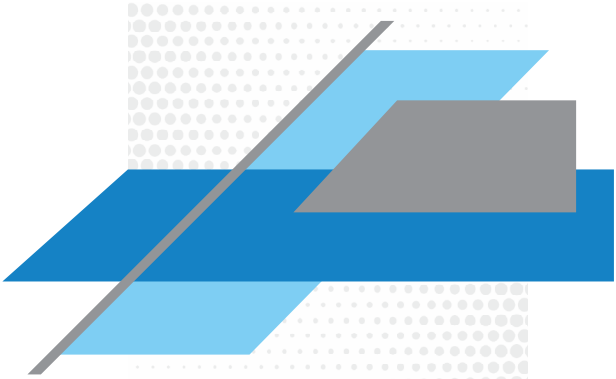
anti-Lamblia

ELISA kit for the qualitative detection of antibodies to *Giardia lamblia (intestinalis)*

Instructions for use



IVD

REF EI-606

Σ 96 tests

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EQUI anti-Lamblia

ELISA kit for the qualitative detection of antibodies to
до *Giardia lamblia (intestinalis)*

1. INTENDED USE

The «EQUI anti-Lamblia» is ELISA kit intended to qualitatively detect antibodies to *Giardia lamblia (intestinalis)* in human serum or plasma by enzyme-linked immunosorbent assay (ELISA) to diagnose giardiasis. The testing procedure is designed for both manual arrangement with automatic pipettes and standard equipment, and for automated «open» immunoassay analysers.

Target group: children, pet owners, citizens of rural areas, summer house owners.

Usage: ELISA kit is used in clinical diagnostic laboratories and other institutions engaged in *in vitro* diagnostics.

2. CLINICAL SIGNIFICANCE

Giardiasis is considered one of the most common parasitic diseases of the small intestine in the world. This infection is a major cause of acute and chronic diarrhea, especially in children. The etiological agent of giardiasis is *Giardia lamblia*, which is also called *Giardia intestinalis* or *Giardia duodenalis*.

Giardia lamblia are unicellular flagellate protozoa that parasitize in the intestines of humans and some other mammals. During the life cycle of these parasites, two stages alternate: cysts, resistant to external conditions, and a vegetative form - trophozoites. Infection occurs when cysts enter the human gastrointestinal tract. After experiencing the effects of gastric acid, cysts in the duodenum turn into trophozoites, which parasitize in the upper parts of the small intestine. They absorb nutrients from the intestinal lumen, block parietal digestion and disrupt the motility of the intestine.

Humans get infected via fecal-oral routes through cyst-contaminated food, water, unwashed hands, and so on. Giardia can also be transmitted to humans from infected cats, dogs, and livestock. Giardiasis is especially common in regions with poor sanitation. In addition, human-to-human transmission is common in preschools.

In many cases, the invasion of Giardia occurs without clinical manifestations. In other cases, the first symptoms of giardiasis appear in 1-3 weeks after infection. They are most often manifested by spasms, bloating, nausea and diarrhea, which leads to dehydration and weight loss. The acute form of the disease can last up to two weeks and end in recovery without additional treatment or become chronic. Chronic giardiasis develops when the duration of the invasion is longer than 2 month and the exacerbation of clinical manifestations (diarrhea) is cyclical. *Giardia lamblia* parasitism can lead to malabsorption syndrome, which disrupts the absorption of carbohydrates and fats, as well as the metabolism of vitamins B12, A and C.

Immune response to invasion and non-immune factors are important to control the development of the disease and the severity of clinical manifestations. Both

humoral and cellular immunity play the part in the eradication of the pathogen, the role of which is still subjected to scientific research. In addition, partial resistance to re-infection is formed due to protective mechanisms of the body.

Typically, to diagnose giardiasis, the duodenal contents and feces are examined for trophozoites and cysts of giardiasis. In case of the chronic course of the disease, cysts get excreted periodically, and, considering this, the additional tests should be performed regularly for several weeks. Another method of diagnosing giardiasis is to detect *Giardia lamblia* antigens in the feces. However, serodiagnosis with the detection of specific antibodies to Giardia antigens is an important step in assessing the immune response of patients. Detection of specific IgM antibodies suggests an acute stage of giardiasis. However, the detection of specific IgG and IgA antibodies should be interpreted with caution: in some regions they persist for a long time after infection, while in others their level decreases after eradication of the pathogen.

3. ANALYSIS PRINCIPLE

The procedure of testing for *Giardia lamblia* specific antibodies in «EQUI anti-Lamblia» ELISA kit is based on «indirect» solid-phase ELISA with a two-stage incubation. Recombinant *Giardia lamblia* antigens are entrapped in the wells. During the first step of incubation of the test samples in the wells of the ELISA plate, *Giardia lamblia*-specific antibodies, if present in the samples, bind to the solid phase antigens. The wells are washed to remove unbound antibodies and have only specific antigen-antibody complexes left. Then, a conjugate of anti-species (anti-IgG and anti-IgA) monoclonal antibodies with horseradish peroxidase is added, which binds to solid-phase immune complexes. Unbound components are removed by washing. Antigen-antibody complexes are detected by adding a solution of chromogen 3,3',5,5'-tetramethylbenzidine (TMB) with hydrogen peroxide. After 30-minute incubation, the reaction is stopped by adding the stop solution. The optical density (OD) in the wells is determined using a spectrophotometer at 450/620-695 nm. The intensity of the yellow colour is proportional to the level of antibodies in the sample.

4. MATERIALS AND EQUIPMENT

4.1. Contents of the ELISA kit

		Microplate
STRIPS	1 x 96 wells	Each plate well is coated with *Giardia lamblia* purified antigens. The wells are detachable. After the first opening, store unused strips in the package at 2-8 °C for a maximum of 6 months
		Positive control
CONTROL +	1 x 0,35 ml	Conjugated specific monoclonal antibody solution with preservative (pink). Store at 2-8 °C
		Negative control
CONTROL -	1 x 1,2 ml	Negative human serum with a preservative (yellow). Store at 2-8 °C

DIL SAMPLE	1 x 11 ml	**Serum dilution solution** Buffer solution with a milk extract, a detergent and a preservative (purple). Store at 2-8 °C
SOLN CONJ	1 x 13 ml	**Conjugate solution (ready to use)** Buffer solution of monoclonal antibodies to human IgG and IgA, conjugated with horseradish peroxidase, with stabilizers and preservative (green). Store at 2-8 °C
SOLN TMB	1 x 13 ml	**TMB solution (ready to use)** TMB solution, H_2O_2, a stabilizer, a preservative (colourless). Store at 2-8 °C
TWEEN WASH 20x	1 x 50 ml	**Washing solution TWEEN (20x concentrated)** 20-fold phosphate buffer concentrate with Tween-20 (colourless). Dilute TWEEN detergent (20x) at 1:20 with distilled or deionized water (e. g., 5 mL of concentrate + 95 mL of water for 8 wells) before use. Store the diluted solution at 2-8 °C for a maximum of 7 days
SOLN STOP	1 x 13 ml	**Stop Solution (ready to use)** 0.5 mol H_2SO_4 solution (colourless). Store at 2-8 °C

The ELISA kit also includes adhesive films (2 items), sample application plan (1 item), checklist, and instruction for use.

4.2. Optional reagents, materials and equipment

Automatic single and multichannel pipettes 10–1000 µL, tips, volumetric laboratory glassware (10–1,000 mL), deionized or distilled water, thermostat at 37 °C, automatic or semi-automatic plate washer, spectrophotometer (reader) for microplates at 450/620-695 nm, appropriate containers for potentially contaminated waste, timer, filter paper, disposable powder-free gloves, disinfectants.

5. PRECAUTIONS AND SAFETY

5.1. Precautions

Be sure to read the instructions for use carefully before the test. The validity of the test results depends on strict following of the test procedure.

- do not use the ELISA kit components after the expiry date;
- do not use for analysis or mix components of different batches, components of kits for different nosologies, or reagents from other manufacturers with the «EQUI anti-Lamblia» ELISA kit;
- do not freeze the ELISA kit or its contents;
- after using a reagent, close each vial with its cap;
- when washing, control filling and complete aspiration of solution from the wells;
- use a new pipette tip each time you add samples or reagents;
- prevent direct sunlight from reaching the reagents from the ELISA kit;
- SOLN TMB solution must be colourless before use. Do not use the solution if its colour is blue or yellow. Avoid contact of SOLN TMB with metals or metal ions. Use only clean glassware thoroughly rinsed with distilled water;

– do not use reagents with colour not in line with para. 4.1;
– under no circumstances should the same glassware be used for SOLN|CONJ and SOLN|TMB;
– do not evaluate the test results visually (without a reader);
– any optional equipment that is in direct contact with biological material or kit components should be considered contaminated and requires cleaning and decontamination;
– the ELISA kit includes materials for 96 tests. Dispose of the used components as well as any remaining unused components.

5.2. Safety requirements

– all reagents in the ELISA kit are for laboratory professional use for *in vitro* diagnosis only and may only be used by qualified personnel;
– conduct the tests in disposable powder-free gloves and goggles only;
– do not eat, drink, smoke, or apply make-up in the test room;
– do not mouth-pipette the solutions;
– controls from the «EQUI anti-Lamblia» ELISA kit have been tested and found to be for anti-HIV1/2, anti-HCV and anti-*Treponema pallidum* antibodies and HBsAg negative; however, controls and test samples should be handled as potentially hazardous infectious materials;
– some of the kit components contain low concentrations of harmful substances and can damage skin or mucoga. In case of contact of SOLN|TMB, SOLN|STOP and SOLN|CONJ with mucous membranes or skin, immediately wash the affected area with plenty of water;
– in case of spillage of acid-free solutions, e. g. sera, treat the surface with a disinfectant solution and then wipe dry with filter paper. Otherwise first neutralize acid with sodium bicarbonate solution and then wipe the surface dry as described above.

5.3. Waste inactivation and disposal

– the liquid waste must be inactivated, for example, with hydrogen peroxide solution at the final concentration of 6% for 3 hours at room temperature, or with sodium hypochlorite at the final concentration of 5% for 30 minutes, or with other approved disinfectants;
– the solid waste must be inactivated by autoclaving at a temperature not less than 132°C;
– do not autoclave the solutions that contain sodium azide or sodium hypochlorite;
– disposal of inactivated waste must be conducted due to national laws and regulations.

6. STORAGE AND STABILITY

ELISA kit is stable up to the expiry date stated on the label when stored at 2-8°C. The kit should be transported at 2-8°C. Single transportation at a

temperature up to 23°C for two days is possible.

7. SAMPLE COLLECTION, TRANSPORTATION AND STORAGE GUIDELINES

Collect blood from the vein into the sterile test tube. Test tube must be marked with patient ID and date of sample collecting. Blood before serum separation can be stored at 2-8 ºC for 24 hours, avoiding freezing.

Serum or plasma can be stored at 2-8 °C for maximum 3 days. Frozen serum can be stored for longer periods of time at -20 °C or -70 °C. Thaw frozen samples and keep them at room temperature for 30 minutes before use. After thawing, the stir samples to achieve homogeneity. Avoid repeated freezing-thawing cycles for test samples. If serum (or plasma) is turbid, remove insoluble inclusions by centrifugation at 3000 rpm for 10-15 minutes. Do not use serum samples with hyperlipidemia, hemolysis, and bacterial growth.

Transport serum samples in insulated containers. To do that, put closed labelled tubes in a plastic bag, tightly seal it and place in the centre of an insulated container.Put the frozen cold packs on the bottom, along the side walls of the insulated container and on top of the serum samples.

8. REAGENT PREPARATION

NOTE! It is very important to keep all ELISA kit components for at least 30 min at room temperature 18-25 ºC before the assay!

8.1. Microplate preparation

To prevent water condensation in the wells, keep the STRIPS for 30 minutes at a room temperature before opening. Open the vacuum pack, detach the appropriate number of wells, and carefully pack the remaining wells with a desiccant and store tightly zip-locked at 2-8 °C. Storing the packed plate this way ensures its stability for 6 months.

8.2. Washing solution preparation

To prepare detergent, dilute TWEEN WASH 20x at 1:20 (1+19) with distilled or deionized water and stir. E. g., 5 mL of concentrate + 95 mL of water, which is enough for 8 wells. If there are crystals present in the detergent concentrate, heat the vial at 37 °C until the crystals dissolve completely (15–20 minutes). Store the diluted solution at 2-8 °C for a maximum of 7 days.

9. ASSAY PROCEDURE

9.1. Prepare the necessary number of wells (four wells for controls and a necessary number of wells for test samples) and insert them into the ELISA plate frame. Be sure to add control wells in every test run.

9.2. Fill in the sample application plan.

9.3. Prepare the detergent as per para. 8.2.

9.4. Add 80 µL of DIL SAMPLE into each plate well.

9.5. Add 20 µL of controls and test samples into the wells:
CONTROL + – into well A1,
CONTROL - – into wells B1, C1 and D1,
and test samples into the remaining wells.

At the time of adding, the solution changes its colour from brown to blue. Pipette the mix in the wells carefully to avoid foaming.

9.6. Cover the strips up with adhesive film and incubate for **30 minutes at 37 °C**.

9.7. Remove and discard the adhesive film and wash all wells 5 times with automatic washer or 8-channel pipette as follows:
– aspirate the content of all wells into a liquid waste container;
– add a minimum of 300 µl of diluted washing solution to each well, soak each well for 30 seconds;
– aspirate the content of all wells again. The residual volume after every aspiration should be less than 5 µl;
– repeat the washing step 4 more times;
– after the final aspiration, eliminate extra moisture by tapping the plate against a piece of filter paper.

9.8. Add 100 µL of SOLN CONJ into each well. Cover the strips with a new piece of adhesive film and incubate for **30 minutes at 37 °C**.

9.9. Following incubation, remove the film carefully and wash the wells five times as described in para. 9.7.

9.10. Add 100 µL of SOLN TMB into the wells; do not touch the bottom and the walls of the plate wells.

9.11. Incubate the strips for **30 minutes** in a dark place at a room temperature of 18-25 °C. Do not use adhesive film at this stage.

9.12. Add 100 µL of SOLN STOP into each strip well to stop the enzymatic reaction; adhere to the same sequence of actions as when adding SOLN TMB. At the time of adding, the solution colour changes from blue to yellow, and clear solution slightly changes its shade.

9.13. Measure the optical density (OD) of the wells at 450/620-695 nm wavelength using an ELISA microplate reader within 5 minutes after stopping the reaction. Pay attention to the cleanness of the plate bottom and the absence of bubbles in the wells before reading.

Measurement at the single wavelength of 450 nm is possible, in that case, it is needed to leave one well for blank (only SOLN TMB and SOLN STOP must be added in blank well).

10. CALCULATION AND INTERPRETATION OF RESULTS

10.1. Calculation of results

Calculate the average OD for the negative control ($\overline{Nc}$), Cut off (CO) and a sample positivity index (IP_{sample}).

$$\overline{Nc} = (Nc1 + Nc2 + Nc3)/3; \quad CO = \overline{Nc} + 0{,}25$$

$$IP_{sample} = OD_{sample}/CO, \text{ where } OD_{sample} \text{ is the OD sample.}$$

10.2. Quality control (assay validation)

The test results are considered valid if they meet the following requirements:

CONTROL + OD ≥ 1,0

CONTROL - OD ≤ 0,150

CONTROL - $\overline{Nc} \times 0,5 \leq Ncn \leq \overline{Nc} \times 2,0$ where Ncn is the OD for each Nc run

If any of the OD values for the negative control is beyond the above interval, it should be discarded, and $\overline{Nc}$ is calculated based on the remaining OD values for the negative control. If several OD values for the negative control fail to meet the above requirements, the test is considered invalid and requires a new run.

10.3. Interpretation of results

$IP_{sample} > 1,1$	POSITIVE
$0,9 \leq IP_{sample} \leq 1,1$	BORDERLINE*
$IP_{sample} < 0,9$	NEGATIVE

* Uncertain samples are recommended to be re-examined in two wells of the ELISA kit. If the results are again uncertain, a new sample should be selected and analyzed in 2-4 weeks. In case of repeated indeterminate results, such samples shall be considered negative.

11. PERFORMANCE CHARACTERISTICS

11.1. Analytical performance characteristic

Precision of measurement

Intra assay repeatability

The coefficient of variation (CV) for two sera with different levels of specific antibodies was evaluated in 32 replicates on one series of ELISA kits.

Sample No.	OD_{av}	IP_{av}	CV, %
14L	0,679	2,47	6,5
16L	0,490	1,79	6,6

Inter assay reproducibility

The coefficient of variation (CV) for three sera with different levels of specific antibodies was evaluated for 3 days in 3 sets of analysis, 8 replicates in each analysis.

Sample No.	OD_{av}	IP_{av}	CV, %
14L	0,670	2,39	5,55
16L	0,463	1,65	7,06

Analytical specificity

The test results are not affected by bilirubin at up to 0.21 mg/mL (361.8 µmol/L), haemoglobin at up to 10 mg/mL and triglycerides at up to 10 mg/mL (11.3 mmol/l) present in the sample.

11.2. Diagnostic characteristics

Studies of the characteristics of the method in comparison with a similar commercial ELISA kit were performed on a sample of characterized sera, the target group of children and a group of donors. The relative sensitivity of «EQUI anti-Lamblia» ELISA kits was determined from a group of 23 serum samples that were tested for antibodies to *Giardia lamblia* and characterized as positive in a commercial ELISA kit. All sera were also determined to be positive in «EQUI anti-Lamblia» kits, so the relative sensitivity equals 100%. For 148 serum samples of children that were tested and characterized in commercial analogues, the relative specificity of «EQUI anti-Lamblia» ELISA kits was 92.86%, the percentage of coincidence - 93.24%. According to a similar principle, for 238 serum samples of donor blood, the relative specificity was 97% and the percentage of coincidence was 96.64%.

12. LIMITATIONS OF ASSAY

The final diagnosis cannot be made solely on the basis of serological test results, sunce clinical manifestations of the disease and laboratory data (such as the detection of cysts in faecal samples or trophozoites in duodenal contents; the results of detection of *Giardia lamblia* antigen in faeces) should be taken into account as well.

Addionally, cross-reactions with antibodies to antigens of other parasites cannot be completely ruled out.

Giardia lamblia-specific antibodies may not be detected in case of children with persistent and prolonged giardiasis.

It should be noted that IgG antibodies to *Giardia lamblia* can be detected via ELISA for a long time, even after successful treatment.

13. DIFFICULTIES THAT CAN OCCUR DURING THE ASSAY PROCEDURE

Possible reasons	Solution
High background in all wells	
Contaminated washer	Clean the washer head and rinse according to the instructions for use
Poor quality or contaminated water	Use purified water with specific resistance ≥ 10 MΩ · cm
Use of poorly washed glassware	Use chemically clean utensils
Use of chlorinated disinfectants	Do not use chlorine disinfectants
Use of contaminated tips	Use new tips
Increased incubation times or change in the temperature conditions	Adhere to the incubation regime according to the instructions for use
High background in a row of wells	

Repeat application of TMB solution	TMB solution should be applied once
Contamination of the automatic pipette nozzle with conjugate solution	Clean the pipette and dial carefully liquid
Contamination of one of the washer's channel	Clean the flush channel, rinse washer
Received OD of the positive control is below the border value	
One of the reagents (conjugate solution or TMB solution) was not prepared in a correct way or was not added	Re-conduct ELISA, pay attention to the correctness of the introduction of these reagents
Reduced incubation times at any stage	Incubate according to instructions for use
The colour density of the wells fails to meet the obtained optical density value	
This may suggest that the optical beam has been displaced	Check the correct operation of the reader

14. TECHNICAL ASSISTANCE AND CUSTOMER SERVICE

In case of technical problems, you can obtain assistance by contacting the manufacturer.

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	Manufacturer
	Authorized Representative in the European Community
	In vitro diagnostic medical device
	Catalogue number
	Date of manufacture
	Use by date
	Batch code
	Temperature limit
	Contains sufficient for <n> tests
	Caution
	Non-Sterile
	Consult instructions for use
	Keep away from sunlight
	Keep dry
CE	Compliance with EU safety requirements

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For questions and suggestions regarding the ELISA kit contact:

EC REP

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ASSAY PROCEDURE SCHEME

Keep all reagents for 30 min at temperature18-25°C before use
Dispense 80 µl DIL SAMPLE into the wells (purple)
Add to 20 µl of controls and samples into the wells: A1 – CONTROL +, B1, C1, D1 – CONTROL –, other wells – examined samples (change of colour from purple to blue)
Cover strips with an adhesive film, incubate for **30 min at 37°C**
Rinse the wells 5 times with prepared 1:20 (1+19) washing solution TWEEN (300 µl per well)
Add 100 µl of SOLN CONJ into all wells (green)
Cover strips with an adhesive film, incubate for **30 min at 37°C**
Rinse the wells 5 times with prepared 1:20 (1+19) washing solution TWEEN (300 µl per well)
Add 100 µl of SOLN TMB into all wells
Incubate for **30 min** in the dark at **18-25°C**
Add 100 µl of SOLN STOP into all wells (change of colour from blue to yellow)
Measure the optical density (OD) with an ELISA microplate reader at 450/620-695 nm

CALCULATION OF RESULTS

$\overline{Nc} = (Nc1 + Nc2 + Nc3)/3;$

$CO = \overline{Nc} + 0,25;$

$IP_{sample} = OD_{sample}/CO$

$\overline{Nc}$ - the average value of OD 3-x CONTROL –

CO - Cut off

IP_{sample} - sample positivity index

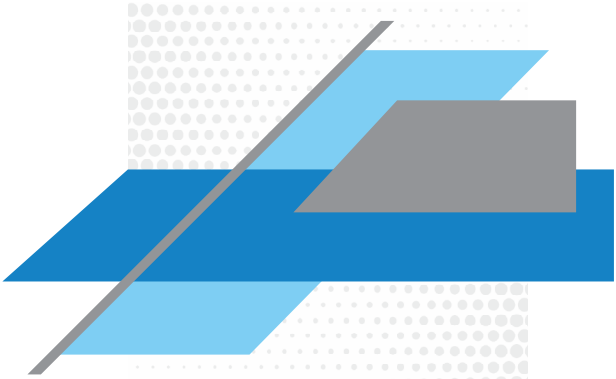
INTERPRETATION OF RESULTS

$IP_{sample} > 1,1$	POSITIVE
$0,9 \leq IP_{sample} \leq 1,1$	BORDERLINE
$IP_{sample} < 0,9$	NEGATIVE

anti-Trichinella spiralis

ELISA kit for the qualitative detection of antibodies to *Trichinella spiralis*

Instructions for use



IVD

REF EI-605

Σ 96 tests

CE

EQUI anti-Trichinella spiralis

ELISA kit for the qualitative detection of antibodies to *Trichinella spiralis*

1. INTENDED USE

The «EQUI anti-Trichinella spiralis» is ELISA kit intended to qualitatively detect anti-*Trichinella spiralis* antibodies in human serum or plasma by enzyme-linked immunosorbent assay (ELISA) in order to diagnose trichinosis. The testing procedure is designed for both manual arrangement with automatic pipettes and standard equipment, and for automated «open» immunoassay analysers.

Target group: villagers, hunters and their families, people whose diet includes meat, particularly pork.

Usage: ELISA kit is used in clinical diagnostic laboratories and other institutions engaged in *in vitro* diagnostics.

2. CLINICAL SIGNIFICANCE

Among helminthiases, which are often caused by meat consumption, trichinosis has a special place. The causative agent of this disease is the *Trichinella spiralis* roundworm, the larvae of which were found even in the mummies of Ancient Egypt. The greatest danger of this helminthiasis is the possibility of severe damage to the central nervous system, which leads to death.

Adult *Trichinella spiralis* have a filamentous body up to 0.5 mm in diameter and 2 mm in length. Trichinella is characterized by live birth – while in the human body, one female can give birth to about one and a half thousand rod-shaped larvae. The larvae actively migrate to other organs, but only settle massively in the striated muscles. Connective tissue capsule with feeding vessels gets formed around the larva after 1-1.5 months. About a year later, the capsule begins to calcify, but the larva inside remains alive and invasive for many years. Most mammals can become infected with trichinosis in the natural environment by eating fresh or deconposed meat that contains Trichinella larvae.

Trichinella enters the human body through the meat infested with encapsulated larvae (pork, horse meat, nutria's meat, wild boar, bear, etc.). Acute (intestinal) trichinosis may be asymptomatic or show symptoms 1-2 days after eating raw or undercooked meat. This stage of the disease is characterized by abdominal pain, nausea or diarrhea. However, the classic symptoms of trichinosis appear at the stage of larval migration about 2 weeks after the invasion and can last up to two months. These include fever, fatigue, muscle aches and pain, swelling of the face and eyes, skin rashes, and more. Trichinella spiralis affects the most active human muscles: diaphragm, intercostal and abdominal muscles, tongue, as well as facial and skeletal muscles. The overall extent of clinical manifestations is directly correlated with the quantity of consumed parasites.

During larval migration, the organism is hyper-sensitized by highly immunogenic products of Trichinella metabolism. Patients have eosinophilia and an increase in IgE antibodies. However, a strong immune response does not lead to the elimination of parasites, although it may be crucial for protection from re-infection.

Thus, recurring cases of trichinosis are much easier to tolerate than the primary ones.

To diagnose this helminthiasis, specific tests are conducted on meat that could be a source of invasion, to test for the presence of encapsulated larvae of Trichinella spiralis. During the early stages of infection, PCR is used to detect Trichinella DNA. A few weeks after the probable invasion, a biopsy of the affected patient's muscle tissue may be performed to detect the parasite. But from the second week of the invasion, serodiagnosis with the detection of specific antibodies to antigens of larvae of the pathogen trichinosis and an increase in the titer of these antibodies in paired sera is particularly useful. 4-5 months after the invasion, the number of specific antibodies decreases, but may remain at a level sufficient for detection for several years. Immunoblotting is used to confirm the positive result of the detection of specific IgG antibodies. However, for a comprehensive diagnosis of trichinosis we should use not only laboratory-generated data but also clinical and epidemiological studies.

3. ANALYSIS PRINCIPLE

The procedure of testing for anti-*Trichinella spiralis* antibodies in «EQUI anti-Trichinella spiralis» ELISA kit is based on «indirect» solid-phase ELISA with a two-stage incubation. Antigens of *Trichinella spiralis* larvae are entrapped in the wells. During the first step of incubation of ELISA plate wells with test samples, specific anti-*Trichinella spiralis* antibodies (if present in the samples) bind to the solid-phase antigens. The wells are washed to remove unbound antibodies and have only specific antigen-antibody complexes left. Then, a conjugate of anti-species (anti-IgG and anti-IgA) monoclonal antibodies with horseradish peroxidase is added, which binds to solid-phase immune complexes. Unbound components are removed by washing. Antigen-antibody complexes are detected by adding a solution of chromogen 3,3',5,5'-tetramethylbenzidine (TMB) with hydrogen peroxide. After 30-minute incubation, the reaction is stopped by adding the stop solution. The optical density (OD) in the wells is determined using a spectrophotometer at 450/620-695 nm. The intensity of the yellow colour is proportional to the level of antibodies in the sample.

4. MATERIALS AND EQUIPMENT

4.1. Contents of the ELISA kit

STRIPS	1 x 96 wells	**Microplate** Each plate well is coated with *Trichinella spiralis* larvae antigens. The wells are detachable. After the first opening, store unused strips in the package at 2-8 °C for a maximum of 6 months
CONTROL +	1 x 0,25 ml	**Positive control** Conjugated specific monoclonal antibody solution with preservative (pink). Store at 2-8 °C

		Negative control
CONTROL -	1 x 0,6 ml	Negative human serum with a preservative (yellow). Store at 2-8 °C
		Serum dilution solution
DIL SAMPLE	1 x 13 ml	Buffer solution with a milk extract, a detergent and a preservative (purple). Store at 2-8 °C
		Conjugate solution (ready to use)
SOLN CONJ	1 x 13 ml	Buffer solution of monoclonal antibodies to human IgG and IgA, conjugated with horseradish peroxidase, with stabilizers and preservative (green). Store at 2-8 °C
		TMB solution (ready to use)
SOLN TMB	1 x 13 ml	TMB solution, H_2O_2, a stabilizer, a preservative (colourless). Store at 2-8 °C
		Washing solution TWEEN (20x concentrated)
TWEEN WASH 20x	1 x 50 ml	20-fold phosphate buffer concentrate with Tween-20 (colourless). Dilute TWEEN detergent (20x) at 1:20 with distilled or deionized water (e. g., 5 mL of concentrate + 95 mL of water for 8 wells) before use. Store the diluted solution at 2-8 °C for a maximum of 7 days
		Stop Solution (ready to use)
SOLN STOP	1 x 13 ml	0.5 mol H_2SO_4 solution (colourless). Store at 2-8 °C

The ELISA kit also includes adhesive films (2 items), sample application plan (1 item), checklist, and instruction for use.

4.2. Optional reagents, materials and equipment

Automatic single and multichannel pipettes 10–1000 µL, tips, volumetric laboratory glassware (10–1,000 mL), deionized or distilled water, thermostat at 37 °C, automatic or semi-automatic plate washer, spectrophotometer (reader) for microplates at 450/620-695 nm, appropriate containers for potentially contaminated waste, timer, filter paper, disposable powder-free gloves, disinfectants.

5. PRECAUTIONS AND SAFETY

5.1. Precautions

Be sure to read the instructions for use carefully before the test. The validity of the test results depends on strict following of the test procedure.

– do not use the ELISA kit components after the expiry date;
– do not use for analysis or mix components of different batches, components of kits for different nosologies, or reagents from other manufacturers with the «EQUI anti-Trichinella spiralis» ELISA kit;
– do not freeze the ELISA kit or its contents;
– after using a reagent, close each vial with its cap;
– when washing, control filling and complete aspiration of solution from the wells;
– use a new pipette tip each time you add samples or reagents;
– prevent direct sunlight from reaching the reagents from the ELISA kit;

– SOLN TMB solution must be colourless before use. Do not use the solution if its colour is blue or yellow. Avoid contact of SOLN TMB with metals or metal ions. Use only clean glassware thoroughly rinsed with distilled water;
– do not use reagents with colour not in line with para. 4.1;
– under no circumstances should the same glassware be used for SOLN CONJ and SOLN TMB;
– do not evaluate the test results visually (without a reader);
– any optional equipment that is in direct contact with biological material or kit components should be considered contaminated and requires cleaning and decontamination;
– the ELISA kit includes materials for 96 tests. Dispose of the used components as well as any remaining unused components.

5.2. Safety requirements

– all reagents in the ELISA kit are for laboratory professional use for *in vitro* diagnosis only and may only be used by qualified personnel;
– conduct the tests in disposable powder-free gloves and goggles only;
– do not eat, drink, smoke, or apply make-up in the test room;
– do not mouth-pipette the solutions;
– controls from the «EQUI anti-Trichinella spiralis» kit have been tested and found to be for anti-HIV1/2, anti-HCV and anti-*Treponema pallidum* antibodies and HBsAg negative; however, controls and test samples should be handled as potentially hazardous infectious materials;
– some of the kit components contain low concentrations of harmful substances and can damage skin or mucoga. In case of contact of SOLN TMB, SOLN STOP and SOLN CONJ with mucous membranes or skin, immediately wash the affected area with plenty of water;
– in case of spillage of acid-free solutions, e. g. sera, treat the surface with a disinfectant solution and then wipe dry with filter paper. Otherwise first neutralize acid with sodium bicarbonate solution and then wipe the surface dry as described above.

5.3. Waste inactivation and disposal

– the liquid waste must be inactivated, for example, with hydrogen peroxide solution at the final concentration of 6% for 3 hours at room temperature, or with sodium hypochlorite at the final concentration of 5% for 30 minutes, or with other approved disinfectants;
– the solid waste must be inactivated by autoclaving at a temperature not less than 132°C;
– do not autoclave the solutions that contain sodium azide or sodium hypochlorite;
– disposal of inactivated waste must be conducted due to national laws and regulations.

6. STORAGE AND STABILITY

ELISA kit is stable up to the expiry date stated on the label when stored at 2-8°C. The kit should be transported at 2-8°C. Single transportation at a temperature up to 23°C for two days is possible.

7. SAMPLE COLLECTION, TRANSPORTATION AND STORAGE GUIDELINES

Collect blood from the vein into the sterile test tube. Test tube must be marked with patient ID and date of sample collecting. Blood before serum separation can be stored at 2-8 ºC for 24 hours, avoiding freezing.

Serum or plasma can be stored at 2-8 °C for maximum 3 days. Frozen serum can be stored for longer periods of time at -20 °C or -70 °C. Thaw frozen samples and keep them at room temperature for 30 minutes before use. After thawing, the stir samples to achieve homogeneity. Avoid repeated freezing-thawing cycles for test samples. If serum (or plasma) is turbid, remove insoluble inclusions by centrifugation at 3000 rpm for 10-15 minutes. Do not use serum samples with hyperlipidemia, hemolysis, and bacterial growth.

Transport serum samples in insulated containers. To do that, put closed labelled tubes in a plastic bag, tightly seal it and place in the centre of an insulated container.Put the frozen cold packs on the bottom, along the side walls of the insulated container and on top of the serum samples.

8. REAGENT PREPARATION

NOTE! It is very important to keep all ELISA kit components for at least 30 min at room temperature 18-25 ºC before the assay!

8.1. Microplate preparation

To prevent water condensation in the wells, keep the STRIPS for 30 minutes at a room temperature before opening. Open the vacuum pack, detach the appropriate number of wells, and carefully pack the remaining wells with a desiccant and store tightly zip-locked at 2-8 °C. Storing the packed plate this way ensures its stability for 6 months.

8.2. Washing solution preparation

To prepare detergent, dilute TWEEN|WASH|20x at 1:20 (1+19) with distilled or deionized water and stir. E. g., 5 mL of concentrate + 95 mL of water, which is enough for 8 wells. If there are crystals present in the detergent concentrate, heat the vial at 37 °C until the crystals dissolve completely (15–20 minutes). Store the diluted solution at 2-8 °C for a maximum of 7 days.

9. ASSAY PROCEDURE

9.1. Prepare the necessary number of wells (four wells for controls and a necessary number of wells for test samples) and insert them into the ELISA plate frame. Be sure to add control wells in every test run.

9.2. Fill in the sample application plan.

9.3. Prepare the detergent as per para. 8.2.

9.4. Add 90 µL of DIL SAMPLE into each plate well.

9.5. Add 10 µL of controls and test samples into the wells:
CONTROL + – into well A1,
CONTROL - – into wells B1, C1 and D1,
and test samples into the remaining wells.
At the time of adding, the solution changes its colour from purple to blue. Pipette the mix in the wells carefully to avoid foaming.

9.6. Cover the strips up with adhesive film and incubate for **30 minutes at 37 °C**.

9.7. Remove and discard the adhesive film and wash all wells 5 times with automatic washer or 8-channel pipette as follows:
– aspirate the content of all wells into a liquid waste container;
– add a minimum of 300 µl of diluted washing solution to each well, soak each well for 30 seconds;
– aspirate the content of all wells again. The residual volume after every aspiration should be less than 5 µl;
– repeat the washing step 4 more times;
– after the final aspiration, eliminate extra moisture by tapping the plate against a piece of filter paper.

9.8. Add 100 µL of SOLN CONJ into each well. Cover the strips with a new piece of adhesive film and incubate for **30 minutes at 37 °C**.

9.9. Following incubation, remove the film carefully and wash the wells five times as described in para. 9.7.

9.10. Add 100 µL of SOLN TMB into the wells; do not touch the bottom and the walls of the plate wells.

9.11. Incubate the strips for **30 minutes** in a dark place at a room temperature of 18-25 °C. Do not use adhesive film at this stage.

9.12. Add 100 µL of SOLN STOP into each strip well to stop the enzymatic reaction; adhere to the same sequence of actions as when adding SOLN TMB. At the time of adding, the solution colour changes from blue to yellow, and clear solution slightly changes its shade.

9.13. Measure the optical density (OD) of the wells at 450/620-695 nm wavelength using an ELISA microplate reader within 5 minutes after stopping the reaction. Pay attention to the cleanness of the plate bottom and the absence of bubbles in the wells before reading.

Measurement at the single wavelength of 450 nm is possible, in that case, it is needed to leave one well for blank (only SOLN TMB and SOLN STOP must be added in blank well).

10. CALCULATION AND INTERPRETATION OF RESULTS

10.1. Calculation of results

Calculate the average OD for the negative control ($\overline{Nc}$), Cut off (CO) and a sample positivity index (IP_{sample}).

$$\overline{Nc} = (Nc1 + Nc2 + Nc3)/3; \quad CO = \overline{Nc} + 0,3$$

$IP_{sample} = OD_{sample}/CO$, where OD_{sample} is the OD sample.

10.2. Quality control (assay validation)

The test results are considered valid if they meet the following requirements:

CONTROL + $OD \geq 1,0$

CONTROL - $OD \leq 0,150$

CONTROL - $\overline{Nc} \times 0,5 \leq Ncn \leq \overline{Nc} \times 2,0$ where Ncn is the OD for each Nc run

If any of the OD values for the negative control is beyond the above interval, it should be discarded, and $\overline{Nc}$ is calculated based on the remaining OD values for the negative control. If several OD values for the negative control fail to meet the above requirements, the test is considered invalid and requires a new run.

10.3. Interpretation of results

$IP_{sample} > 1,1$	POSITIVE
$0,9 \leq IP_{sample} \leq 1,1$	BORDERLINE*
$IP_{sample} < 0,9$	NEGATIVE

* Uncertain samples are recommended to be re-examined in two wells of the ELISA kit. If the results are again uncertain, a new sample should be selected and analyzed in 2-4 weeks. In case of repeated indeterminate results, such samples shall be considered negative.

11. PERFORMANCE CHARACTERISTICS

11.1. Analytical performance characteristics

Precision of measurement

Intra assay repeatability

The coefficient of variation (CV) for two sera with different levels of specific antibodies was evaluated in 32 replicates on one series of ELISA kits.

Sample No.	OD_{av}	IP_{av}	CV, %
G12	0,554	1,59	4,1
E5	0,999	2,87	6,5

Inter assay reproducibility

The coefficient of variation (CV) for three sera with different levels of specific antibodies was evaluated for 4 days in 4 sets of analysis, 8 replicates in each analysis.

Sample No.	OD_{av}	IP_{av}	CV, %
G12	0,561	1,56	4,7
E5	1,042	2,89	7,1

Analytical specificity

The test results are not affected by bilirubin at up to 0.21 mg/mL (361.8 μmol/L), haemoglobin at up to 10 mg/mL and triglycerides at up to 10 mg/mL (11.3 mmol/l) present in the sample.

11.2. Diagnostic characteristics

Studies of the characteristics of the method in comparison with a similar commercial ELISA kit were performed on a sample of characterized serum and a sample of donor blood serum. The relative sensitivity of «EQUI anti-Trichinella spiralis» ELISA kits was determined from 24 serum samples that were tested for antibodies to anti-*Trichinella spiralis* and characterized as positive in a commercial ELISA kit. According to the results of the analysis, the relative sensitivity of the sets «EQUI anti-Trichinella spiralis» is 96%. Studies of the relative specificity of ELISA kits were performed on a complex of 260 samples of donor blood serum. According to research, the relative specificity of ELISA kits «EQUI anti-Trichinella spiralis» was 98%. The coincidence percentage is 97.86%.

12. LIMITATIONS OF ASSAY

A positive result in the «EQUI anti-Trichinella spiralis» ELISA kit is an indication that the patient has antibodies of IgG and / or IgA specific to Trichinella spiralis. The presence of IgG antibodies in newborns is not an evidence of Trichinella spiralis invasion.

Uncertain results may indicate a history of Trichinella spiralis invasion. A negative result in the «EQUI anti-Trichinella spiralis» ELISA kit indicates the absence of antibodies specific for Trichinella spiralis in the test sample or the concentration of specific antibodies below the sensitivity limit of the analysis. Specific antibodies may not be detected at the beginning of the clinical manifestations of the invasion. In this case, it is recommended to re-obtain and test serum samples from patients with clinical signs of trichinosis in one or two weeks.

The final diagnosis cannot be established solely on the basis of serological test results. When making a diagnosis the results of a set of laboratory and instrumental studies, should be taken into account as well as clinical manifestations of the disease. Cross-reactions with antibodies to antigens of other helminths cannot be completely ruled out. To exclude a false-positive result, it is recommended to conduct a verification study of positive samples by immunoblotting.

13. DIFFICULTIES THAT CAN OCCUR DURING THE ASSAY PROCEDURE

Possible reasons	Solution
High background in all wells	
Contaminated washer	Clean the washer head and rinse according to the instructions for use
Poor quality or contaminated water	Use purified water with specific resistance ≥ 10 MΩ · cm

Use of poorly washed glassware	Use chemically clean utensils
Use of chlorinated disinfectants	Do not use chlorine disinfectants
Use of contaminated tips	Use new tips
Increased incubation times or change in the temperature conditions	Adhere to the incubation regime according to the instructions for use
High background in a row of wells	
Repeat application of TMB solution	TMB solution should be applied once
Contamination of the automatic pipette nozzle with conjugate solution	Clean the pipette and dial carefully liquid
Contamination of one of the washer's channel	Clean the flush channel, rinse washer
Received OD of the positive control is below the border value	
One of the reagents (conjugate solution or TMB solution) was not prepared in a correct way or was not added	Re-conduct ELISA, pay attention to the correctness of the introduction of these reagents
Reduced incubation times at any stage	Incubate according to instructions for use
The colour density of the wells fails to meet the obtained optical density value	
This may suggest that the optical beam has been displaced	Check the correct operation of the reader

14. TECHNICAL ASSISTANCE AND CUSTOMER SERVICE

In case of technical problems, you can obtain assistance by contacting the manufacturer.

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	Manufacturer
	Authorized Representative in the European Community
	In vitro diagnostic medical device
	Catalogue number
	Date of manufacture
	Use by date
	Batch code
	Temperature limit
	Contains sufficient for <n> tests
	Caution
	Non-Sterile
	Consult instructions for use
	Keep away from sunlight
	Keep dry
	Compliance with EU safety requirements

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For questions and suggestions regarding the ELISA kit contact:

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Ekvitestlab LLC
Velyka Vasylkivska St. 114, Kyiv, Ukraine, 03150
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e-mail: info@equitest.com.ua, www.equitest.com.ua

ASSAY PROCEDURE SCHEME

Keep all reagents for 30 min at temperature18-25°C before use
Dispense 90 µl DIL SAMPLE into the wells (purple)
Add to 10 µl of controls and samples into the wells: A1 – CONTROL +, B1, C1, D1 – CONTROL –, other wells – examined samples (change of colour from purple to blue)
Cover strips with an adhesive film, incubate for **30 min at 37°C**
Rinse the wells 5 times with prepared 1:20 (1+19) washing solution TWEEN (300 µl per well)
Add 100 µl of SOLN CONJ into all wells (green)
Cover strips with an adhesive film, incubate for **30 min at 37°C**
Rinse the wells 5 times with prepared 1:20 (1+19) washing solution TWEEN (300 µl per well)
Add 100 µl of SOLN TMB into all wells
Incubate for **30 min** in the dark at **18-25°C**
Add 100 µl of SOLN STOP into all wells (change of colour from blue to yellow)
Measure the optical density (OD) with an ELISA microplate reader at 450/620-695 nm

CALCULATION OF RESULTS

$\overline{Nc} = (Nc1 + Nc2 + Nc3)/3;$

$CO = \overline{Nc} + 0,3;$

$IP_{sample} = OD_{sample}/CO$

$\overline{Nc}$ - the average value of OD 3-x CONTROL –

CO - Cut off

IP_{sample} - sample positivity index

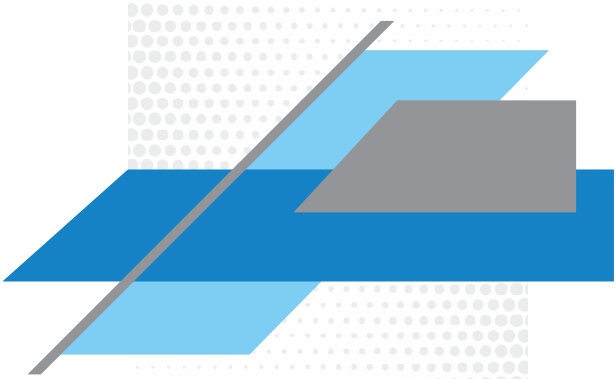
INTERPRETATION OF RESULTS

$IP_{sample} > 1,1$	POSITIVE
$0,9 \leq IP_{sample} \leq 1,1$	BORDERLINE
$IP_{sample} < 0,9$	NEGATIVE

Ascaris lumbricoides IgG

ELISA kit for the qualitative detection of IgG antibodies to *Ascaris lumbricoides*

Instructions for use



IVD

REF EI-601

Σ 96 tests

CE

EQUI Ascaris lumbricoides IgG

ELISA kit for the qualitative detection of
IgG antibodies to *Ascaris lumbricoides*

1. INTENDED USE

The «EQUI Ascaris lumbricoides IgG» is ELISA kit intended to qualitatively detect anti-*Ascaris lumbricoides* IgG in human serum or plasma by enzyme-linked immunosorbent assay (ELISA) in order to diagnose lumbricosis. The testing procedure is designed for both manual arrangement with automatic pipettes and standard equipment, and for automated «open» immunoassay analysers.

Target group: children, rural people, summer visitors.

Usage: ELISA kit is used in clinical diagnostic laboratories and other institutions engaged in *in vitro* diagnostics.

2. CLINICAL SIGNIFICANCE

Ascaris lumbricoides is a human parasite resulting in lumbricosis — one of the most common helminthiases in the world. By some estimates, over a milliard of people infested with acaricides are on earth.

Human ascaris belongs to *Nematoda* roundworms infesting the small intestine of a man who is its exclusive host. *Ascaris lumbricoides* eggs are excreted in the environment with faeces of the infested man. In a warm, wet soil, ascaris larvae develops in the eggs, therefore eggs become invasive only after a maturation period (2 to 3 weeks at 25–30 °C, lower temperatures require longer term). After infestation, larvae leave eggs in the human intestine, penetrates blood circulation and migrate to the liver and lungs with blood flow. The larvae move to the pharynx from the lungs, and here they are re-ingested and further enter the small intestine. In 2 to 3 months, adult ascaris able to propagate develops from larvae in the small intestine.

The helminths are transferred by faecal-oral route upon injection of mature eggs of *Ascaris lumbricoides* with soil-contaminated vegetables, fruits, water, as well as through dirty hands after contact with soil. Lumbricosis is conditionally divided into the early stage (migration of larvae) and late stage (parasitism of adults in the intestine). Invasion is asymptomatic in most cases. Primary feeling of being unwell occurs as early as several days after infestation and is accompanied by weakness, abdominal pain, nausea. Migration of larvae to the lungs may manifest as rales and cough. In some cases, intense invasion may result in pneumonia and liver damage. However, the most common symptom of early lumbricosis are allergic reactions due to hypersensitivity to metabolic products of larvae.

Late stage manifests as decreases appetite, abdominal pain, vomiting, diarrhoea, constipation. Massive ascaris invasion may result in the intestinal obstruction with a lump of helminths or rupture of the walls with peritonitis. When ascarides penetrate other organs, complications may develop such as

hepatitis, cholangitis, pancreatitis and even asphyxia. Cases of neurological disorders sometimes develop in lumbricosis, namely: headache, irritability, sleep impairment, inattention, etc. If no timely treatment is started for intense invasion, it may lead to death, especially in younger children.

Strong immune response to *Ascaris lumbricoides* invasion develops as early as at the early stage. It includes cellular and humoral immunity. Antigens of ascaris larvae stimulate secretion of all-class specific immunoglobulins, however, the level of specific and total IgE antibodies is the highest. The intensity of the immune response (including increased IgG titres) correlates with the massiveness of the invasion.

For diagnosis of lumbricosis, parasitologic stool test for presence of ascaris larvae and eggs is the most common. X-ray imaging of the lungs is additionally applied at the early stage of invasion. Complete blood count (eosinophilia develops in lumbricosis) and detection of serum anti-*Ascaris lumbricoides* antibodies also is included in the set of exams. The presence of specific anti-ascaris antibodies may suggest asymptomatic invasion, and allows initiation of treatment before complications develop in conjunction with other diagnostic instruments.

3. ANALYSIS PRINCIPLE

The procedure of testing for anti-*Ascaris lumbricoides* IgG in «EQUI Ascaris lumbricoides IgG» ELISA kit is based on «indirect» solid-phase ELISA with a two-stage incubation. Antigens of *Ascaris lumbricoides* larvae are entrapped in the wells. During the first step of incubation of ELISA plate wells with test samples, specific anti-*Ascaris lumbricoides* antibodies (if present in the samples) bind to the solid-phase antigens. The wells are washed to remove unbound antibodies and have only specific antigen-antibody complexes left. Then, a conjugate of anti-species IgG monoclonal antibodies with horseradish peroxidase is added, which binds to solid-phase immune complexes. Unbound components are removed by washing. Antigen-antibody complexes are detected by adding a solution of chromogen 3,3',5,5'-tetramethylbenzidine (TMB) with hydrogen peroxide. After 30-minute incubation, the reaction is stopped by adding the stop solution. The optical density (OD) in the wells is determined using a spectrophotometer at 450/620-695 nm. The intensity of the yellow colour is proportional to the level of antibodies in the sample.

4. MATERIALS AND EQUIPMENT

4.1. Contents of the ELISA kit

STRIPS	1 x 96 wells	**Microplate** Each plate well is coated with *Ascaris lumbricoides* antigen. The wells are detachable. After the first opening, store unused strips in the package at 2-8 °C for a maximum of 6 months

CONTROL +	1 x 0,25 ml	**Positive control** Conjugated specific monoclonal antibody solution with preservative (pink). Store at 2-8 °C
CONTROL -	1 x 0,6 ml	**Negative control** Negative human serum with a preservative (yellow). Store at 2-8 °C
DIL SAMPLE	1 x 13 ml	**Serum dilution solution** Buffer solution with a milk extract, a detergent and a preservative (brown). Store at 2-8 °C
SOLN CONJ	1 x 13 ml	**Conjugate solution (ready to use)** Buffer solution of monoclonal antibodies to human IgG, conjugated with horseradish peroxidase, with stabilizers and preservative (green). Store at 2-8 °C
SOLN TMB	1 x 13 ml	**TMB solution (ready to use)** TMB solution, H_2O_2, a stabilizer, a preservative (colourless). Store at 2-8 °C
TWEEN WASH 20x	1 x 50 ml	**Washing solution TWEEN (20x concentrated)** 20-fold phosphate buffer concentrate with Tween-20 (colourless). Dilute TWEEN detergent (20x) at 1:20 with distilled or deionized water (e. g., 5 mL of concentrate + 95 mL of water for 8 wells) before use. Store the diluted solution at 2-8 °C for a maximum of 7 days
SOLN STOP	1 x 13 ml	**Stop Solution (ready to use)** 0.5 mol H_2SO_4 solution (colourless). Store at 2-8 °C

The ELISA kit also includes adhesive films (2 items), sample application plan (1 item), checklist, and instruction for use.

4.2. Optional reagents, materials and equipment

Automatic single and multichannel pipettes 10–1000 µL, tips, volumetric laboratory glassware (10–1,000 mL), deionized or distilled water, thermostat at 37 °C, automatic or semi-automatic plate washer, spectrophotometer (reader) for microplates at 450/620-695 nm, appropriate containers for potentially contaminated waste, timer, filter paper, disposable powder-free gloves, disinfectants.

5. PRECAUTIONS AND SAFETY

5.1. Precautions

Be sure to read the instructions for use carefully before the test. The validity of the test results depends on strict following of the test procedure.

– do not use the ELISA kit components after the expiry date;
– do not use for analysis or mix components of different batches, components of kits for different nosologies, or reagents from other manufacturers with the «EQUI Ascaris lumbricoides IgG» ELISA kit;
– do not freeze the ELISA kit or its contents;
– after using a reagent, close each vial with its cap;

– when washing, control filling and complete aspiration of solution from the wells;
– use a new pipette tip each time you add samples or reagents;
– prevent direct sunlight from reaching the reagents from the ELISA kit;
– SOLN TMB solution must be colourless before use. Do not use the solution if its colour is blue or yellow. Avoid contact of SOLN TMB with metals or metal ions. Use only clean glassware thoroughly rinsed with distilled water;
– do not use reagents with colour not in line with para. 4.1;
– under no circumstances should the same glassware be used for SOLN CONJ and SOLN TMB;
– do not evaluate the test results visually (without a reader);
– any optional equipment that is in direct contact with biological material or kit components should be considered contaminated and requires cleaning and decontamination;
– the ELISA kit includes materials for 96 tests. Dispose of the used components as well as any remaining unused components.

5.2. Safety requirements

– all reagents in the ELISA kit are for laboratory professional use for *in vitro* diagnosis only and may only be used by qualified personnel;
– conduct the tests in disposable powder-free gloves and goggles only;
– do not eat, drink, smoke, or apply make-up in the test room;
– do not mouth-pipette the solutions;
– controls from the «EQUI Ascaris lumbricoides IgG» ELISA kit have been tested and found to be for anti-HIV1/2, anti-HCV and anti-*Treponema pallidum* antibodies and HBsAg negative; however, controls and test samples should be handled as potentially hazardous infectious materials;
– some of the kit components contain low concentrations of harmful substances and can damage skin or mucoga. In case of contact of SOLN TMB, SOLN STOP and SOLN CONJ with mucous membranes or skin, immediately wash the affected area with plenty of water;
– in case of spillage of acid-free solutions, e. g. sera, treat the surface with a disinfectant solution and then wipe dry with filter paper. Otherwise first neutralize acid with sodium bicarbonate solution and then wipe the surface dry as described above.

5.3. Waste inactivation and disposal

– the liquid waste must be inactivated, for example, with hydrogen peroxide solution at the final concentration of 6% for 3 hours at room temperature, or with sodium hypochlorite at the final concentration of 5% for 30 minutes, or with other approved disinfectants;
– the solid waste must be inactivated by autoclaving at a temperature not less than 132°C;

– do not autoclave the solutions that contain sodium azide or sodium hypochlorite;
– disposal of inactivated waste must be conducted due to national laws and regulations.

6. STORAGE AND STABILITY

ELISA kit is stable up to the expiry date stated on the label when stored at 2-8°C. The kit should be transported at 2-8°C. Single transportation at a temperature up to 23°C for two days is possible.

7. SAMPLE COLLECTION, TRANSPORTATION AND STORAGE GUIDELINES

Collect blood from the vein into the sterile test tube. Test tube must be marked with patient ID and date of sample collecting. Blood before serum separation can be stored at 2-8 ºC for 24 hours, avoiding freezing.

Serum or plasma can be stored at 2-8 °C for maximum 3 days. Frozen serum can be stored for longer periods of time at -20 °C or -70 °C. Thaw frozen samples and keep them at room temperature for 30 minutes before use. After thawing, the stir samples to achieve homogeneity. Avoid repeated freezing-thawing cycles for test samples. If serum (or plasma) is turbid, remove insoluble inclusions by centrifugation at 3000 rpm for 10-15 minutes. Do not use serum samples with hyperlipidemia, hemolysis, and bacterial growth.

Transport serum samples in insulated containers. To do that, put closed labelled tubes in a plastic bag, tightly seal it and place in the centre of an insulated container.Put the frozen cold packs on the bottom, along the side walls of the insulated container and on top of the serum samples.

8. REAGENT PREPARATION

NOTE! It is very important to keep all ELISA kit components for at least 30 min at room temperature 18-25 ºC before the assay!

8.1. Microplate preparation

To prevent water condensation in the wells, keep the STRIPS for 30 minutes at a room temperature before opening. Open the vacuum pack, detach the appropriate number of wells, and carefully pack the remaining wells with a desiccant and store tightly zip-locked at 2-8 °C. Storing the packed plate this way ensures its stability for 6 months.

8.2. Washing solution preparation

To prepare detergent, dilute TWEEN WASH 20x at 1:20 (1+19) with distilled or deionized water and stir. E. g., 5 mL of concentrate + 95 mL of water, which is enough for 8 wells. If there are crystals present in the detergent concentrate, heat the vial at 37 °C until the crystals dissolve completely (15–20 minutes). Store the diluted solution at 2-8 °C for a maximum of 7 days.

9. ASSAY PROCEDURE

9.1. Prepare the necessary number of wells (four wells for controls and a necessary number of wells for test samples) and insert them into the ELISA plate frame. Be sure to add control wells in every test run.

9.2. Fill in the sample application plan.

9.3. Prepare the detergent as per para. 8.2.

9.4. Add 90 µL of DIL SAMPLE into each plate well.

9.5. Add 10 µL of controls and test samples into the wells:
CONTROL + – into well A1,
CONTROL - – into wells B1, C1 and D1,
and test samples into the remaining wells.
At the time of adding, the solution changes its colour from brown to blue. Pipette the mix in the wells carefully to avoid foaming.

9.6. Cover the strips up with adhesive film and incubate for **30 minutes at 37 °C**.

9.7. Remove and discard the adhesive film and wash all wells 5 times with automatic washer or 8-channel pipette as follows:
– aspirate the content of all wells into a liquid waste container;
– add a minimum of 300 µl of diluted washing solution to each well, soak each well for 30 seconds;
– aspirate the content of all wells again. The residual volume after every aspiration should be less than 5 µl;
– repeat the washing step 4 more times;
– after the final aspiration, eliminate extra moisture by tapping the plate against a piece of filter paper.

9.8. Add 100 µL of SOLN CONJ into each well. Cover the strips with a new piece of adhesive film and incubate for **30 minutes at 37 °C**.

9.9. Following incubation, remove the film carefully and wash the wells five times as described in para. 9.7.

9.10. Add 100 µL of SOLN TMB into the wells; do not touch the bottom and the walls of the plate wells.

9.11. Incubate the strips for **30 minutes** in a dark place at a room temperature of 18-25 °C. Do not use adhesive film at this stage.

9.12. Add 100 µL of SOLN STOP into each strip well to stop the enzymatic reaction; adhere to the same sequence of actions as when adding SOLN TMB. At the time of adding, the solution colour changes from blue to yellow, and clear solution slightly changes its shade.

9.13. Measure the optical density (OD) of the wells at 450/620-695 nm wavelength using an ELISA microplate reader within 5 minutes after stopping the reaction. Pay attention to the cleanness of the plate bottom and the absence of bubbles in the wells before reading.

Measurement at the single wavelength of 450 nm is possible, in that case, it is needed to leave one well for blank (only SOLN TMB and SOLN STOP must be added

in blank well).

10. CALCULATION AND INTERPRETATION OF RESULTS

10.1. Calculation of results

Calculate the average OD for the negative control ($\overline{Nc}$), Cut off (CO) and a sample positivity index (IP_{sample}).

$$\overline{Nc} = (Nc1 + Nc2 + Nc3)/3; \quad CO = \overline{Nc} + 0,3$$

$IP_{sample} = OD_{sample}/CO$, where OD_{sample} is the OD sample.

10.2. Quality control (assay validation)

The test results are considered valid if they meet the following requirements:

CONTROL + $OD \geq 1,0$

CONTROL - $OD \leq 0,150$

CONTROL - $\overline{Nc} \times 0,5 \leq Ncn \leq \overline{Nc} \times 2,0$ where Ncn is the OD for each Nc run

If any of the OD values for the negative control is beyond the above interval, it should be discarded, and $\overline{Nc}$ is calculated based on the remaining OD values for the negative control. If several OD values for the negative control fail to meet the above requirements, the test is considered invalid and requires a new run.

10.3. Interpretation of results

$IP_{sample} > 1,1$	POSITIVE
$0,9 \leq IP_{sample} \leq 1,1$	BORDERLINE*
$IP_{sample} < 0,9$	NEGATIVE

* Uncertain samples are recommended to be re-examined in two wells of the ELISA kit. If the results are again uncertain, a new sample should be selected and analyzed in 2-4 weeks. In case of repeated indeterminate results, such samples shall be considered negative.

11. PERFORMANCE CHARACTERISTICS

11.1. Analytical performance characteristics

Precision of measurement

Intra assay repeatability

The coefficient of variation (CV) for three sera with different levels of specific antibodies was evaluated in 24 replicates on one series of ELISA kits.

Sample No.	OD_{av}	IP_{av}	CV, %
547	0,504	1,43	2,9
671	0,753	2,13	3,6
413	1,165	3,30	3,1

Inter assay reproducibility

The coefficient of variation (CV) for three sera with different levels of specific antibodies was evaluated for 4 days in 4 sets of analysis, 8 replicates in each analysis.

Sample No.	OD_{av}	IP_{av}	CV, %
547	0,534	1,55	5,0
671	0,750	2,17	4,6
413	1,159	3,36	3,6

Analytical specificity

The test results are not affected by bilirubin at up to 0.21 mg/mL (361.8 μmol/L), haemoglobin at up to 10 mg/mL and triglycerides at up to 10 mg/mL (11.3 mmol/l) present in the sample.

11.2. Diagnostic characteristics

To evaluate clinical sensitivity and specificity of «EQUI Ascaris lumbricoides IgG» ELISA kits, 55 serum samples from patients with clinical symptoms typical for lumbricosis and 60 serum samples from patients without clinical manifestations (seronegative in terms of *Ascaris lumbricoides*) were used. Clinical sensitivity of «EQUI Ascaris lumbricoides IgG» ELISA kits was 94.55 % and clinical specificity — 93.3 %.

Method characteristics in comparison with equal commercial ELISA kit was studied in target paediatric population (160 samples) and population of donors (346 samples). For paediatric population serum, relative specificity of «EQUI Ascaris lumbricoides IgG» ELISA kits was established at the level of 97.92 % and percent agreement was 95.51 %. For donor population serum, relative specificity of was 89.74 %, relative specificity — 96.30 % and percent agreement was 95.47 %.

12. LIMITATIONS OF ASSAY

Positive result in «EQUI Ascaris lumbricoides IgG» ELISA kit supports presence of anti-*Ascaris lumbricoides* specific IgG antibodies. Presence of this class antibodies in newborns is not an evidence of *Ascaris lumbricoides* invasion.

Inconclusive results may suggest a history of *Ascaris lumbricoides* invasion.

Negative result of «EQUI Ascaris lumbricoides IgG» ELISA kit supports the absence of anti- *Ascaris lumbricoides* IgG specific antibodies in the test sample or concentration of specific antibodies is below the sensitivity limit of the assay.

The results of serological test only are not the basis for final diagnosis. When establishing the diagnosis, the results of complex laboratory and instrumental tests, as well as clinical manifestations should be considered. Cross-reactions with antibodies to antigens of other helminths cannot be fully ruled out.

13. DIFFICULTIES THAT CAN OCCUR DURING THE ASSAY PROCEDURE

Possible reasons	Solution
High background in all wells	
Contaminated washer	Clean the washer head and rinse according to the instructions for use
Poor quality or contaminated water	Use purified water with specific resistance ≥ 10 MΩ · cm
Use of poorly washed glassware	Use chemically clean utensils
Use of chlorinated disinfectants	Do not use chlorine disinfectants
Use of contaminated tips	Use new tips
Increased incubation times or change in the temperature conditions	Adhere to the incubation regime according to the instructions for use
High background in a row of wells	
Repeat application of TMB solution	TMB solution should be applied once
Contamination of the automatic pipette nozzle with conjugate solution	Clean the pipette and dial carefully liquid
Contamination of one of the washer's channel	Clean the flush channel, rinse washer
Received OD of the positive control is below the border value	
One of the reagents (conjugate solution or TMB solution) was not prepared in a correct way or was not added	Re-conduct ELISA, pay attention to the correctness of the introduction of these reagents
Reduced incubation times at any stage	Incubate according to instructions for use
The colour density of the wells fails to meet the obtained optical density value	
This may suggest that the optical beam has been displaced	Check the correct operation of the reader

14. TECHNICAL ASSISTANCE AND CUSTOMER SERVICE

In case of technical problems, you can obtain assistance by contacting the manufacturer.

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	Manufacturer
	Authorized Representative in the European Community
	In vitro diagnostic medical device
	Catalogue number
	Date of manufacture
	Use by date
	Batch code
	Temperature limit
	Contains sufficient for <n> tests
	Caution
	Non-Sterile
	Consult instructions for use
	Keep away from sunlight
	Keep dry
	Compliance with EU safety requirements

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For questions and suggestions regarding the ELISA kit contact:

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ASSAY PROCEDURE SCHEME

Keep all reagents for 30 min at temperature18-25°C before use
Dispense 90 µl DIL SAMPLE into the wells (brown)
Add to 10 µl of controls and samples into the wells: A1 – CONTROL +, B1, C1, D1 – CONTROL –, other wells – examined samples (change of colour from brown to blue)
Cover strips with an adhesive film, incubate for **30 min at 37°C**
Rinse the wells 5 times with prepared 1:20 (1+19) washing solution TWEEN (300 µl per well)
Add 100 µl of SOLN CONJ into all wells (green)
Cover strips with an adhesive film, incubate for **30 min at 37°C**
Rinse the wells 5 times with prepared 1:20 (1+19) washing solution TWEEN (300 µl per well)
Add 100 µl of SOLN TMB into all wells
Incubate for **30 min** in the dark at **18-25°C**
Add 100 µl of SOLN STOP into all wells (change of colour from blue to yellow)
Measure the optical density (OD) with an ELISA microplate reader at 450/620-695 nm

CALCULATION OF RESULTS

$\overline{Nc} = (Nc1 + Nc2 + Nc3)/3;$

$CO = \overline{Nc} + 0,3;$

$IP_{sample} = OD_{sample}/CO$

$\overline{Nc}$ - the average value of OD 3-x CONTROL –

CO - Cut off

IP_{sample} - sample positivity index

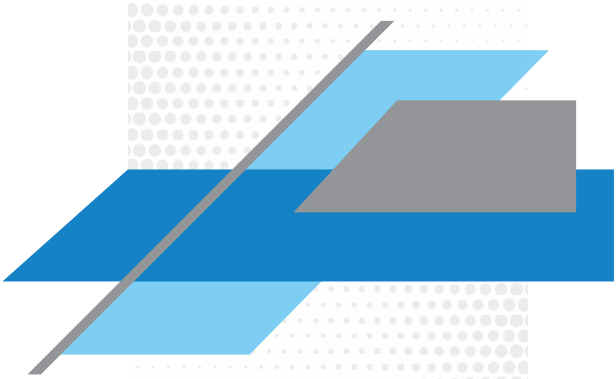
INTERPRETATION OF RESULTS

$IP_{sample} > 1,1$	POSITIVE
$0,9 \leq IP_{sample} \leq 1,1$	BORDERLINE
$IP_{sample} < 0,9$	NEGATIVE

HAV IgM

ELISA kit for the qualitative detection of IgM antibodies to hepatitis A virus

Instruction for use



IVD

REF EI-031

Σ 96 tests

CE

EQUI HAV IgM

ELISA kit for the qualitative detection
of IgM antibodies to hepatitis A virus

1. INTENDED USE

The «EQUI HAV IgM» is ELISA kit intended for the qualitative detection of IgM antibodies to hepatitis A virus in human serum or plasma by enzyme-linked immunosorbent assay (ELISA) to diagnose acute hepatitis A. The testing procedure is designed for both manual arrangement with automatic pipettes and standard equipment, and for automated «open» immunoassay analysers.

Target group: blood or organ donors; pregnant women and children born to infected mothers; patients with symptoms of liver disease.

Usage: ELISA kit is used in clinical diagnostic laboratories, blood transfusion stations, as well as in other institutions working in the field of *in vitro* diagnostics.

2. CLINICAL SIGNIFICANCE

One of the most common foodborne infections is hepatitis A. The hepatitis A virus (HAV) causes acute liver disease, which can be mild or severe. Unlike hepatitis B and C, this hepatitis does not become chronic, but can lead to acute liver failure, which is characterized by high mortality.

Hepatitis A virus is a small shellless RNA virus from the *Picornaviridae* family. It is characterized by being highly stable in different environments and can be stored at + 4 ° C for several months, but becomes inactive after 5 minutes at 100 ° C. The virus replicates in liver cells, and then is released through the bile into the environment with the fecal masses of the infected person. The cellular immune response to HAV infection leads to the destruction of hepatocytes, liver dysfunction and the development of symptoms, typical for other types of hepatitis.

Acute hepatitis A, even with the clinical manifestations, does not differ from other viral hepatitis. Therefore, serological markers of infection are used for diagnosis, namely the detection of specific antibodies to HAV antigens. IgM antibodies are detected in the serum 1-2 weeks after infection, with the onset of symptoms or a few days before. In the maximum titer of anti-CAA IgM are detected in the jaundice period, after that their level gradually decreases. In most patients, specific IgM ceases to be detected after 6 months, and may occasionally circulate in the blood for more than a year. IgG antibodies to HAV antigens begin to be released shortly after IgM antibodies and stay in the blood throughout life. Also, specific IgG is produced after a vaccination. Detection of IgG antibodies to CAA indicates the formation of a stable immunity due to infection or immunization.

3. ANALYSIS PRINCIPLE

Detection of specific IgM antibodies to hepatitis A virus in the «EQUI HAV IgM» ELISA kit is based on the principle of «IgM capture» of solid-phase ELISA in a two-stage incubation. Monoclonal antibodies specific for human IgM immunoglobulins are adsorbed into the wells of the plate. During the first step of incubation of the

test samples in the wells of the ELISA plate, IgM immunoglobulins, if present in the samples, bind to monoclonal antibodies in the solid phase. The wells are washed to remove unbound components, leaving only specific antibody-antibody complexes. A mixture of HAV antigen and peroxidase conjugate of HAV-specific antibodies that bind to solid-phase immune complexes is then added. Unbound components are removed during washing. Immune complexes are detected by adding a solution of chromogen 3,3', 5,5'-tetramethylbenzidine (TMB) with hydrogen peroxide. After a 30-minute incubation, the reaction is halted by adding a stop solution. Optical density (OG) in the wells is determined on a spectrophotometer at a wavelength of 450 / 620-695 nm. The intensity of the yellow color is proportional to the number of antibodies in the sample.

4. MATERIALS AND EQUIPMENT

4.1. Contents of the ELISA kit

STRIPS	1 x 96 wells	**Microplate** Each plate well is coated with monoclonal antibodies specific for human IgM immunoglobulins. The wells are detachable. After the first opening, store unused strips in the package at 2-8 °C for a maximum of 6 months
CONTROL +	1 x 0,25 ml	**Positive control** The solution of human IgM immunoglobulins crosslinked with monoclonal antibodies specific for horseradish peroxidase, with a preservative (pink). Store at 2-8 °C
CONTROL -	1 x 0,6 ml	**Negative control** Negative human serum with a preservative (yellow). Store at 2-8 °C
DIL SAMPLE	1 x 13 ml	**Serum dilution solution** Buffer solution with monoclonal antibodies to human IgG, milk extract, detergent and preservative (brown). Store at 2-8 °C
CONJ 11x	1 x 1,3 ml	**Conjugate (11x concentrated)** 11-fold concentrate of conjugate of antibodies to hepatitis A virus with horseradish peroxidase in buffer solution with stabilizers (purple). Dilute the conjugate (11x) 1:11 with the conjugate dilution solution before use (eg 100 µl concentrate + 1 ml conjugate dilution solution, enough for 8 wells). Diluted solution should be stored at 2-8 ° C for no more than 1 day
DIL CONJ	1 x 13 ml	**Conjugate dilution solution** Buffer solution of inactivated hepatitis A virus antigen with detergent and preservative (yellow). Store at 2-8 °C
SOLN TMB	1 x 13 ml	**TMB solution (ready to use)** TMB solution, H_2O_2, a stabilizer, a preservative (colourless). Store at 2-8 °C

TWEEN WASH 20x	1 x 50 ml	**Washing solution TWEEN (20x concentrated)** 20-fold phosphate buffer concentrate with Tween-20 (colourless). Dilute TWEEN detergent (20x) at 1:20 with distilled or deionized water (e. g., 5 mL of concentrate + 95 mL of water for 8 wells) before use. Store the diluted solution at 2-8 °C for a maximum of 7 days
SOLN STOP	1 x 13 ml	**Stop Solution (ready to use)** 0.5 mol H_2SO_4 solution (colourless). Store at 2-8 °C

The ELISA kit also includes adhesive films (2 items), sample application plan (1 item), checklist, and instruction for use.

4.2. Optional reagents, materials and equipment

Automatic single and multichannel pipettes 10–1000 µL, tips, volumetric laboratory glassware (10–1,000 mL), deionized or distilled water, thermostat at 37 °C, automatic or semi-automatic plate washer, spectrophotometer (reader) for microplates at 450/620-695 nm, appropriate containers for potentially contaminated waste, timer, filter paper, disposable powder-free gloves, disinfectants.

5. PRECAUTIONS AND SAFETY

5.1. Precautions

Be sure to read the instructions for use carefully before the test. The validity of the test results depends on strict following of the test procedure.

– do not use the ELISA kit components after the expiry date;
– do not use for analysis or mix components of different batches, components of kits for different nosologies, or reagents from other manufacturers with the «EQUI HAV IgM» ELISA kit;
– do not freeze the ELISA kit or its contents;
– after using a reagent, close each vial with its cap;
– when washing, control filling and complete aspiration of solution from the wells;
– use a new pipette tip each time you add samples or reagents;
– prevent direct sunlight from reaching the reagents from the ELISA kit;
– SOLN TMB solution must be colourless before use. Do not use the solution if its colour is blue or yellow. Avoid contact of SOLN TMB with metals or metal ions. Use only clean glassware thoroughly rinsed with distilled water;
– do not use reagents with colour not in line with para. 4.1;
– under no circumstances should the same glassware be used for conjugate solution and SOLN TMB;
– do not evaluate the test results visually (without a reader);
– any optional equipment that is in direct contact with biological material or kit components should be considered contaminated and requires cleaning and decontamination;
– the ELISA kit includes materials for 96 tests. Dispose of the used components as well as any remaining unused components.

5.2. Safety requirements

– all reagents in the ELISA kit are for laboratory professional use for *in vitro* diagnosis only and may only be used by qualified personnel;
– conduct the tests in disposable powder-free gloves and goggles only;
– do not eat, drink, smoke, or apply make-up in the test room;
– do not mouth-pipette the solutions;
– controls from the «EQUI HAV IgM» ELISA kit have been tested and found to be for anti-HIV1/2, anti-HCV and anti-*Treponema pallidum* antibodies and HBsAg negative; however, controls and test samples should be handled as potentially hazardous infectious materials;
– some of the kit components contain low concentrations of harmful substances and can damage skin or mucoga. In case of contact of SOLN TMB, SOLN STOP and conjugate solution with mucous membranes or skin, immediately wash the affected area with plenty of water;
– in case of spillage of acid-free solutions, e. g. sera, treat the surface with a disinfectant solution and then wipe dry with filter paper. Otherwise first neutralize acid with sodium bicarbonate solution and then wipe the surface dry as described above.

5.3. Waste inactivation and disposal

– the liquid waste must be inactivated, for example, with hydrogen peroxide solution at the final concentration of 6% for 3 hours at room temperature, or with sodium hypochlorite at the final concentration of 5% for 30 minutes, or with other approved disinfectants;
– the solid waste must be inactivated by autoclaving at a temperature not less than 132°C;
– do not autoclave the solutions that contain sodium azide or sodium hypochlorite;
– disposal of inactivated waste must be conducted due to national laws and regulations.

6. STORAGE AND STABILITY

ELISA kit is stable up to the expiry date stated on the label when stored at 2-8°C. The kit should be transported at 2-8°C. Single transportation at a temperature up to 23°C for two days is possible.

7. SAMPLE COLLECTION, TRANSPORTATION AND STORAGE GUIDELINES

Collect blood from the vein into the sterile test tube. Test tube must be marked with patient ID and date of sample collecting. Blood before serum separation can be stored at 2-8 ºC for 24 hours, avoiding freezing.

Serum or plasma can be stored at 2-8 °C for maximum 3 days. Frozen serum can be stored for longer periods of time at -20 °C or -70 °C. Thaw frozen samples and keep them at room temperature for 30 minutes before use. After thawing, the stir samples to achieve homogeneity. Avoid repeated freezing-thawing cycles for test samples. If serum (or plasma) is turbid, remove insoluble inclusions by

centrifugation at 3000 rpm for 10-15 minutes. Do not use serum samples with hyperlipidemia, hemolysis, and bacterial growth.

Transport serum samples in insulated containers. To do that, put closed labelled tubes in a plastic bag, tightly seal it and place in the centre of an insulated container.Put the frozen cold packs on the bottom, along the side walls of the insulated container and on top of the serum samples.

8. REAGENT PREPARATION

NOTE! It is very important to keep all ELISA kit components for at least 30 min at room temperature 18-25 ºC before the assay!

8.1. Microplate preparation

To prevent water condensation in the wells, keep the STRIPS for 30 minutes at a room temperature before opening. Open the vacuum pack, detach the appropriate number of wells, and carefully pack the remaining wells with a desiccant and store tightly zip-locked at 2-8 °C. Storing the packed plate this way ensures its stability for 6 months.

8.2. Washing solution preparation

To prepare detergent, dilute TWEEN WASH 20x at 1:20 (1+19) with distilled or deionized water and stir. E. g., 5 mL of concentrate + 95 mL of water, which is enough for 8 wells. If there are crystals present in the detergent concentrate, heat the vial at 37 °C until the crystals dissolve completely (15–20 minutes). Store the diluted solution at 2-8 °C for a maximum of 7 days.

8.3. Conjugate solution preparation

Working dilution of the conjugate is prepared as follows: dilute CONJ 11x (purple) in a clean vial of solution DIL CONJ (yelow) in the ratio 1:11 (ie, 1 + 10), the solution turns green. For example, for 8 well analysis add to 1 ml DIL CONJ 100 µl CONJ 11x. The solution of the conjugate in the working dilution is stable during the day when stored at 2-8 °C.

9. ASSAY PROCEDURE

9.1. Prepare the necessary number of wells (four wells for controls and a necessary number of wells for test samples) and insert them into the ELISA plate frame. Be sure to add control wells in every test run.

9.2. Fill in the sample application plan.

9.3. Prepare the detergent as per para. 8.2.

9.4. Add 90 µL of DIL SAMPLE into each plate well.

9.5. Add 10 µL of controls and test samples into the wells:
CONTROL + – into well A1,
CONTROL - – into wells B1, C1 and D1,
and test samples into the remaining wells.
At the time of adding, the solution changes its colour from brown to blue. Pipette the mix in the wells carefully to avoid foaming.

9.6. Cover the strips up with adhesive film and incubate for **30 minutes at 37 °C**.

9.7. Remove and discard the adhesive film and wash all wells 5 times with automatic washer or 8-channel pipette as follows:
– aspirate the content of all wells into a liquid waste container;
– add a minimum of 300 µl of diluted washing solution to each well, soak each well for30 seconds;
– aspirate the content of all wells again. The residual volume after every aspiration should be less than 5 µl;
– repeat the washing step 4 more times;
– after the final aspiration, eliminate extra moisture by tapping the plate against a piece of filter paper.

9.8. Prepare conjugate solution as per para. 8.3.

9.9. Add 100 µL of conjugate solution into each well. Cover the strips with a new piece of adhesive film and incubate for **60 minutes at 37 °C**.

9.10. Following incubation, remove the film carefully and wash the wells five times as described in para. 9.7.

9.11. Add 100 µL of SOLN TMB into the wells; do not touch the bottom and the walls of the plate wells.

9.12. Incubate the strips for **30 minutes** in a dark place at a room temperature of 18-25 °C. Do not use adhesive film at this stage.

9.13. Add 100 µL of SOLN STOP into each strip well to stop the enzymatic reaction; adhere to the same sequence of actions as when adding SOLN TMB. At the time of adding,the solution colour changes from blue to yellow, and clear solution slightly changes its shade.

9.14. Measure the optical density (OD) of the wells at 450/620-695 nm wavelength using an ELISA microplate reader within 5 minutes after stopping the reaction. Pay attention to the cleanness of the plate bottom and the absence of bubbles in the wells before reading.

Measurement at the single wavelength of 450 nm is possible, in that case, it is needed to leave one well for blank (only SOLN TMB and SOLN STOP must be added in blank well).

10. CALCULATION AND INTERPRETATION OF RESULTS

10.1. Calculation of results

Calculate the average OD of the negative control ($\overline{Nc}$), Cut off (CO) and a sample positivity index (IP_{sample}).

$$\overline{Nc} = (Nc1 + Nc2 + Nc3)/3; \quad CO = \overline{Nc} + 0,3$$

$$IP_{sample} = OD_{sample}/CO, \text{ where } OD_{sample} \text{ is the OD sample.}$$

10.2. Quality control (assay validation)

The test results are considered valid if they meet the following requirements:

CONTROL +	OD ≥ 1,2	
CONTROL -	OD ≤ 0,150	
CONTROL -	$\overline{Nc} \times 0,5 \leq Ncn \leq \overline{Nc} \times 2,0$	where Ncn is the OD for each Nc run

If any of the OD values for the negative control is beyond the above interval, it should be discarded, and $\overline{Nc}$ is calculated based on the remaining OD values for the negative control. If several OD values for the negative control fail to meet the above requirements, the test is considered invalid and requires a new run.

10.3. Interpretation of results

$IP_{sample} > 1,1$	POSITIVE
$0,9 \leq IP_{sample} \leq 1,1$	BORDERLINE*
$IP_{sample} < 0,9$	NEGATIVE

* Uncertain samples are recommended to be re-examined in two wells of the ELISA kit. If the results are again uncertain, a new sample should be selected and analyzed in 2-4 weeks. In case of repeated indeterminate results, such samples shall be considered negative.

11. PERFORMANCE CHARACTERISTICS

11.1. Analytical performance characteristics

Precision of measurement

Intra assay repeatability

The coefficient of variation (CV) for two sera with different levels of specific antibodies was evaluated in 32 replicates on one series of ELISA kits.

Sample No.	OD_{av}	IP_{av}	CV, %
37s/2	1,576	4,85	5,3
24s	2,462	7,57	4,3

Inter assay reproducibility

The coefficient of variation (CV) for two sera with different levels of specific antibodies was evaluated for 4 days in 4 sets of analysis, 8 replicates in each analysis.

Sample No.	OD_{av}	IP_{av}	CV, %
37s/2	1,600	4,78	6,3
24s	2,463	7,36	7,1

Analytical specificity

The test results are not affected by bilirubin at up to 0.21 mg/mL (361.8 µmol/L), haemoglobin at up to 10 mg/mL and triglycerides at up to 10 mg/mL (11.3 mmol/l) present in the sample.

11.2. Diagnostic characteristics

The diagnostic characteristics of the ELISA kit were evaluated by examining a set of 30 samples containing IgM antibodies to hepatitis A virus, a set of donor serum samples (188 samples) and samples of PHT202 Anti-Hepatitis A Virus (HAVed) panel. Performance Panel (contains 8 positive and 13 negative samples) - a total of 239 samples - was compared to similar commercial kits. For this set (239 samples) the relative sensitivity of the «EQUI HAV IgM» ELISA kit is 100%, the relative specificity - 100%, the percentage of coincidence - 100%.

12. LIMITATIONS OF ASSAY

A positive result in the «EQUI HAV IgM» ELISA kit is the evidence that the patient has IgM antibodies specific for hepatitis A virus. Anti-CAA specific IgM antibodies are usually markers of active replication of hepatitis A virus.

In order to counteract the false-positive results caused by the presence of autoantibodies specific for class G immunoglobulins (rheumatoid factor) in human serum samples, the kit uses a special block component that prevents the formation of immune complexes with anti-human antibodies in the solid phase.

The final diagnosis cannot be established solely on the basis of serological test results. When making a diagnosis the results of a set of laboratory and instrumental studies, as well as clinical manifestations of the disease should all be taken into account.

13. DIFFICULTIES THAT CAN OCCUR DURING THE ASSAY PROCEDURE

Possible reasons	Solution
High background in all wells	
Contaminated washer	Clean the washer head and rinse according to the instructions for use
Poor quality or contaminated water	Use purified water with specific resistance ≥ 10 MΩ · cm
Use of poorly washed glassware	Use chemically clean utensils
Use of chlorinated disinfectants	Do not use chlorine disinfectants
Use of contaminated tips	Use new tips
Increased incubation times or change in the temperature conditions	Adhere to the incubation regime according to the instructions for use
High background in a row of wells	
Repeat application of TMB solution	TMB solution should be applied once
Contamination of the automatic pipette nozzle with conjugate solution	Clean the pipette and dial carefully liquid
Contamination of one of the washer's channel	Clean the flush channel, rinse washer

Received OD of the positive control is below the border value	
One of the reagents (conjugate solution or TMB solution) was not prepared in a correct way or was not added	Re-conduct ELISA, pay attention to the correctness of the introduction of these reagents
Reduced incubation times at any stage	Incubate according to instructions for use
The colour density of the wells fails to meet the obtained optical density value	
This may suggest that the optical beam has been displaced	Check the correct operation of the reader

14. TECHNICAL ASSISTANCE AND CUSTOMER SERVICE

In case of technical problems, you can obtain assistance by contacting the manufacturer.

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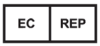
	Manufacturer
	Authorized Representative in the European Community
	In vitro diagnostic medical device
	Catalogue number
	Date of manufacture
	Use by date
	Batch code
	Temperature limit
	Contains sufficient for <n> tests
	Caution
	Non-Sterile
	Consult instructions for use
	Keep away from sunlight
	Keep dry
	Compliance with EU safety requirements

Edition 6, 01.11.2021

For questions and suggestions regarding the ELISA kit contact:



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ASSAY PROCEDURE SCHEME

Keep all reagents for 30 min at temperature18-25°C before use
Dispense 90 µl [DIL SAMPLE] into the wells (brown)
Add to 10 µl of controls and samples into the wells: A1 – [CONTROL +], B1, C1, D1 – [CONTROL –], other wells – examined samples (change of colour from brown to blue)
Cover strips with an adhesive film, incubate for **30 min at 37°C**
Rinse the wells 5 times with prepared 1:20 (1+19) washing solution TWEEN (300 µl per well)
Add 100 µl of prepared 1:11 (1+10) conjugate solution into all wells (green)
Cover strips with an adhesive film, incubate for **60 min at 37°C**
Rinse the wells 5 times with prepared 1:20 (1+19) washing solution TWEEN (300 µl per well)
Add 100 µl of [SOLN TMB] into all wells
Incubate for **30 min in the dark at 18-25°C**
Add 100 µl of [SOLN STOP] into all wells (change of colour from blue to yellow)
Measure the optical density (OD) with an ELISA microplate reader at 450/620-695 nm

CALCULATION OF RESULTS

$\overline{Nc} = (Nc1 + Nc2 + Nc3)/3;$

$CO = \overline{Nc} + 0,3;$

$IP_{sample} = OD_{sample}/CO$

$\overline{Nc}$ - the average value of OD 3-x [CONTROL –]

CO - Cut off

IP_{sample} - sample positivity index

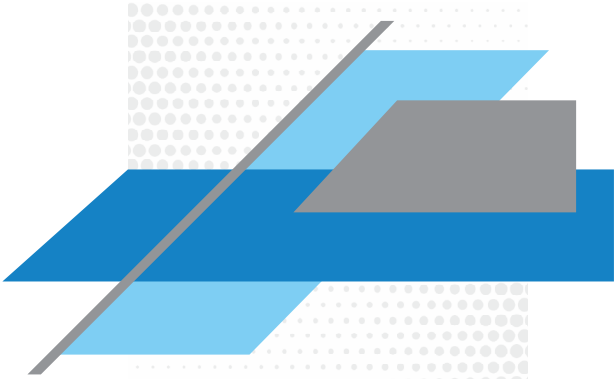
INTERPRETATION OF RESULTS

$IP_{sample} > 1,1$	POSITIVE
$0,9 \leq IP_{sample} \leq 1,1$	BORDERLINE
$IP_{sample} < 0,9$	NEGATIVE

Toxocara canis IgG

ELISA kit for the qualitative detection of IgG antibodies to *Toxocara canis*

Instructions for use



IVD

REF EI-603

Σ 96 tests

CE

EQUI Toxocara canis IgG

ELISA kit for the qualitative detection of
IgG antibodies to *Toxocara canis*

1. INTENDED USE

The «EQUI Toxocara canis IgG» is ELISA kit intended to qualitatively detect anti-*Toxocara canis* IgG in human serum or plasma by enzyme-linked immunosorbent assay (ELISA) in order to diagnose toxocariasis. The testing procedure is designed for both manual arrangement with automatic pipettes and standard equipment, and for automated «open» immunoassay analysers.

Target group: children, pet owners, rural people, summer visitors, forest guards, veterinarians.

Usage: ELISA kit is used in clinical diagnostic laboratories and other institutions engaged in *in vitro* diagnostics.

2. CLINICAL SIGNIFICANCE

Toxocariasis is a common disease induced by *Toxocara* helminth which is transmitted from animals to human. Toxocariasis is spread throughout the world, however, it is more common in depressed areas with poor hygienic conditions. In some regions, up to 90 % of puppies and up to 10 % of adult domesticated dogs are infested with toxocara. The risk of infestation is higher for owners of cats and dogs and for children due to playing in the sandpits and on the playgrounds contaminated with animal faeces.

Toxocara are threadworms belonging to *Nematoda*. Human conditions are mostly caused by *Toxocara canis*, which infested canids, rare - *Toxocara cati*, which is more common in felids. Adult toxocara in the body of infested animals reaches 5–15 cm in length; their propagation takes place here. Female helminths lay about 200 thous eggs daily, which are excreted in the environment with faeces. If conditions are favourable, following several weeks of maturation in the soil they become invasive — a larva is developed in the eggs. In the paratenic host (mice, poultry, cows, pigs, etc.). larva develops without propagation. If the conditions are unfavourable, larvae are encapsulated and may maintain viability for a long time (up to 10 years). They may also be the source of invasion.

People are infested through faecal-oral route when ingesting *Toxocara canis* mature eggs with soil-contaminated vegetables, fruits, berries, via dirty hands or when consuming meat of paratenic hosts. In the small intestine, larvae leave their cover and penetrates blood circulation through the intestinal walls. The larvae migrate to other organs and tissues with blood, namely: liver, lungs, muscles, eyes, CNS, etc. In the most of the infested, toxocariasis is asymptomatic. Clinical manifestations of this disease are associated with the site of larvae migration and depend on the intensity of invasion and age of the host. Visceral syndrome larva migrans is typical after infestation of the internal organs with *Toxocara canis* and ocular

toxocariasis, when eye and optic nerve are involved. Symptoms of visceral toxocariasis: fever, fatigue, abdominal pain, anorexia, hepatomegaly, cough and others. Heart and respiratory failure may develop in severe cases. Due to a strong immune response to larvae antigens, immediate and delayed hypersensitivity reactions develop. Granulomatosis in occular toxocariasis may result in retinal detachment and loss of vision.

Diagnosis of toxocariasis is complicated due to the lack of specific manifestations of the disease, even upon intense invasion. Furthermore, a man is an intermediate host of *Toxocara canis* and does not excrete parasites in the environment, whereas it is difficult to localise larvae in certain organs via non-invasive methods. Eosinophilia may appear in blood tests, however, serological tests are more common to detect toxocariasis (immunofluorescence reaction, ELISA and immunoblotting). Detection of specific anti-*Toxocara canis* IgG to larvae antigens may suggest current or previous invasion. High titter of IgE antibodies is also typical for active invasion. However, the combination of clinical manifestations and laboratory findings are necessary for diagnosis.

3. ANALYSIS PRINCIPLE

The procedure of testing for anti-*Toxocara canis* IgG in «EQUI Toxocara canis IgG» ELISA kit is based on «indirect» solid-phase ELISA with a two-stage incubation. Antigens of *Toxocara canis* larvae are entrapped in the wells. During the first step of incubation of ELISA plate wells with test samples, specific anti-*Toxocara canis* antibodies (if present in the samples) bind to the solid-phase antigens. The wells are washed to remove unbound antibodies and have only specific antigen-antibody complexes left. Then, a conjugate of anti-species IgG monoclonal antibodies with horseradish peroxidase is added, which binds to solid-phase immune complexes. Unbound components are removed by washing. Antigen-antibody complexes are detected by adding a solution of chromogen 3,3',5,5'-tetramethylbenzidine (TMB) with hydrogen peroxide. After 30-minute incubation, the reaction is stopped by adding the stop solution. The optical density (OD) in the wells is determined using a spectrophotometer at 450/620-695 nm. The intensity of the yellow colour is proportional to the level of antibodies in the sample.

4. MATERIALS AND EQUIPMENT

4.1. Contents of the ELISA kit

STRIPS	1 x 96 wells	**Microplate** Each plate well is coated with *Toxocara canis* larval antigens. The wells are detachable. After the first opening, store unused strips in the package at 2-8 °C for a maximum of 6 months

CONTROL +	1 x 0,25 ml	**Positive control** Conjugated specific monoclonal antibody solution with preservative (pink). Store at 2-8 °C
CONTROL -	1 x 0,6 ml	**Negative control** Negative human serum with a preservative (yellow). Store at 2-8 °C
DIL SAMPLE	1 x 13 ml	**Serum dilution solution** Buffer solution with a milk extract, a detergent and a preservative (brown). Store at 2-8 °C
SOLN CONJ	1 x 13 ml	**Conjugate solution (ready to use)** Buffer solution of monoclonal antibodies to human IgG, conjugated with horseradish peroxidase, with stabilizers and preservative (green). Store at 2-8 °C
SOLN TMB	1 x 13 ml	**TMB solution (ready to use)** TMB solution, H_2O_2, a stabilizer, a preservative (colourless). Store at 2-8 °C
TWEEN WASH 20x	1 x 50 ml	**Washing solution TWEEN (20x concentrated)** 20-fold phosphate buffer concentrate with Tween-20 (colourless). Dilute TWEEN detergent (20x) at 1:20 with distilled or deionized water (e. g., 5 mL of concentrate + 95 mL of water for 8 wells) before use. Store the diluted solution at 2-8 °C for a maximum of 7 days
SOLN STOP	1 x 13 ml	**Stop Solution (ready to use)** 0.5 mol H_2SO_4 solution (colourless). Store at 2-8 °C

The ELISA kit also includes adhesive films (2 items), sample application plan (1 item), checklist, and instruction for use.

4.2. Optional reagents, materials and equipment

Automatic single and multichannel pipettes 10–1000 µL, tips, volumetric laboratory glassware (10–1,000 mL), deionized or distilled water, thermostat at 37 °C, automatic or semi-automatic plate washer, spectrophotometer (reader) for microplates at 450/620-695 nm, appropriate containers for potentially contaminated waste, timer, filter paper, disposable powder-free gloves, disinfectants.

5. PRECAUTIONS AND SAFETY

5.1. Precautions

Be sure to read the instructions for use carefully before the test. The validity of the test results depends on strict following of the test procedure.

– do not use the ELISA kit components after the expiry date;
– do not use for analysis or mix components of different batches, components of kits for different nosologies, or reagents from other manufacturers with the «EQUI Toxocara canis IgG» ELISA kit;

– do not freeze the ELISA kit or its contents;
– after using a reagent, close each vial with its cap;
– when washing, control filling and complete aspiration of solution from the wells;
– use a new pipette tip each time you add samples or reagents;
– prevent direct sunlight from reaching the reagents from the ELISA kit;
– SOLN|TMB solution must be colourless before use. Do not use the solution if its colour is blue or yellow. Avoid contact of SOLN|TMB with metals or metal ions. Use only clean glassware thoroughly rinsed with distilled water;
– do not use reagents with colour not in line with para. 4.1;
– under no circumstances should the same glassware be used for SOLN|CONJ and SOLN|TMB;
– do not evaluate the test results visually (without a reader);
– any optional equipment that is in direct contact with biological material or kit components should be considered contaminated and requires cleaning and decontamination;
– the ELISA kit includes materials for 96 tests. Dispose of the used components as well as any remaining unused components.

