ТОВ «ХЕМА» код ЄДРПОУ 36038442
Адреса 03179, м. Київ, вул. Академіка Єфремова, 23
Для кореспонденції: 03179, а/с 49
З питань замовлення продукції: 050-422-62-16, 067-422-62-16
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STATEMENT

We, XEMA LLC, as a manufacturer of in vitro diagnostic medical devices, having a registered office at Akademika Yefremova St. 23, Kyiv, Ukraine assign SRL SANMEDICO having a registered office at A. Corobceanu Street 7A, apt. 9, Chişinău MD-2012, Moldova, as authorized representative in correspondence with legislative requirements of the Republic of 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.

This Statement shall come into force on the date of its signing. The duration of this Statement is 3 years from the date of signing.

Date: 06.09.2023

Signature:

Director Xema LLC
Oleksandra Zavaliei

Digitally signed by Dolgopol Iulian
Date: 2025.03.04 10:51:17 EET
Reason: MoldSign Signature
Location: Moldova
MOLDOVA EUROPEANĂ

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Vertretung und Repräsentanz

Certificate

Of Marketing Authorization of Medical Product

within Germany, the member states of the European Union
and the other states having a contractual agreement with the European Economic Area

Nr. AR/IVD/XEMA LLC/01/2023

Issued on the basis of the Declaration of conformity and registration taking into account account Article 11 of Regulation (EU) 2017/746 (IVDR) on In Vitro Diagnostic, and Medical Device Implementing Act (MPDG)

Ausgestellt auf Grund der Konformitätserklärung und Registrierung unter Berücksichtigung der der Verordnung (EU) 2017/746 (IVDR) über In-vitro-Diagnostika und Medizinprodukterecht-Durchführungsgesetz (MPDG)

Manufacturer / Hersteller	**XEMA LLC** **SRN: UA-MF-000032959** UKRAINE, 03179 KYIV Akademika Yefremova St. 23 qa@xema.com.ua; www.xema.in.ua
Product name / Produkt	**See annex to the Certificate** Siehe Anhang zum Zertifikat
Product Classification: Produktklassifizierung	**In Vitro Diagnostic Medical Devices** In-vitro-Diagnostikum (IVD) Medizinprodukte
Category: Kategorie	**Common/ Other IVD** Sonstige IVD-Produkte
Conformity assessment procedure: Konformitatsbewertungsverfahren:	**EC DECLARATION OF CONFORMITY** **(Annex III, except point 6, Directive 98/79/EC)** **in connection with article 110(3) IVDR** **EU- KONFORMITATSERKLARUNG** (Anhang III, außer Nummer 6, Richtlinie 98/79 / EG) in Verbindung mit Artikel 110 (3) IVDR
State Competent Authority: Staatliche Zuständige Behörde	**BfArM** Federal Institute for Drugs and Medical Devices DMIDS (German Medical Device Information and Database System) **BfArM** Das Bundesinstitut für Arzneimittel und Medizinprodukte DMIDS (Deutsches Medizinprodukte-Informations- und Datenbanksystem)

Date of issue : **2023-03-07**
Das Ausstellungsdatum

Valid to : **2025-05-31**
Gültig bis

Represented in the EC by:

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Valid with the Extract from the database www.dimdi.de (German Medical Device Information and Database System (DMIDS))
Gilt nur mit :Auszug aus der Datenbank www.dimdi.de (Deutsches Medizinprodukte-Informations- und Datenbanksystem (DMIDS))

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Vertretung und Repräsentanz

Annex to the Certificate No.:

Anhang zum Zertifikat Nr.:

AR/IVD/XEMA LLC/01/2023

The following medical devices can be placed on the market in the Federal Republic of Germany, in the member states of the European Economic Community (EEC) and in the other contract states of the agreement about the European Economic Area.

Die folgenden Medizinprodukte in der Bundesrepublik Deutschland, in den Mitgliedsstaaten der Europäischen Wirtschaftsgemeinschaft (EG) und in den Vertragsstaaten der EG in den Verkehr gebracht werden dürfen.

