscles where they encyst. Encystment is completed in 4 to 5 weeks and the encysted larvae may remain viable for several years. Ingestion of the encysted larvae perpetuates the cycle.
Trichinosis infection occurs worldwide, but is most common in parts of Europe and the United States. Light infections may be asymptomatic. For mild to moderate infections, most symptoms subside within a few months whereas fatigue, weakness, and diarrhoea may last for months. In severe cases, death can occur.

Species	Disease	Symptoms	Mechanism of Infection
Trichinella spiralis	Trichinosis	Nausea, diarrhoea, vomiting, fatigue, fever and abdominal discomfort. Larval migration into muscle tissue can cause edema, conjunctivitis, myalgias, splinter hemorrhages, rashes and blood eosinophilia.	Infection can only occur by eating raw or undercooked pork and wild game products infected with the larvae of Trichinella worms.

The presence of pathogen or Infection may be identified by
- Microscopy: muscle biopsy
- Serology: Detection of antibodies by ELISA

2. INTENDED USE

The IBL America Trichinella spiralis IgG-ELISA is intended for the determination of IgG class antibodies against Trichinella spiralis in human serum or plasma (citrate). For in-vitro diagnostic use only.

3. PRINCIPLE OF THE ASSAY

The immunoenzymatic determination of antibodies against Trichinella spiralis 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 color. Absorbance at 450/620 nm is read using an ELISA microwell plate reader.

4. MATERIALS

4.1. Reagents supplied

- **SORB | MT Trichinella spiralis Coated Microplate (IgG):** 12 breakapart 8-well snap-off strips coated with Trichinella spiralis antigens; in resealable aluminium foil.
- **SAM | DIL IgG Sample Diluent:** 1 bottle containing 100 ml of phosphate buffer (10mM) for sample dilution; pH 7.2 ± 0.2; coloured yellow; ready to use; white cap.
- **STOP | SOLN Stop Solution**: 1 bottle containing 15 ml acidic solution, 0.2 mol/L; ready to use; red cap.
- **WASH | SOLN 20x Washing Buffer (20x conc.):** 1 bottle containing 50 ml of a 20-fold concentrated phosphate buffer (0.2M), pH 7.2 ± 0.2, for washing the wells; white cap.
- **ENZ | CONJ Trichinella spiralis Protein A Conjugate:** 1 bottle containing 20 ml of peroxidase labelled Protein A in phosphate buffer (10mM); colored blue, ready to use; black cap.
- **SUB | TMB TMB Substrate Solution:** 1 bottle containing 15 ml 3,3',5,5'-tetramethylbenzidine (TMB), <0.1% ready to use; yellow cap; <5% NMP.
- **CAL | C Trichinella spiralis Positive Control:** 1 vial containing 2 ml control (human serum or plasma); coloured yellow; ready to use; red cap.
- **CAL | B Trichinella spiralis Cut-off Control:** 1 vial containing 3 ml control (human serum or plasma); coloured yellow; ready to use; green cap.
- **CAL | A Trichinella spiralis 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 kits 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 to 25°C) and mix t hem before starting the test run!

6.1. Coated Snap-off Strips

The break-apart snap-off strips are coated with Trichinella spiralis 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 Solution (20xconc.)

Dilute washing solution 1+19; e.g. 10 ml Washing Buffer + 190 ml distilled water. The diluted buffer is stable for 5 days at room temperature (20 to 25°C). In case crystals appear in the concentrate, warm up the solution to 37ºC 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 have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away.

7. SAMPLE COLLECTION AND PREPARATION

Use human serum or plasma (citrate, heparin) samples with this assay. If the assay is performed within 5 days after sample collection, the sample should be kept at 2-8°C; otherwise they should be aliquoted and stored deep-frozen (-20 to -70°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 test protocol carefully **before** performing the assay. Result reliability depends on strict adherence to the test protocol as described. The following test procedure is only validated for manual procedure. If performing the test on ELISA automatic systems we recommend to increase the washing steps from three to five and the volume of washing solution from 300µl to 350µl to avoid washing effects. Prior to commencing the assay, the distribution and identification plan for all samples and 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 control and sample.

Adjust the incubator to 37° ± 1°C.

1. Dispense 100µl controls and diluted samples into their respective wells. Leave well A1 for 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 300µl of Washing Buffer. Avoid overflows from the reaction wells. The soak time between each wash cycle 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 100µl Conjugate into all wells except for the Substrate Blank well A1.
6. **Incubate for 30 min at room temperature (20-25ºC).** 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 (20-25ºC) in the dark.** A blue colour occurs due to an enzymatic reaction.
10. Dispense 100µl 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 control and sample in the plate layout. Bichromatic measurement using a reference wavelength of 620nm 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 – 1.30.**
- **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 [U}

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

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

9.3. Interpretation of Results

Cut-off	10 U	
Positive	> 11 U	Antibodies against the pathogen are present. There has been a contact with the antigen (pathogen resp. vaccine).
Equivocal	9 – 11 U	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 U	The sample contains no antibodies against the pathogen. A previous contact with the antigen (pathogen resp. vaccine) is unlikely.
Determination of an infectious disease should not be established on the basis of a single test result. A precise determination should take into consideration clinical history, symptomatology as well as serological data.		

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 IBL-America.

10.1. Precision

Intraassay	n	Mean	Cv (%)
#1	24	0.748	6.37
#2	24	1.225	4.21
#3	24	1.570	5.13

Interassay	n	Mean	Cv (%)
#1	12	17.50	8.38
#2	12	21.33	13.08
#3	12	3.69	10.38

10.2. Specificity

The specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte. It is 94.81 % (95% confidence interval: 87.23% - 98.57%)

10.3. Sensitivity

The 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: 79.41% - 100.0%)

10.4. Interferences

Interferences with hemolytic, lipemic or icteric sera 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 a 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 test kits 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.
- For *in-vitro* diagnostic use only.
- 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. Nevertheless, all materials should still be regarded and handled as potentially infectious.
- 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 expiration 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 control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense conjugate without splashing accurately to the bottom of 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.

13.0 BIBLIOGRAPHY

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14.0 Summary of the Test Procedure

SCHEME OF THE ASSAY

Trichinella spiralis IgG-ELISA

Test Preparation

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

Assay Procedure

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

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SYMBOLS USED WITH IBL-AMERICA ASSAYS

Symbol	English	Deutsch	Francais	Espanol	Italiano
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conforme aux normes européennes	Conformidad europea	Conformità europea
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las Instrucciones	Consultare le istruzioni per l'uso
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Ussage Diagnostic in vitro	Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Référence	Número de catálogo	No. di Cat.
LOT	Lot. No. / Batch code	Chargen-Nr.	No. de lot	Número de lote	Lotto no
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Note warnings and precautions	Warnhinweise und Vorsichtsmaßnahmen beachten	Avertissements et mesures de précaution font attention	Tiene en cuenta advertencias y precauciones	Annoti avvisi e le precauzioni
	Storage Temperature	Lagerungstemperatur	Temperature de conservation	Temperatura de conservacion	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributtore