Vitrotest® Anti-Ascaris

ELISA test-kit for the detection of antibodies to *Ascaris lumbricoides*

TK051
96 tests

1. INTENDED USE

ELISA test-kit «Vitrotest Anti-Ascaris» is an enzyme linked immunosorbent assay (ELISA) for the detection of IgG antibodies to *Ascaris lumbricoides* in human serum or plasma. The test-kit might be applied for the ELISA using both automatic pipettes and standard equipment as well as open system automated ELISA analyzers.

2. CLINICAL VALUE

Ascariasis is prevalent worldwide, especially in tropical and subtropical countries. The ascariasis pathogen in humans, *Ascaris lumbricoides* is a roundworm of the Nematoda phylum. Adult ascaris parasitizes in the small intestine, has a length of 15-40 cm, a diameter of 5 mm and produces 200 000 eggs per day.

Infections happen when a human swallows water or food contaminated with eggs which hatch into juveniles in the duodenum and enter the blood stream. From there parasites go to the liver and heart and enter the pulmonary circulation to break free in the alveoli, where they grow and molt. In three weeks, the larva passes from the respiratory system to be coughed up, swallowed and thus returned to the small intestine, where it matures to an adult male or female worm.

Often, no symptoms are visible with an *A. lumbricoides* infection. However, in the case of a particularly severe infection bloody sputum, cough, fever, abdominal discomfort, intestinal ulcer and the passing worms can be observed. Ascariasis is also the most common cause of Löffler's syndrome worldwide. Accompanying symptoms include pulmonary infiltration and eosinophilia.

The presence of an infection can be identified by microscopy (detection of eggs in faces) and serology (detection of antibodies by ELISA).

Most diagnoses are made by identifying the appearance of the worm or eggs in faeces. This method is effective when the adult roundworms parasitize in the intestine. During the larvae migration the efficiency of ascariasis diagnosis can be increased with ELISA for the detection of antibodies to helminth antigens. The results of the serological analysis coupled with anamnesis and clinical symptoms facilitate diagnosis of the Ascaris invasion at an early stage and enable therapy to begin the before complications of the disease appear.

3. PRINCIPLE OF THE TEST

«Vitrotest Anti-Ascaris» ELISA is a solid phase, indirect ELISA method for detection of IgG antibodies to *Ascaris lumbricoides* in a two-step incubation procedure. Microwells are coated with *A. lumbricoides* antigens. During the first incubation step, the specific antibodies to *A. lumbricoides*, if present, will be bound to the solid phase precoated antigens. The wells are washed to remove unbound antibodies, leaving only the specific antigen-antibody complexes. Secondary antibodies (anti-IgG) which are conjugated to horseradish peroxidase (HRP) added next and bind to the immune complexes on the solid phase. Unbound components are removed by washing. Chromogen solution containing 3,3',5,5'- tetramethylbenzidine (TMB) and hydrogen peroxide is added. TMB is catalysed by the HRP to produce a blue colour product that changes to yellow after adding stop solution. Absorbance at 450/620-695nm is read using a plate reader. The density of yellow colouration is directly proportional to the amount of the antibodies present in the sample.

4. MATERIALS AND EQUIPMENT

4.1. Composition of the test-kit

ELISA STRIPS	1x96 wells	Microplate (12 strips x 8 wells) Each well is coated with *A. lumbricoides* antigens. The wells can be separated.
CONTROL +	1x0.3 ml	Positive control Solution of specific antibodies to A. lumbricoides with preservative (pink).
CONTROL –	1x0.5 ml	Negative control Negative human serum with preservative (yellow).
SAMPLE DILUENT	1x12 ml	Sample diluent Buffer solution with detergent and preservative (brown-green).
CONJUGATE SOLUTION	1x12 ml	Conjugate solution (ready to use) Buffer solution of monoclonal antibodies to human IgG conjugated to HRP with stabilizers and preservative (green).

TMB SOLUTION	1x12 ml	**TMB solution (ready to use)** TMB, H_2O_2, stabilizers and preservative (colourless).
WASH TWEEN 20X	1x50 ml	**Washing solution Tw20 (20x concentrated)** 20X concentrate of PBS buffer with Tween-20 and NaCl (colourless)
STOP SOLUTION	1x12 ml	**Stop Solution (ready to use)** 0.5 M H_2SO_4 (colourless).

Adhesive films (2), sera identification plan (1) and instruction for use.

4.2. Material required but not provided

- Variable volume automatic pipettes (10µl–1000µl) and disposable pipette tips;
- plate reader (single wavelength 450 nm or dual wavelength 450/620–695 nm);
- volumetric laboratory glassware (10–1000ml);
- distilled/DI water;
- incubator thermostatically controlled at 37°C;
- automatic/semiautomatic plate washer;
- appropriate waste containers for potentially contaminated materials;
- timer;
- absorbent paper;
- disposable gloves;
- disinfectants;
- protective clothes.

5. PRECAUTIONS AND SAFETY

5.1. Precautions

The ELISA assays are time and temperature sensitive. Strictly follow the test procedure and do not modify it.

- do not use expired reagents;
- do not use for analyses and do not mix reagents from different lots or from test-kits of different nosology as well as other manufacturer's reagents with Vitrotest® kits;

Note: it is possible to use WASH TWEEN 20X, TMB SOLUTION *and* STOP SOLUTION *from other Vitrotest® ELISA kits.*

- close reagents after use only with appropriate caps;
- control the filling and full aspiration of the solution in the wells;
- use a new tip for each sample and reagent;
- avoid exposure of kit reagents to direct sunlight;
- TMB SOLUTION must be colourless before use. If TMB SOLUTION is blue or yellow it cannot be used. Avoid any contact of TMB SOLUTION with metals or metal ions. Use glassware thoroughly washed and rinsed with distilled/DI water;
- never use the same glassware for CONJUGATE SOLUTION and TMB SOLUTION.

The manufacturer is not responsible or liable for any incorrect results and/or incidents taking place as a result of any violation of the instruction. The manufacturer is not responsible for visual readings of samples (without using a plate reader).

5.2. Safety

- all reagents included in the kit are intended for in vitro diagnostic use only;
- the test-kit is designed for use by qualified personnel only;
- disposable gloves and safety glasses must be worn at all times while performing analysis;
- never eat, drink, smoke or apply cosmetics in the assay laboratory;
- never pipette solutions by mouth;
- positive control does not contain of human origin components;
- negative control of test-kit «Vitrotest Anti-Ascaris» was tested and found negative for anti-HIV1/2, anti-HCV, anti-T.pallidum antibodies and HBsAg. Nevertheless, all controls and patient samples should be regarded and handled as potentially infectious;
- 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 121°C for 1 hour;
- dispose of inactivated waste in accordance of national laws and regulations;
- do not autoclave the solutions that contain sodium azide or sodium hypochlorite;
- some components of the test-kit contain low concentrations of harmful compounds and could cause irritation of the skin and the mucosa. In the case of contact of TMB SOLUTION, STOP SOLUTION or CONJUGATE SOLUTION with skin or mucosa, the place of contact should be immediately rinsed with large amounts of water;
- in case of spilling of solutions that do not contain acid, e.g. sera, rinse the surface with disinfectant, then dry it with absorbent paper. In other case acid first must be neutralized by sodium bicarbonate and then wiped out as described above.

6. STORAGE AND STABILITY

Reagents are stable until stated expiration date on the label when stored refrigerated (2-8°C). Do not freeze. The kit should be shipped at 2-8°C. Single transportation at the temperature up to 23°C for two days is acceptable.

7. SPECIMEN COLLECTION

The fresh serum or plasma samples can be stored for 3 days at 2-8 ºC or frozen for longer periods at -20 – -70°C. Frozen samples must be thawed and kept at room temperature for at least 30 minutes before use. Do not use preheated samples. Mix thawed samples thoroughly to homogeneity. Avoid repeated freezing/thawing. Samples containing aggregates must be clarified by centrifugation (3000rpm for 10-15min). Do not use hyperlipeamic, hyperhaemolysed or contaminated by microorganisms serum specimens. The presence of bilirubin up to concentration of 0.21 mg/ml (361.8 µM/l), haemoglobin up to concentration of 10 mg/ml and triglycerides up to concentration of 10 mg/ml (11.3 mM/l) are allowed.

8. REAGENT PREPARATION

It is very important to keep all test components for at least 30 min at room temperature (18-25 ºC) before the assay!

8.1. ELISA STRIPS preparation

Before opening the bag with ELISA STRIPS, keep it at room temperature for 30 minutes to avoid water condensation inside the wells. Open the vacuum bag and take out the necessary number of the wells. Once opened the bag with the remaining strips must be *resealed with zip-lock* immediately and kept refrigerated at 2-8°C for no more than 3 months.

8.2. Washing solution preparation

Check the WASH TWEEN 20X for the presence of salt crystals. If crystals have formed, re-solubilise by warming at 37°C, until crystals dissolve (15-20min). Dilute the WASH TWEEN 20X 1:20 (1+19) with distilled/DI water before use. For example, 4 ml concentrate + 76 ml water is sufficient for 8 wells. Once diluted it is stable at 2-8°C for 1 week.