#	Nomenclature term Nomenklaturbezeichnung	Catalog No. Katalog-Nr.	Name of device Produktbezeichnung	DMIDS Registration number Registriernummer
1.	ASPERGILLUS	K021	GalMAg EIA	**DE/CA64/00115824**
2.	HSV IgG	K104	HSV 1/2 IgG EIA	**DE/CA64/00115826**
3.	HSV IgM	K104M	HSV 1, 2 IgM EIA	**DE/CA64/00115833**
4.	HSV 2 IgG	K104B	HSV 2 IgG EIA	**DE/CA64/00115836**
5.	MYCOPLASMA ANTIBODY ASSAYS	K106	Mycoplasma IgG EIA	**DE/CA64/00115837**
6.	SYPHILIS ANTIBODY ASSAYS TOTAL	K111	anti-Treponema pallidum EIA	**DE/CA64/00115839**
7.	SYPHILIS ANTIBODY IGG	K111G	Treponema pallidum IgG EIA	**DE/CA64/00115840**
8.	H. PYLORI ANTIBODY ASSAYS	K119G	Helicobacter pylori IgG EIA	**DE/CA64/00115850**
9.	OTHER OTHER BACTERIOLOGY IMMUNOASSAY	K126	Ureaplasma IgG EIA	**DE/CA64/00115851**
10.	THYROID PEROXIDASE (INCL. MICROSOMAL) ANTIBODIES	K131	aTPO EIA	**DE/CA64/00115852**
11.	THYROGLOBULIN AUTOANTIBODIES	K132	aTG EIA	**DE/CA64/00115853**
12.	MPO ANCA	K133	aMPO EIA	**DE/CA64/00115854**
13.	TISSUE TRANSGLUTAMINASE ANTIBODIES	K160 K161	anti-TGlu IgG EIA anti-TGlu IgA EIA	**DE/CA64/00115855**
14.	GIARDIA LAMBLIA	K171	anti-Giardia lamblia EIA	**DE/CA64/00115856**
15.	OTHER PARASITOLOGY	K174	Ascaris IgG EIA	**DE/CA64/00115857**
16.	ECHINOCOCCUS	K175	Echinococcus IgG EIA	**DE/CA64/00115858**
17.	DISTOMATOSIS	K176	Opisthorchis IgG EIA	**DE/CA64/00115859**
18.	GLIADIN ANTIBODIES	K180 K181	Gliadin IgG EIA Gliadin IgA EIA	**DE/CA64/00115860**
19.	IMMUNOGLOBULIN E – TOTAL	K200	Total IgE EIA	**DE/CA64/00115861**
20.	THYROID STIMULATING HORMONE	K201	TSH EIA	**DE/CA64/00115863**
21.	LUTEINISING HORMONE	K202	LH EIA	**DE/CA64/00115864**
22.	FOLLICLE STIMULATING HORMONE	K203	FSH EIA	**DE/CA64/00115865**
23.	HUMAN GROWTH HORMONE	K204	GH EIA	**DE/CA64/00115866**
24.	HUMAN CHORIONIC GONADOTROPIN TOTAL	K205	hCG EIA	**DE/CA64/00115867**
25.	PROLACTIN	K206	Prolactin EIA	**DE/CA64/00115868**

The above-mentioned medical products are marked with the CE symbol.
Die oben genannten medizinischen Produkte sind mit dem CE-Zeichen gekennzeichnet.

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#	Nomenclature term Nomenklaturbezeichnung	Catalog No. Katalog-Nr.	Name of device Produktbezeichnung	DMIDS Registration number Registriernummer
26.	PROGESTERONE	K207	Progesterone EIA	**DE/CA64/00115869**
27.	ESTRADIOL	K208	Estradiol EIA	**DE/CA64/00115870**
28.	TESTOSTERONE (WITH DEHYDRO AND FREE TESTOSTERONE)	K209	Testosterone EIA	**DE/CA64/00115871**
29.	CORTISOL	K210	Cortisol EIA	**DE/CA64/00115872**
30.	TRIIODOTHYRONINE	K211	T3 EIA	**DE/CA64/00115873**
31.	THYROXINE	K212	T4 EIA	**DE/CA64/00115874**
32.	FREE TRIIODOTHYRONINE	K213	fT3 EIA	**DE/CA64/00115875**
33.	FREE THYROXINE	K214	fT4 EIA	**DE/CA64/00115876**
34.	DEHYDRO-EPIANDROSTERONE SULPHATE (INCL. DHEA)	K215	DHEAS EIA	**DE/CA64/00115877**
35.	17 OH PROGESTERONE	K217	17-OH-progesterone EIA	**DE/CA64/00115878**
36.	ESTRIOL	K218	free Estriol EIA	**DE/CA64/00115880**
37.	TESTOSTERONE (WITH DEHYDRO AND FREE TESTOSTERONE)	K219	free Testosterone EIA	**DE/CA64/00115881**
38.	CANCER ANTIGEN 125	K222	CA 125 EIA	**DE/CA64/00115882**
39.	CANCER ANTIGEN 19-9	K223	CA 19-9 EIA	**DE/CA64/00115883**
40.	CARCINOEMBRYONIC ANTIGEN	K224	CEA EIA	**DE/CA64/00115884**
41.	ALPHAFETOPROTEIN	K225	AFP EIA	**DE/CA64/00115885**
42.	CANCER ANTIGEN 15-3	K226	CA 15-3 (M12) EIA	**DE/CA64/00115886**
43.	OTHER OTHER TUMOUR MARKERS	K232	Thyroglobulin EIA	**DE/CA64/00115887**
44.	ß HUMAN CHORIONIC GONADOTROPIN (INCL. SUBUNIT)	K235	free β-HCG EIA	**DE/CA64/00115888**
45.	CYFRA 21-1	K236	CYFRA 21-1 EIA	**DE/CA64/00115889**
46.	SQUAMOUS CELL CARCINOMA ANTIGEN	K237	SCC (A) EIA	**DE/CA64/00115890**
47.	PREGNANCY ASSOCIATED PLASMA PROTEIN - A (DOWNS)	K238	PAPP-A EIA	**DE/CA64/00115892**
48.	OTHER OTHER TUMOUR MARKERS	K239	HE4 EIA	**DE/CA64/00115893**
49.	CANCER ANTIGEN 242	K243	CA242 EIA	**DE/CA64/00115894**
50.	OTHER PREGNANCY TESTING HORMONES	K245	AMH EIA	**DE/CA64/00115896**

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#	Nomenclature term Nomenklaturbezeichnung	Catalog No. Katalog-Nr.	Name of device Produktbezeichnung	DMIDS Registration number Registriernummer
51.	HUMAN PLACENTAL LACTOGEN HPL	K246	Placental lactogen EIA	DE/CA64/00115897
52.	C-REACTIVE PROTEIN	K250	CRP EIA	DE/CA64/00115898
53.	C-PEPTIDE	K267C	C-peptide EIA	DE/CA64/00115900
54.	INSULIN	K267N	Insulin EIA	DE/CA64/00115901
55.	SEX HORMONE BINDING GLOBULIN	K268	SHBG EIA	DE/CA64/00115902
56.	TROPONIN (T + I)	K291	Troponin I EIA	DE/CA64/00115903
57.	LYME ANTIBODY IGG	K118G	Borelia burgdorferi IgG EIA	DE/CA64/00115904
58.	LYME ANTIBODY IGM	K118M	Borelia burgdorferi IgM EIA	DE/CA64/00115905
59.	EBV ANTIBODIES	K108V K108VM K108N	Epstein-Barr virus VCA IgG EIA Epstein-Barr virus VCA IgM EIA Epstein-Barr virus EBNA IgG EIA	DE/CA64/00115906

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Date: **March 07, 2023**

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UkrMedCert

8О047
Сертифікація
систем менеджменту

CERTIFICATE

on compliance of Quality Management System

Registration Date:
August 02, 2024
No. UA.SM.214-21
Expiry Date: August 01, 2027
First edition: August 04, 2021

THIS IS TO CERTIFY THAT
QUALITY MANAGEMENT SYSTEM CONCERNING

The Design and Development, Manufacture, Storage and Distribution medical devices for in vitro diagnostics

was implemented by: XEMA LLC
at the address: Akademika Yefremova St. 23, Kyiv, Ukraine, 03179

meets the requirements of DSTU EN ISO 13485:2018 (EN ISO 13485:2016, IDT; ISO 13485:2016, IDT); ISO 13485:2016.

Compliance control of the certified quality management system with the requirements of the specified standard is carried out through supervision, the frequency and procedures of which are regulated by the procedures of the conformity assessment body.

The conformity assessment body UKRMEDCERT LLC, address: str. Drahomanova, building 1-A, office 2, Kyiv, 02059, Ukraine, phone: +38-067-595-02-30, https://ukrmedcert.org.ua

Head of CAB **Tetiana SUKHENKO**

The validity of a certificate of compliance can be verified in the online Register https://ukrmedcert.org.ua or by phone +38-067-595-02-30.
The original version of this Certificate is issued in Ukrainian.