5.2. Safety requirements

– all reagents in the ELISA kit are for laboratory professional use for *in vitro* diagnosis only and may only be used by qualified personnel;
– conduct the tests in disposable powder-free gloves and goggles only;
– do not eat, drink, smoke, or apply make-up in the test room;
– do not mouth-pipette the solutions;
– controls from the «EQUI Toxocara canis IgG» ELISA kit have been tested and found to be for anti-HIV1/2, anti-HCV and anti-*Treponema pallidum* antibodies and HBsAg negative; however, controls and test samples should be handled as potentially hazardous infectious materials;
– some of the kit components contain low concentrations of harmful substances and can damage skin or mucoga. In case of contact of SOLN|TMB, SOLN|STOP and SOLN|CONJ with mucous membranes or skin, immediately wash the affected area with plenty of water;
– in case of spillage of acid-free solutions, e. g. sera, treat the surface with a disinfectant solution and then wipe dry with filter paper. Otherwise first neutralize acid with sodium bicarbonate solution and then wipe the surface dry as described above.

5.3. Waste inactivation and disposal

– the liquid waste must be inactivated, for example, with hydrogen peroxide solution at the final concentration of 6% for 3 hours at room temperature, or with sodium hypochlorite at the final concentration of 5% for 30 minutes, or with other approved disinfectants;

– the solid waste must be inactivated by autoclaving at a temperature not less than 132°C;
– do not autoclave the solutions that contain sodium azide or sodium hypochlorite;
– disposal of inactivated waste must be conducted due to national laws and regulations.

6. STORAGE AND STABILITY

ELISA kit is stable up to the expiry date stated on the label when stored at 2-8°C. The kit should be transported at 2-8°C. Single transportation at a temperature up to 23°C for two days is possible.

7. SAMPLE COLLECTION, TRANSPORTATION AND STORAGE GUIDELINES

Collect blood from the vein into the sterile test tube. Test tube must be marked with patient ID and date of sample collecting. Blood before serum separation can be stored at 2-8 ºC for 24 hours, avoiding freezing.

Serum or plasma can be stored at 2-8 °C for maximum 3 days. Frozen serum can be stored for longer periods of time at -20 °C or -70 °C. Thaw frozen samples and keep them at room temperature for 30 minutes before use. After thawing, the stir samples to achieve homogeneity. Avoid repeated freezing-thawing cycles for test samples. If serum (or plasma) is turbid, remove insoluble inclusions by centrifugation at 3000 rpm for 10-15 minutes. Do not use serum samples with hyperlipidemia, hemolysis, and bacterial growth.

Transport serum samples in insulated containers. To do that, put closed labelled tubes in a plastic bag, tightly seal it and place in the centre of an insulated container.Put the frozen cold packs on the bottom, along the side walls of the insulated container and on top of the serum samples.

8. REAGENT PREPARATION

NOTE! It is very important to keep all ELISA kit components for at least 30 min at room temperature 18-25 ºC before the assay!

8.1. Microplate preparation

To prevent water condensation in the wells, keep the STRIPS for 30 minutes at a room temperature before opening. Open the vacuum pack, detach the appropriate number of wells, and carefully pack the remaining wells with a desiccant and store tightly zip-locked at 2-8 °C. Storing the packed plate this way ensures its stability for 6 months.

8.2. Washing solution preparation

To prepare detergent, dilute TWEEN WASH 20x at 1:20 (1+19) with distilled or deionized water and stir. E. g., 5 mL of concentrate + 95 mL of water, which is enough for 8 wells. If there are crystals present in the detergent concentrate, heat the vial at 37 °C until the crystals dissolve completely (15–20 minutes). Store the diluted solution at 2-8 °C for a maximum of 7 days.

9. ASSAY PROCEDURE

9.1. Prepare the necessary number of wells (four wells for controls and a necessary number of wells for test samples) and insert them into the ELISA plate frame. Be sure to add control wells in every test run.

9.2. Fill in the sample application plan.

9.3. Prepare the detergent as per para. 8.2.

9.4. Add 90 µL of DIL SAMPLE into each plate well.

9.5. Add 10 µL of controls and test samples into the wells:
CONTROL + – into well A1,
CONTROL - – into wells B1, C1 and D1,
and test samples into the remaining wells.
At the time of adding, the solution changes its colour from brown to blue. Pipette the mix in the wells carefully to avoid foaming.

9.6. Cover the strips up with adhesive film and incubate for **30 minutes at 37 °C**.

9.7. Remove and discard the adhesive film and wash all wells 5 times with automatic washer or 8-channel pipette as follows:
– aspirate the content of all wells into a liquid waste container;
– add a minimum of 300 µl of diluted washing solution to each well, soak each well for 30 seconds;
– aspirate the content of all wells again. The residual volume after every aspiration should be less than 5 µl;
– repeat the washing step 4 more times;
– after the final aspiration, eliminate extra moisture by tapping the plate against a piece of filter paper.

9.8. Add 100 µL of SOLN CONJ into each well. Cover the strips with a new piece of adhesive film and incubate for **30 minutes at 37 °C**.

9.9. Following incubation, remove the film carefully and wash the wells five times as described in para. 9.7.

9.10. Add 100 µL of SOLN TMB into the wells; do not touch the bottom and the walls of the plate wells.

9.11. Incubate the strips for **30 minutes** in a dark place at a room temperature of 18-25 °C. Do not use adhesive film at this stage.

9.12. Add 100 µL of SOLN STOP into each strip well to stop the enzymatic reaction; adhere to the same sequence of actions as when adding SOLN TMB. At the time of adding, the solution colour changes from blue to yellow, and clear solution slightly changes its shade.

9.13. Measure the optical density (OD) of the wells at 450/620-695 nm wavelength using an ELISA microplate reader within 5 minutes after stopping the reaction. Pay attention to the cleanness of the plate bottom and the absence of bubbles in the wells before reading.

Measurement at the single wavelength of 450 nm is possible, in that case, it is needed to leave one well for blank (only SOLN TMB and SOLN STOP must be added

in blank well).

10. CALCULATION AND INTERPRETATION OF RESULTS

10.1. Calculation of results

Calculate the average OD for the negative control ($\overline{Nc}$), Cut off (CO) and a sample positivity index (IP_{sample}).

$$\overline{Nc} = (Nc1 + Nc2 + Nc3)/3; \quad CO = \overline{Nc} + 0,3$$

$IP_{sample} = OD_{sample}/CO$, where: OD_{sample} is the OD sample.

10.2. Quality control (assay validation)

The test results are considered valid if they meet the following requirements:

CONTROL + $OD \geq 1,0$

CONTROL - $OD \leq 0,150$

CONTROL - $\overline{Nc} \times 0,5 \leq Ncn \leq \overline{Nc} \times 2,0$ where Ncn is the OD for each Nc run

If any of the OD values for the negative control is beyond the above interval, it should be discarded, and $\overline{Nc}$ is calculated based on the remaining OD values for the negative control. If several OD values for the negative control fail to meet the above requirements, the test is considered invalid and requires a new run.

10.3. Interpretation of results

$IP_{sample} > 1,1$	POSITIVE
$0,9 \leq IP_{sample} \leq 1,1$	BORDERLINE*
$IP_{sample} < 0,9$	NEGATIVE

* Uncertain samples are recommended to be re-examined in two wells of the ELISA kit. If the results are again uncertain, a new sample should be selected and analyzed in 2-4 weeks. In case of repeated indeterminate results, such samples shall be considered negative.

11. PERFORMANCE CHARACTERISTICS

11.1. Analytical performance characteristics

Precision of measurement

Intra assay repeatability

The coefficient of variation (CV) for three sera with different levels of specific antibodies was evaluated in 24 replicates on one series of ELISA kits.

Sample No.	OD_{av}	IP_{av}	CV, %
669	0,927	2,81	4,8
544	1,503	4,56	1,4
666	1,694	5,14	4,5

Inter assay reproducibility

The coefficient of variation (CV) for three sera with different levels of specific antibodies was evaluated for 4 days in 4 sets of analysis, 8 replicates in each analysis.

Sample No.	OD_{av}	IP_{av}	CV, %
669	1,016	3,04	4,7
544	1,516	4,54	1,9
666	1,683	5,04	4,1

Analytical specificity

The test results are not affected by bilirubin at up to 0.21 mg/mL (361.8 μmol/L), haemoglobin at up to 10 mg/mL and triglycerides at up to 10 mg/mL (11.3 mmol/l) present in the sample.

11.2. Diagnostic characteristics

To evaluate diagnostic characteristics of «EQUI Toxocara canis IgG» ELISA kits, 78 serum samples from patients with clinical symptoms typical for toxocariasis and 60 serum samples from patients without clinical manifestations (seronegative in terms of *Toxocara canis*) were used. Clinical sensitivity of «EQUI Toxocara canis IgG» ELISA kits was 98.7 %, clinical specificity — 96.7 %.

Method characteristics in comparison with equal commercial ELISA kit was studied in target paediatric population (160 samples) and population of donors (298 samples). For paediatric population serum, relative specificity of «EQUI Toxocara canis IgG» ELISA kits was established at the level of 99.28 % and percent agreement was 97.45 %. For donor population serum, relative specificity of was 89.19 %, relative specificity — 93.55 % and percent agreement was 91.73 %.

12. LIMITATIONS OF ASSAY

Positive result in «EQUI Toxocara canis IgG» ELISA kit supports presence of anti-*Toxocara canis* specific IgG antibodies. Presence of this class antibodies in newborns is not an evidence of *Toxocara canis* invasion.

Inconclusive results may suggest a history of *Toxocara canis* invasion.

Negative result of «EQUI Toxocara canis IgG» ELISA kit supports the absence of anti-*Toxocara canis* specific IgG antibodies in the test sample or concentration of specific antibodies is below the sensitivity limit of the assay.

The results of serological test only are not the basis for final diagnosis. When establishing the diagnosis, the results of complex laboratory and instrumental tests, as well as clinical manifestations should be considered. Cross-reactions with antibodies to antigens of other helminths cannot be fully ruled out.

13. DIFFICULTIES THAT CAN OCCUR DURING THE ASSAY PROCEDURE

Possible reasons	Solution
High background in all wells	
Contaminated washer	Clean the washer head and rinse according to the instructions for use
Poor quality or contaminated water	Use purified water with specific resistance ≥ 10 MΩ · cm
Use of poorly washed glassware	Use chemically clean utensils
Use of chlorinated disinfectants	Do not use chlorine disinfectants
Use of contaminated tips	Use new tips
Increased incubation times or change in the temperature conditions	Adhere to the incubation regime according to the instructions for use
High background in a row of wells	
Repeat application of TMB solution	TMB solution should be applied once
Contamination of the automatic pipette nozzle with conjugate solution	Clean the pipette and dial carefully liquid
Contamination of one of the washer's channel	Clean the flush channel, rinse washer
Received OD of the positive control is below the border value	
One of the reagents (conjugate solution or TMB solution) was not prepared in a correct way or was not added	Re-conduct ELISA, pay attention to the correctness of the introduction of these reagents
Reduced incubation times at any stage	Incubate according to instructions for use
The colour density of the wells fails to meet the obtained optical density value	
This may suggest that the optical beam has been displaced	Check the correct operation of the reader

14. TECHNICAL ASSISTANCE AND CUSTOMER SERVICE

In case of technical problems, you can obtain assistance by contacting the manufacturer.

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	Manufacturer
EC REP	Authorized Representative in the European Community
IVD	In vitro diagnostic medical device
REF	Catalogue number
	Date of manufacture
	Use by date
LOT	Batch code
	Temperature limit
Σ	Contains sufficient for <n> tests
	Caution
NON STERILE	Non-Sterile
	Consult instructions for use
	Keep away from sunlight
	Keep dry
CE	Compliance with EU safety requirements

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For questions and suggestions regarding the ELISA kit contact:

EC REP

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ASSAY PROCEDURE SCHEME

Keep all reagents for 30 min at temperature18-25°C before use
Dispense 90 µl DIL SAMPLE into the wells (brown)
Add to 10 µl of controls and samples into the wells: A1 – CONTROL +, B1, C1, D1 – CONTROL –, other wells – examined samples (change of colour from brown to blue)
Cover strips with an adhesive film, incubate for **30 min at 37°C**
Rinse the wells 5 times with prepared 1:20 (1+19) washing solution TWEEN (300 µl per well)
Add 100 µl of SOLN CONJ into all wells (green)
Cover strips with an adhesive film, incubate for **30 min at 37°C**
Rinse the wells 5 times with prepared 1:20 (1+19) washing solution TWEEN (300 µl per well)
Add 100 µl of SOLN TMB into all wells
Incubate for **30 min** in the dark at **18-25°C**
Add 100 µl of SOLN STOP into all wells (change of colour from blue to yellow)
Measure the optical density (OD) with an ELISA microplate reader at 450/620-695 nm

CALCULATION OF RESULTS

$\overline{Nc} = (Nc1 + Nc2 + Nc3)/3;$

$CO = \overline{Nc} + 0,3;$

$IP_{sample} = OD_{sample}/CO$

$\overline{Nc}$ - the average value of OD 3-x CONTROL –

CO - Cut off

IP_{sample} - sample positivity index

INTERPRETATION OF RESULTS

$IP_{sample} > 1,1$	POSITIVE
$0,9 \leq IP_{sample} \leq 1,1$	BORDERLINE
$IP_{sample} < 0,9$	NEGATIVE