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Wrocław, 02.06.2022

To whom it may concern

STATEMENT

Herewith we, Vitrotest Europe Sp. z O.O. with registered address at Krakowska str., 139-155, 50-428, Wroclaw, Poland, acting as a manufacturer, hereby assign SRL SANMEDICO having a registered office at A. Corobceanu street 7A, apt. 9, Chişinău MD-2012, Moldova , as authorized representative in Republic Moldova.

We declare that the company mentioned above is authorized to register, notify, renew or modify the registration of medical devices on the territory of the Republic of Moldova.

June 2, 2022

Galyna Rayevska, Chief of the board

Vitrotest Europe Sp. z O.O.

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Vitrotest®
ELISA kits & components

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CLARIFICATION LETTER

Hereby, we Vitrotest Europe Sp. Z O.O. with legal address at ul. Krakowska 139-155, 50-428, Wroclaw, Poland, inform that Vitrotest ELISA kits are produced according to Directive 98/78 EC. They are classified as in vitro medical device, other device (not applicable to list A or B of Annex II of Directive 98/79/EC, not a product for self-testing, not for performance evaluation). ISO certificate isn't mandatory for manufacturing of this group of IVD according to requirements of Directive 98/79/EC. Although we are not certified to standard 13485, all its requirements had been implemented and apply to the production of Vitrotest ELISA kits since 2022.

Ihor Nikolaienko, Ph.D.
Vice Chairman of the Board

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08.12.2023
Wroclaw, Poland

MANUFACTURER:	**Vitrotest Europe Sp. z O.O.**
ADDRESS:	**Krakowska str., 139-155, 50-428, Wroclaw, Poland**
PRODUCT NAME:	**Vitrotest Echinococcus granulosus IgG** ELISA test kit for the detection of IgG class antibodies to *Echinococcus granulosus*
PRODUCT CATALOGUE NUMBER:	**EL066-96**
GMDN CODE:	**52210**

We hereby declare that the above mentioned product meet the provisions of the Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998 on in vitro diagnostic medical devices.

CLASSIFICATION:	In vitro medical device, other device (not applicable to list A or B of Annex II of Directive 98/79/EC, not a product for self-testing, not for performance evaluation).
CONFORMITY ROUTE:	Annex III of Directive 98/79/EC.
APPLICABLE STANDARDS:	EN ISO 13485:2016; EN ISO 14971:2019; EN 13612:2002; EN ISO 15223-1:2016; EN ISO 18113-1:2011; EN ISO 18113-2:2011; EN ISO 23640:2015.

This Declaration of conformity is issued under the responsibility of the manufacturer.

Edition 1

Wroclaw, Poland
Issued in

15.02.2022
Date

Vitrotest Europe Sp. z o.o.
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Galyna Rayevska, Ph.D.
Chief of the Board

INSTRUCTION FOR USE

Vitrotest Echinococcus granulosus IgG

ELISA test kit for the detection of IgG class antibodies to *Echinococcus granulosus*

REF EL066-96

IVD Σ 96

1. INTENDED USE

The test kit Vitrotest Echinococcus granulosus IgG is an enzyme linked immunosorbent assay (ELISA) for the detection of IgG class antibodies to *Echinococcus granulosus* 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

Echinococcosis is a chronic disease of humans and animals caused by parasitizing the larvae of the helminth *Echinococcus*. The causative agent of this helminthiasis is most often the larva of *Echinococcus granulosus*. Echinococcosis is quite common all over the world, especially in southern countries, where livestock breeding, mainly sheep breeding, is widespread.

Echinococcus eggs enter the human body through dirty hands after contacting dogs (less often - cats). Also, infection is not excluded when eating unwashed vegetables, berries, fruits, water that are contaminated with helminth eggs.

In the digestive canal of the intermediate host, the egg of the echinococcus is freed from the membrane, and the embryo (oncosphere) deepens into the mucous membrane of the small intestine, entering the internal organs, where, in most cases, they linger and develop into echinococcal cysts. More often, echinococcus affects the liver (in 44-85 % of cases) and lungs (10 % of cases).

The pathological effect of echinococcus is due to the sensitization of the body by the metabolic products of the parasite and mechanical damage to the affected organs and tissues. The sizes of cysts are from 1-5 cm in diameter to large blisters, which can contain several liters of fluid. The mechanical effect of such a cyst leads to dysfunction of the affected organ, its hypertrophy.