Instruction for use
A solid-phase enzyme immunoassay kit
for the qualitative detection of IgG antibodies
to *Borelia burgdorferi sensu lato*
in human serum or plasma

Borelia burgdorferi IgG EIA

Catalogue number REF **K118G**

Σ 96 For 96 determinations

IVD *In vitro* diagnostic medical device

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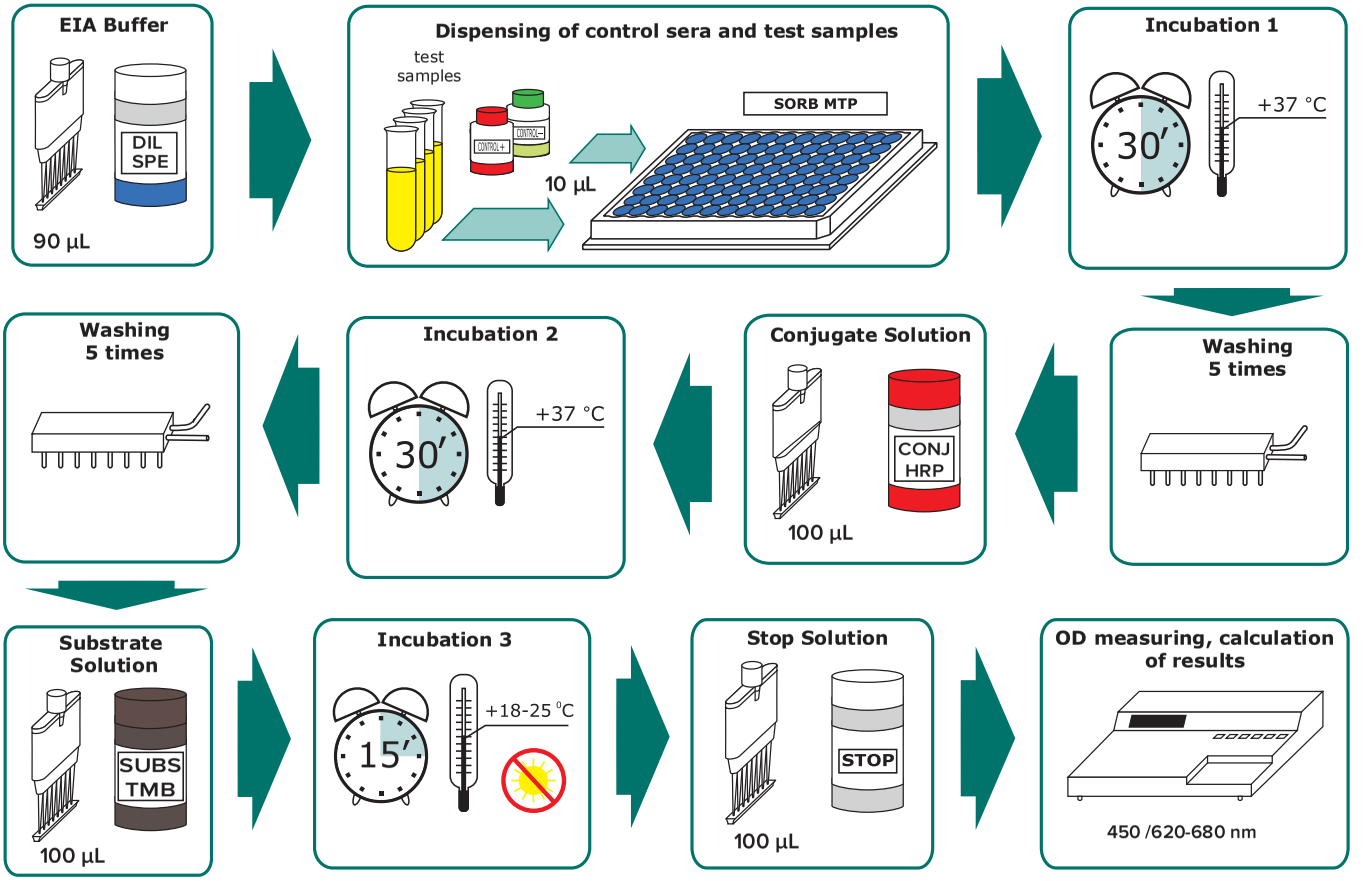
ASSAY PROCEDURE



During performing several independent series of tests, Positive and Negative Control Serum should be used **each time**.

CONTENT

Instruction for use
A solid-phase enzyme immunoassay kit for the qualitative detection of IgG antibodies to *Borelia burgdorferi sensu lato* in human serum or plasma

Borelia burgdorferi IgG EIA

1. INTENDED USE

ELISA reagent kit Borelia burgdorferi IgG EIA is a solid-phase enzyme immunoassay for the qualitative detection of IgG antibodies to *Borelia burgdorferi sensu lato* in human serum or plasma.