9. ASSAY PROCEDURE

9.1. Take out from the protective bag the support frame and the necessary number of the wells (the number of specimens + 4 for controls). Place the wells into the frame. Wells with the controls must be included in every test.
9.2. Complete the sera identification plan.
9.3. Prepare washing solution (see 8.2.).
9.4. Dispense 90 µl of SAMPLE DILUENT into each well.
9.5. Dispense 10 µl of controls and patient samples into the wells in the following order: A1 – CONTROL +, B1, C1 and D1 – CONTROL -, other wells – patient samples. Mix gently to avoid foaming. The colour of the sample diluent changes from brown-green to blue.
9.6. Cover strips with an adhesive film and incubate for 30 min at 37°C.
9.7. At the end of the incubation period, remove and discard the adhesive film and wash the well 5 times with automatic washer or 8-channel pipette as follows:
- aspirate the contents of all wells into a liquid waste container and add immediately a minimum of 300 µl of diluted washing solution to each well;
- soak each well for 30 seconds between each wash cycle;
- aspirate again. The residual volume must be lower than 5 µl.
- repeat the washing step 4 times;
- after the final washing cycle, turn down the plate onto an absorbent paper and tap it to remove any residual buffer.

9.8. Dispense 100 µl of CONJUGATE SOLUTION. per well. Cover strips with a new adhesive film, incubate for 30 min at 37°C.
9.9. At the end of the incubation period, remove and discard the adhesive film and wash the wells five times as described above (see 9.7).
9.10. Dispense 100 µl TMB SOLUTION into all wells. Do not touch the walls and bottoms of the wells to avoid contamination.
9.11. Incubate the strips for 30 minutes at room temperature (18-25°C) in the dark. Do not use adhesive film in this step.
9.12. Dispense 100 µl STOP SOLUTION into all wells in the same order and at the same rate as for TMB SOLUTION.
9.13. Read the optical density (OD) of the wells at 450/620-695 nm using a microplate reader within 5 minutes after adding the STOP SOLUTION. Pay attention to the cleanness of the plate bottom and absence of bubbles in the wells before reading.

Measurement in the single-wave procedure at 450 nm is possible. Reserve blank well to adjust spectrophotometer in such analysis. Only TMB SOLUTION *and* STOP SOLUTION *must be added in blank well).*

10. CALCULATION AND INTERPRETATION OF RESULTS

10.1. Calculation of results

Calculate the mean absorbance value for 3 negative controls (Nc), Cut off value (CO) and Sample Index of Positivity (IP_{sample}),

$$Nc = (Nc1 + Nc2 + Nc3)/3; \quad CO = Nc + 0.3; \quad IP_{sample} = OD_{sample}/CO$$

10.2. Validation of the test

The test run may be considered valid provided the following criteria are met:

CONTROL +	OD ≥ 1.200
CONTROL –	OD ≤ 0.150
CONTROL –	Nc × 0.5 ≤ Ncn ≤ Nc × 2.0

If one of the negative control absorbances does not match the above criteria, this value should be discarded and a mean value should be calculated using the other two values. If more than one negative control absorbance does not meet the criteria, the test is invalid and must be re-tested.

10.3. Interpretation of results

$IP_{sample} > 1.1$	POSITIVE
$0.9 \leq IP_{sample} \leq 1.1$	DOUBTFUL*
$IP_{sample} < 0.9$	NEGATIVE

* If the result is doubtful, repeat the test. If it remains doubtful, collect a new serum sample.

11. PERFORMANCE CHARACTERISTICS

11.1. Specificity and sensitivity

Relative sensitivity of the «Vitrotest Anti-Ascaris» ELISA kit was 92 % while evaluating it by using of 64 sera positive to *Ascaris lumbricoides* antibodies in other commercial test-kit.

In the comparative studies with other commercial test-kit using 224 negative sera for antibodies to *Ascaris lumbricoides* specificity of the «Vitrotest Anti-Ascaris» was 93.3 %.

11.2. Accuracy

Intra assay repeatability

Coefficient of variation (CV) was calculated by measuring 3 samples with various specific antibody levels in 24-replicate determinations using 1 lot of the test-kit.

Serum No.	OD_{mean}	IP_{mean}	CV, %
102L	0.636	1.83	5.3
133L	1.349	3.88	1.0
948	2.593	7.45	1.0

Inter assay reproducibility

Coefficient of variation (CV) was calculated by measuring 3 samples with various specific antibody levels in 4 ELISA performances during 4 days, in 8-replicate determinations.

Serum No.	OD_{mean}	IP_{mean}	CV, %
102L	0.637	1.76	3.7
133L	1.329	3.67	2.5
948	2.539	7.01	2.5

12. LIMITATIONS OF THE PROCEDURE

A positive result in the «Vitrotest Anti-Ascaris» indicates the presence of specific antibodies IgG to Ascaris lumbricoides. The presence of the antibodies in newborn infants cannot be held as proof of Ascaris lumbricoides invasion.