To diagnose echinococcosis, cysts visualization methods are used: X-ray and ultrasound studies, computed and magnetic resonance imaging. Puncture biopsy of a cyst is considered dangerous due to the possibility of spreading parasites into adjacent tissues.

The detection of antibodies specific to the antigens of echinococcus in the blood is a reliable indicator of parasite invasion. The level of the immune response largely depends on the organ localization of the cyst and its morphology. Low antibody levels are observed at the onset of cyst formation or at a late inoperable stage of the disease.

Today, methods of indirect hemagglutination and fluorescence, enzyme immunoassay are used to detect specific antibodies to *Echinococcus granulosus*. These methods are characterized by a sensitivity of 60-90 %, therefore, the best information content is achieved using a combination of serological methods.

Serological methods are also quite informative for monitoring the patient's postoperative state - a gradual decrease in the level of specific antibodies 4-6 months after surgical removal of the cyst indicates a successful result of the surgical intervention. With relapses of cyst formation, specific antibodies are kept at a high level for years.

3. PRINCIPLE OF THE TEST

Vitrotest Echinococcus granulosus IgG ELISA is a solid phase, indirect ELISA method for the detection of IgG antibodies to *Echinococcus granulosus* in a two-step incubation procedure. Microwells are coated with the *E. granulosus* antigens. During the first incubation step, the specific antibodies to *E. granulosus*, if present in the sample, will be bound to the solid phase precoated antigens. The wells are washed to remove unbound antibodies leaving only the specific antigen-antibody complexes. A secondary antibody (anti-IgG), which is conjugated to horseradish peroxidase (HRP), is added next and binds to the immune complexes on the solid phase. Unbound components are removed by washing. Antigen-antibody complexes are revealed by addition of chromogen solution containing 3,3',5,5'- tetramethylbenzidine (TMB) and hydrogen peroxide. After 15 min the reaction has been stopped, the absorbance values are read using a spectrophotometer at 450/620-695 nm. The colour intensity is 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 ELISA (12 strips x 8 wells) Each well is coated with *E. granulosus* antigens. The wells can be separated.

CONTROL +	1x0.5 ml	**Positive control** Solution of specific monoclonal immunoglobulins with preservative (pink).
CONTROL –	1x0.5 ml	**Negative control** Buffer solution with detergent and preservative (yellow).
CONTROL CUT-OFF	1x0.5 ml	**Cut-off control** Solution of specific monoclonal immunoglobulins with preservative (orange).
SAMPLE DILUENT	1x12 ml	**Sample diluent** Buffer solution with detergent and preservative (brown-green).
CONJUGATE SOLUTION	1x12 ml	**Conjugate solution** Buffer solution of monoclonal antibodies to human IgG conjugated to HRP with stabilizers and preservative (green), ready to use.
TMB SOLUTION	1x12 ml	**TMB solution** TMB, H_2O_2, stabilizers, preservative (colourless), ready to use.
WASH TWEEN 20X	1x50 ml	**Washing solution Tw20 (20x concentrate)** 20X concentrated of PBS buffer with Tween-20 and NaCl (colourless).
STOP SOLUTION	1x12 ml	**Stop Solution** 0.5 mol/l H_2SO_4 (colourless), ready to use.

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

4.2. Material required but not provided

- variable volume automatic pipettes (10 µl–1000 µl) and disposable pipette tips;
- plate reader (single wavelength 450 nm or dual wavelength 450/620–695 nm);
- volumetric laboratory glassware (10–1000 ml);
- distilled or deionized 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;
- must be colourless before use. If is blue or yellow it cannot be used. Avoid any contact of with metals or metal ions. Use glassware thoroughly washed and rinsed with distilled or deionized water;
- never use the same glassware for and .

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 components of test kit are intendent for *in vitro* diagnostic use only;
- all materials of human or animal origin should be regarded and handled as potentially infectious;
- the ELISA is only designed for qualified personnel;
- 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;
- controls do not contain of human origin components;
- avoid contact with STOP SOLUTION containing 0.5 mol/l H_2SO_4. It may cause skin irritation and burns;
- 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;
- for information on hazardous substances included in the kit please refer to Safety Data Sheets. Safety Data Sheets for this product are available upon request.

5.3. Waste treatment

Patient specimens, controls and incubated microplate strips should be treated as infectious waste, residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

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.