The field of application is clinical laboratory diagnostics.

2. GENERAL INFORMATION

Borrelia burgdorferi sensu lato - is a group of borreliosis or Lyme disease pathogens, a common infection, the main host and vector of which is the ixodid tick. The disease is transmitted only through a tick bite.

In the early stages of borreliosis, fatigue, chills and headaches may be observed, and later more serious symptoms may occur, such as joint pain, meningitis, numbness in the extremities, facial nerve paralysis, memory disorders, and eye and heart damage. After the spirochete penetrates the skin, a creeping erythema occurs, and after several days or weeks, it reaches many organs by haematogenous or lymphatic means. In general, the incubation period is from 3 to 45 days.

Early diagnosis of the disease is based on clinical and epidemiological data. The diagnosis is confirmed by laboratory, usually by serological methods - the detection of specific antibodies to *Borrelia burgdorferi* in the blood.

IgM antibodies appear in the blood first, a few days after infection, but can be detected by laboratory tests in 2-3 weeks. After about 6 weeks, the concentration of antibodies reaches a maximum and then gradually decreases. IgG antibodies begin to be detected 4-6 weeks after infection and the maximum amount of IgG antibodies is synthesised 2-3 months after the onset of early symptoms of the disease. Then their number gradually decreases, but they remain in the body for several years.

3. TEST PRINCIPLE

The detection of IgG antibodies to *Borrelia burgdorferi* is based on the indirect enzyme immunoassay principle. On the inner surface of the microplate wells are immobilized recombinant *Borrelia burgdorferi* antigen. The analysis procedure includes three stages of incubation:

- during the first stage specific to *Borrelia burgdorferi* antibodies from the specimen are bound onto the microwell surface;
- during the second stage horseradish peroxidase-conjugated specific monoclonal anti-IgG antibodies bind to the antigen-antibody complexes, fixed in the formed at the previous stage complexes;
- during the third stage, the complexes formed due to the reaction with the chromogen 3,3',5,5'-tetramethylbenzidine are visualized.

After stopping the reaction with a stop solution, the intensity of the color of the microwells is measured. The optical density (OD) in the microwell is directly related to the concentration of the measured IgG antibodies to *Borrelia burgdorferi* in test specimen.

4. KIT COMPONENTS

Code of component	Symbol	Name	Volume	Qty, pcs.	Description
P118GZ	SORB MTP	**Microplate**	-	1	96-well polystyrene strip microplate coated with recombinant antigen of *Borrelia burgdorferi*, ready to use
CN118GZ	CONTROL -	**Negative Control Serum K-**	0.5 mL	1	Solution based on human serum, free of IgG antibodies to *Borrelia burgdorferi*, with preservative, ready to use (yellow liquid)
CP118GZ	CONTROL +	**Positive Control Serum K+**	0.2 mL	1	Solution based on human serum, containing of IgG antibodies to *Borrelia burgdorferi*, with preservative, ready to use (red liquid)
T118GZ	CONJ HRP	**Conjugate Solution**	12 mL	1	Solution of monocnoclonal antibodies to IgG conjugated to the horseradish peroxidase, ready to use (red liquid)
SP118GZ	DIL SPE	**EIA Buffer**	12 mL	1	Buffer solution with detergent and preservative, ready to use (purple liquid)
R055Z	SUBS TMB	**Substrate Solution**	12 mL	1	Tetramethylbenzidine (TMB) substrate solution, ready to use (colourless liquid)
S008Z	BUF WASH 26X	**26x Concentrate Washing Solution**	30 mL	1	Buffer solution with detergent, 26x concentrate (colourless liquid)
R050Z	STOP	**Stop Solution**	12 mL	1	5.0% solution of sulphuric acid, ready to use (colourless liquid)
The kit also includes instruction for use, quality control data sheet and plate sealing tape (2 pcs).					

5. EQUIPMENT AND MATERIAL REQUIRED BUT NOT PROVIDED

– microplate photometer with 450\620-680 nm wavelength;
– dry thermostat for +37°C±1°C;
– automatic plate washer (optional);
– micropipettes with variable volume, range volume 5-1000 µL;
– graduated cylinder of 1000 mL capacity;
– distilled or deionized water;
– timer;
– vortex mixer;
– disposable gloves;
– absorbent paper.