Indeterminate results might indicate the invasion of Ascaris lumbricoides in anamnesis.

A negative result in the «Vitrotest Anti-Ascaris» test-kit indicates either the absence of antibodies to Ascaris lumbricoides in the sample tested, or that the concentration of specific antibodies is below the detection threshold of the test.

Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis, in fact, should take into consideration as well as clinical history, symptomatology and serological data. It is impossible to completely eliminate cross-reactions of antibodies and antigens of other worms.

13. TROUBLESHOOTING

Possible causes	*Solutions*
High background in all wells	
Contaminated washer	Clean the washer head, then rinse it with 30% ethanol and distilled water
Low quality water or contaminated water	Use distilled/DI with resistivity ≥ 10 MΩ·cm.
Using contaminated glassware	Use clean glassware
Using chlorine based disinfectants	Use disinfectants without chlorine
Using contaminated tips	Use new tips
Increased time of incubation or temperature regimen was changed	Follow incubation regimen according to instruction for use
High background in a few wells	
TMB solution was added more than once	Add TMB solution once
Pipette shaft was contaminated with conjugate solution	Clean the pipette; pipette the liquids carefully
One the channels of the washer was contaminated	Clean the washer channel, clean the washer
OD of the positive control below normal	
Conjugate solution/tmb solution was prepared improperly or not added	Run ELISA repeatedly, prepare conjugate solution / TMB solution properly
Reduced incubation time in one of the stages	Follow incubation regimen according to the instruction for use
Visual colour intensity of the wells does not correspond to optical density	
The optical beam or another component of the reader is misaligned or malfunctioning	Test the absorbance reader's performance

13. REFERENCE

1. Gildner TE, Cepon-Robins TJ, et. al. Regional variation in Ascaris lumbricoides and Trichuris trichiura infections by age cohort and sex: effects of market integration among the indigenous Shuar of Amazonian Ecuador.//J Physiol Anthropol.– 2016.– V.35, N. 1.– P. 28.
2. Guadalupe I., Mitre E., Benitez S. et.al. Evidence for in utero sensitization to Ascaris lumbricoides in newborns of mothers with ascariasis. // J Infect Dis. - 2009. – V. 199 N.12 – p.1846–1850.
3. Khuroo M.S., Rather A.A., Khuroo N.S., Khuroo M.S. Hepatobiliary and pancreatic ascariasis// World J. Gastroenterol. -2016.- V 22, N 33.- P. 7507-7517.

SYMBOLS

Catalogue number


Consult instructions for use

In vitro diagnostic medical device


Manufacturer


Caution, consult accompanying documents

Contains sufficient for <n> tests


Temperature limitation

Batch code


Use by


Date of manufacture


Keep away from direct sun light / Не допускать воз-действия солнечного света

Authorized representative in the European Community / Уполномоченный представитель в ЕС


Mark of conformity to the technical regulations / Знак соответствия техническим регламентам

ТУ У 24.4-36555928-001:2011
Inst_Anti-Ascaris_TK058_V01

Edition 1st, 12.09.2019.

For questions and suggestions regarding the kit, contact the manufacturer:


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ASSAY PROCEDURE

Keep all reagents and specimens for at least 30 min at 18-25°C before use

Dispense 90µl of SAMPLE DILUENT into the wells
(brown-green colour)

Dispense 10µl of controls and samples into the wells:
A1 – CONTROL +,
B1, C1, D1 – CONTROL –,
E1 and other wells – patient samples
(colour changes from brown-green to blue)

Cover wells with an adhesive film, incubate for 30 min at 37°C

Rinse the wells 5 times with diluted 1:20 (1+19) washing solution Tween-20 (300µl per well)

Add 100µl of CONJUGATE SOLUTION into the wells
(green colour)

Cover wells with an adhesive film, incubate for 30 min at 37°C

Rinse the wells 5 times with diluted 1:20 (1+19) washing solution Tween-20 (300µl per well)

Add 100µl of TMB SOLUTION into the wells

Cover wells with an adhesive film, incubate for 30 min at 37°C

Add 100µl of STOP SOLUTION
(colour changes from blue to yellow)

Determine the optical density (OD) at 450/620-695nm

CALCULATION

Nc = (Nc1 + Nc2 + Nc3)/3;

CO = Nc + 0.3;

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

Nc - OD_{mean} for 3 CONTROL –.
CO - Cut off, IP- Index of Positivity

INTERPRETATION

$IP_{sample} > 1.1$	POSITIVE
$0.9 \leq IP_{sample} \leq 1.1$	DOUBTFUL
$IP_{sample} < 0.9$	NEGATIVE