After the first opening of the packaging, the components of the ELISA kits are stable within 3 months, except for those specified in p. 8 of this Instruction.

7. SPECIMEN COLLECTION

The fresh serum or plasma (EDTA, lithium-heparin, sodium citrate, potassium fluoride) samples can be stored for 3 days at 2-8 °C, or frozen for longer periods at -20 – -70 °C. Frozen samples must be thawed and kept at room temperature for at least 30 min 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 (3000 rpm for 10-15 min). 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 µmol/l), haemoglobin up to concentration of 10 mg/ml and triglycerides up to concentration of 10 mg/ml (11.3 mmol/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 min 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 and desiccant 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, resolubilise by warming at 37 °C, until crystals have been fully dissolved (15-20 min).

Dilute the WASH TWEEN 20X 1:20 (1+19) with distilled or deionized water before use and mix. For example, 4 ml concentrate + 76 ml water is sufficient for 8 wells. Once diluted it is stable at 2-8 °C for 7 days.

9. ASSAY PROCEDURE

9.1. Take out from the protective bag the support frame and the necessary number of the wells ELISA STRIPS (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 – CONTROL CUT-OFF, 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 s 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 15 min 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 min 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. Validation of the test

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

CONTROL +	OD ≥ 1.2
CONTROL CUT-OFF	OD in a range 0.25-0.65
CONTROL –	OD ≤ 0.150

If one of the control cut-off absorbances does not match the above criteria, this value should be discarded and a cut-off value should be calculated using the remaining cut-off control. If both control cut-off absorbance do not meet the criteria, the test is invalid and must be re-tested.

10.2. Calculation of results

The cut-off (CO) is the mean optical density (OD) of the wells containing CONTROL CUT-OFF:

$$CO = (OD_{CONTROL\ CUT\text{-}OFF\ 1} + OD_{CONTROL\ CUT\text{-}OFF\ 2})/2;$$

The sample result is reported as a $Ratio_{sample}$:

$$Ratio_{sample} = OD_{sample}/CO, \quad OD_{sample} – \text{optical density of the well containing sample}$$

10.3. Interpretation of results

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

In comparative studies of the Vitrotest Echinococcus granulosus IgG test kit with another test kit, 366 serum samples were analyzed. The relative sensitivity of the kit was 92.3 %, and the relative specificity - 99.7 %

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	Ratio	CV, %
1399	0.880	2.78	6.2
38S	0.452	1.42	7.5
24S	1.914	6.04	6.3

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	Ratio	CV, %
28S	2.030	5.99	1.4
38S	0.528	1.56	4.6
39S	1.228	3.62	2.1

12. LIMITATIONS OF THE PROCEDURE

A positive result in the test kit Vitrotest Echinococcus granulosus IgG indicates the presence of specific antibodies IgG to *E. granulosus*. The presence of the antibodies in newborn infants cannot be held as proof of *E. granulosus* invasion.

A negative result in the Vitrotest Echinococcus granulosus IgG test kit indicates either the absence of IgG antibodies to *E. granulosus* in the sample tested, or that the concentration of specific antibodies is below the detection threshold of the test. That is a negative result does not rule out echinococcosis .

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 to antigens of other worms. In addition, the results of patients with cancer and immune system disorders should be interpreted with care.

After successful surgical removal of the cyst, the level of specific antibodies begins to decrease after 4-6 months.

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 or deionized water 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, prepared 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

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Catalogue number


Consult instructions for use

In vitro diagnostic medical device


Manufacturer


Caution


Contains sufficient for <*n*> tests


Temperature limit

Batch code


Use-by date


Date of manufacture


Keep away from sunlight


CE mark

Inst_Echinococcus_EL066-96_V02_ENG
Edition 2nd, 09.02.2022

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Vitrotest Echinococcus granulosus IgG

ASSAY PROCEDURE

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

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

Dispense 10 µl of controls and samples into the wells in the following order:
A1 – CONTROL +,
B1, C1 – CONTROL CUT-OFF,
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 (300 µl per well)

Add 100 µl 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 (300 µl per well)

Add 100 µl of TMB SOLUTION into the wells

Incubate for 15 min in the dark at 18-25 °C

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

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

CALCULATION

$$CO = (OD_{CONTROL\ CUT\text{-}OFF\ 1} + OD_{CONTROL\ CUT\text{-}OFF\ 2})/2;$$

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

INTERPRETATION

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