6. WARNING AND PRECAUTIONS

In order to prevent incorrect results, strictly follow the recommended order and duration of the analysis procedure.

6.1. The kit is for *in vitro* diagnostic use only. For professional laboratory use.

6.2. Follow the rules mentioned below during the kit using:

- do not use kit beyond expire date;
- do not use the kit if its packaging is damaged;
- in order to avoid contamination, use new tips to pipette samples and reagents;
- use only verified equipment;
- close each vial with its own cap, after using the reagent;
- do not use components of other kits or reagents of other manufacturers;
- do not let wells dry after completing the rinsing step; immediately proceed to the next stage;
- avoid bubbles when adding reagents.

ATTENTION! The TMB substrate solution is light sensitive. Avoid prolonged exposure of the component to light.

6.3. Some kit components, such as stop solution, substrate solution, and washing solution, may cause toxic or irritant effects. If they get on the skin or mucosa, the affected area should be washed with plenty of running water.

6.4. All human products, including patient samples, should be considered potentially infectious. Handling and disposal should be in accordance with the procedures defined by an appropriate national biohazard safety guidelines or regulations.

6.5. The positive and negative control serum included in the kit are negative for antibodies to HIV 1,2, hepatitis C virus and HBsAg, but the reagents should be considered as potentially infectious material and handled carefully.

6.6. Specimens must not contain any azide compounds, as they inhibit activity of peroxidase.

6.7. Wear protective gloves, protective clothing, eye protection, face protection.

6.8. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.

6.9. Safety Data Sheet for this product is available upon request directly from XEMA LLC.

6.10. Serious incidents related to the kit must be reported to the manufacturer, Authorized Representative, and to the Competent Authority of the EU member state(s) where the incident has occurred.

7. SPECIMEN COLLECTION, TRANSPORTATION AND STORAGE OF SAMPLES

7.1. Blood sampling should be carried out from the cubital vein with a disposable needle using a vacuum blood sampling system. Serum or plasma specimens should be clearly labeled and identified. Serum must be separated from the clot as early as possible to avoid hemolysis of red blood cells. If there are any visible particles in the sample, they should be removed by centrifugation at 3000-5000 rpm for 20 minutes at room temperature or by filtration.

Don't use samples with high lipidemia, hemolysis as they may give false test results.

7.2. Specimen should be stored at +2…+8°C up to 3 days. Specimen held for a longer time, should be placed in a freezer at -15°C or below, avoid no more than three cycles of thawing-freezing samples.

7.3. For the transportation of samples, it is recommended to use triple packaging. The primary package is the labeled tube containing the sample. Secondary packaging is a polyethylene bag that is hermetically closed with a zip-lock. The outer packaging is a heat-insulating container, while the secondary packaging is placed in the outer packaging for transportation in the center of the thermal container. Frozen refrigerants are placed on the bottom, along the side walls of the thermal container, and cover the samples with them.

8. TRANSPORTATION AND STORAGE TERMS OF KIT, WASTE DISPOSAL

Information about the singularity storage conditions, transportation of the kit, and disposal of waste should be taken into account by all persons who participate in these processes.

8.1. Transportation

The Borrelia burgdorferi IgG EIA kit should be transported in the manufacturer's packaging at +2...+8°C. Single transportation at the temperature up to 25ºC for 5 days is acceptable.

8.2. Storage

The Borrelia burgdorferi IgG EIA kit should be stored in the manufacturer's packaging at +2...+8°C. Do not freeze.

The kit contains reagents sufficient for 96 determinations including Positive and Negative Control Serum.

Once opened test-kit is stable for 2 months when stored properly as intended by manufacturer at 2-8°C.

In case of partial use of the kit, the components should be stored in the following way:

- the remaining strips should be immediately resealed in the bag along with the silica gel, closed with the zip-lock, and stored at +2...+8°C within 2 months;
- EIA Buffer, Substrate Solution, Stop Solution, and Washing Solution concentrate after opening the vial, can be stored tightly closed at +2...+8°C until the kit's shelf life;
- Conjugate Solution, Positive and Negative Control Serum after opening the vial, can be stored tightly closed at +2...+8°C within 2 months;
- diluted Washing Solution can be stored at room temperature (+18…+25°C) for up to 5 days or at +2…+8°C for up to 14 days.

Kits that were stored in violation of the storage condition cannot be used.

8.3. Disposal

Expired kit components, used reagents and materials, as well as residual samples must be inactivated and disposed of in accordance with legal requirements.

9. REAGENTS PREPARATION

9.1. All reagents (including microstrips) and test samples should be allowed to reach room temperature (+18…+25 °C) for at least 30 minutes before use.

9.2. Microplate preparation

Open the package with the microplate and install the required number of strips into the frame. The remaining strips should be immediately resealed in the bag along with the silica gel and closed with the zip-lock to prevent moisture from affecting the plate's strips.

9.3. Washing solution preparation

Add the contents of the 30 mL washing solution concentrate vial to 750 mL of distilled or deionized water and mix thoroughly. In case of partial use of the kit, take the necessary amount of washing solution concentrate and dilute it 26 times with distilled or deionized water.

The spending of the components in case of partial use of the kit is given in the table:

Quantity of strips	1	2	3	4	5	6	7	8	9	10	11	12
Volume of the washing solution concentrate, mL	2.5	5	7.5	10	12.5	15	17.5	20	22.5	25	27.5	30
Volume of water, mL	62.5	125	187.5	250	312.5	375	437.5	500	562.5	625	687.5	750

10. ASSAY PROCEDURE

10.1. Put the desired number of strips into the frame based on the number of test samples and 4 wells for Positive and Negative Control Serum (1 well for Positive Control (CP) and 3 wells for Negative Control Serum (CN)).

10.2. Dispense **90 µL of EIA Buffer** to all wells.

10.3. Dispense **10 µL of Positive and Negative Control Serum as well as 10 µL of test serum/plasma samples** (SAMP) to the wells of the microplate according to the scheme below. The introduction of Positive and Negative Control Serum and test samples should be carried out within 5 minutes to ensure equal incubation time for the first and last samples.

NOTE: during performing several independent series of tests, Positive and Negative Control Serum should be used each time.

Scheme of introduction of samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	CP	SAMP5	SAMP13	SAMP21								
B	CN	SAMP6	SAMP14	SAMP22								
C	CN	SAMP7	SAMP15	SAMP23								
D	CN	SAMP8	SAMP16									
E	SAMP1	SAMP9	SAMP17									
F	SAMP2	SAMP10	SAMP18									
G	SAMP3	SAMP11	SAMP19									
H	SAMP4	SAMP12	SAMP20									

10.4. Carefully mix the contents of the microplate in a circular motion on a horizontal surface, cover strips with a plate sealing tape and incubate for **30 minutes at +37°C**.

10.5. At the end of the incubation period, remove and discard the plate cover. Aspirate and wash each well **5 times** using an automatic washer or an 8-channel dispenser. For each washing, add 300 µL of Washing Solution (see 9.3) to all wells, then remove the liquid by aspiration or decantation. The residual volume of the Washing Solution after each aspiration or decantation should be no more than 5 µL. After washing, carefully remove the remaining liquid from the wells on the absorbent paper. For the automatic washer/analyzer, the Washing Solution volume can be increased to 350 µL.

10.6. Add **100 µL of Conjugate Solution** to all wells.

10.7. Cover strips with a plate sealing tape and incubate for **30 minutes at +37°C**.

10.8. At the end of the incubation period, aspirate and wash each well **5 times** as described in 10.5.

10.9. Add **100 µL of Substrate Solution** to all wells. The introduction of the Substrate Solution into the wells must be carried out within 2-3 minutes. Incubate the microplate in the dark **at room temperature (+18...+25°C) for 15 minutes**.

10.10. Add **100 µL of Stop Solution** to all wells in the same order as the Substrate Solution. After adding the Stop Solution, the contents of the wells turn yellow.

10.11. Read the optical density (OD) of the wells at 450 nm and reference light filters 620–680 nm using a microplate photometer within 5 minutes of adding the stop solution. Set photometer blank on air.

11. TEST VALIDITY AND CALCULATION OF RESULTS

11.1. The test results are valid only if Positive and Negative Control Serum are within the specified ranges and if all other test parameters are also within the given assay specifications, namely:

- OD of CONTROL- < 0.15;
- OD of CONTROL+ > 1.5;
- OD(CN) x 0,5 < OD(CN) < OD(CN) x 2.

11.2. Calculate the mean OD value of the Negative Control Serum:

meanOD(CN) = (OD1(CN) + OD2(CN) + OD3(CN))/3

11.3. Calculate the Cut Off value by adding to the mean OD value of the Negative Control Serum the coeficient 0.3.

Cut off = meanOD(CN) + 0.3

11.4. Calculate Positivity Index (PI) for each sample by dividing the OD of the sample by Cut off value:

PI = ODsample/Cut off

12. INTERPRETATION OF THE RESULTS

If PI value > 1.1 the result is **POSITIVE**,
If PI value is between 0.9 and 1.1 the result is **EQUIVOCAL**,
If PI value < 0.9 the result is **NEGATIVE**.

If equivocal results are obtained, it is recommended to conduct a reexamination of the sample in several replicates. If the result is equivocal again, a new sample should be obtained within 5-7 days and retested. If the result remains equivocal, the sample should be considered negative.

13. PERFORMANCE CHARACTERISTICS

13.1. Analytical performance characteristics

13.1.1. Precision of Measurement

Reproducibility (Intra assay repeatability) was determined by evaluation the coefficient of variation (CV) for 2 different samples, with different levels of IgG antibodies to the *Borrelia burgdorferi sensu lato* antigen, during 1 day in 43 replicates on one series of ELISA kit.

№ serum	mean OD	mean PI	CV PI, %
1	0.31	1.02	8.29
2	1.08	3.59	6.65

Reproducibility (Inter assay reproducibility) was determined by evaluating the coefficients of variation (CV) for 2 samples of each serum for 4 days in 8-replicate determinations.

№ serum	mean OD	mean PI	CV PI, %
1	0.27	0.9	8.1
2	1.1	3.66	6.8

13.1.2. Analytical specificity

For the analysis result is not affected by the presence in the sample of bilirubin in a concentration of up to 0.21 mg/mL, hemoglobin in a concentration of up to 10 mg/mL and triglycerides in a concentration of up to 10 mg/mL.

13.2. Diagnostic performance characteristics

The clinical sensitivity and specificity of the assay were evaluated using a serum panel with 8 positive and 8 negative clinical serum samples and were 100%. The relative sensitivity and specificity of the assay were investigated in a sample of 96 donor sera characterised for the content of IgG antibodies to *Borrelia burgdorferi* antigen in commercical Kits, and the ressults were 99.7% and 97.5%, respectively.

14. LIMITATIONS

A positive result is evidence of the presence of IgG antibodies to *Borrelia burgdorferi sensu lato* antigen. The diagnosis cannot be based on the results of an IgG antibody test to *Borrelia burgdorferi* alone and requires confirmation, including an assessment of the patient's clinical presentation and history, the detection of IgM antibodies to *Borrelia burgdorferi* and conducting an immunoblot test.

A negative result indicates the absence of IgG antibodies to *Borrelia burgdorferi sensu lato* or antibody levels below the limit of sensitivity of the kit.

The results of serum tests in patients with immunosuppression and immunological disorders should be interpreted with caution.

15. REFERENCES

1. Lyme Borreliosis (Lyme disease). In: International travel and health. Geneva: World Health Organization; 2014.
2. M Cinco 1, R Murgia, M Ruscio, B Andriolo. IgM and IgG significant reactivity to Borrelia burgdorferi sensu stricto, Borrelia garinii and Borrelia afzelii among Italian patients affected by Lyme arthritis or neuroborreliosis. FEMS Immunol Med Microbiol . 1996 Jun;14(2-3):159-66. doi: 10.1111/j.1574-695X.1996.tb00283.x.
3. Наказ МОЗ України №325 від 08.06.2015 «Про затвердження Державних санітарно-протиепідемічних правил і норм щодо поводження з медичними відходами».
4. Постанова КМУ від 02 жовтня 2013р. №754 «Про затвердження технічного регламенту щодо медичних виробів для діагностики in vitro».
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SAMPLES IDENTIFICATION PLAN

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
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LOT _____ DATE _____

SAMPLES IDENTIFICATION PLAN

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A												
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LOT _____ DATE _____

	Manufacturer
IVD	*In vitro* diagnistic medical device
REF	Catalogue number
	Use-by date
LOT	Batch code
	Temperature limit
	Contains sufficient for <n> tests
	Caution
	Consult instructions for use
	Conformity Marking with technical regulations in Ukraine
EC REP	Authorized representative in the European Community/European Union
CE	CE Conformity Marking

For any issues related to operation of the kit and technical support, please contact by telefon number

+38 044 294-69-78

or write to:

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Instruction for use
A solid-phase enzyme immunoassay kit
for the qualitative detection of IgM antibodies
to *Borelia burgdorferi sensu lato*
in human serum or plasma

Borelia burgdorferi IgM EIA

Catalogue number REF **K118G**

Σ 96 For 96 determinations

IVD *In vitro* diagnostic medical device

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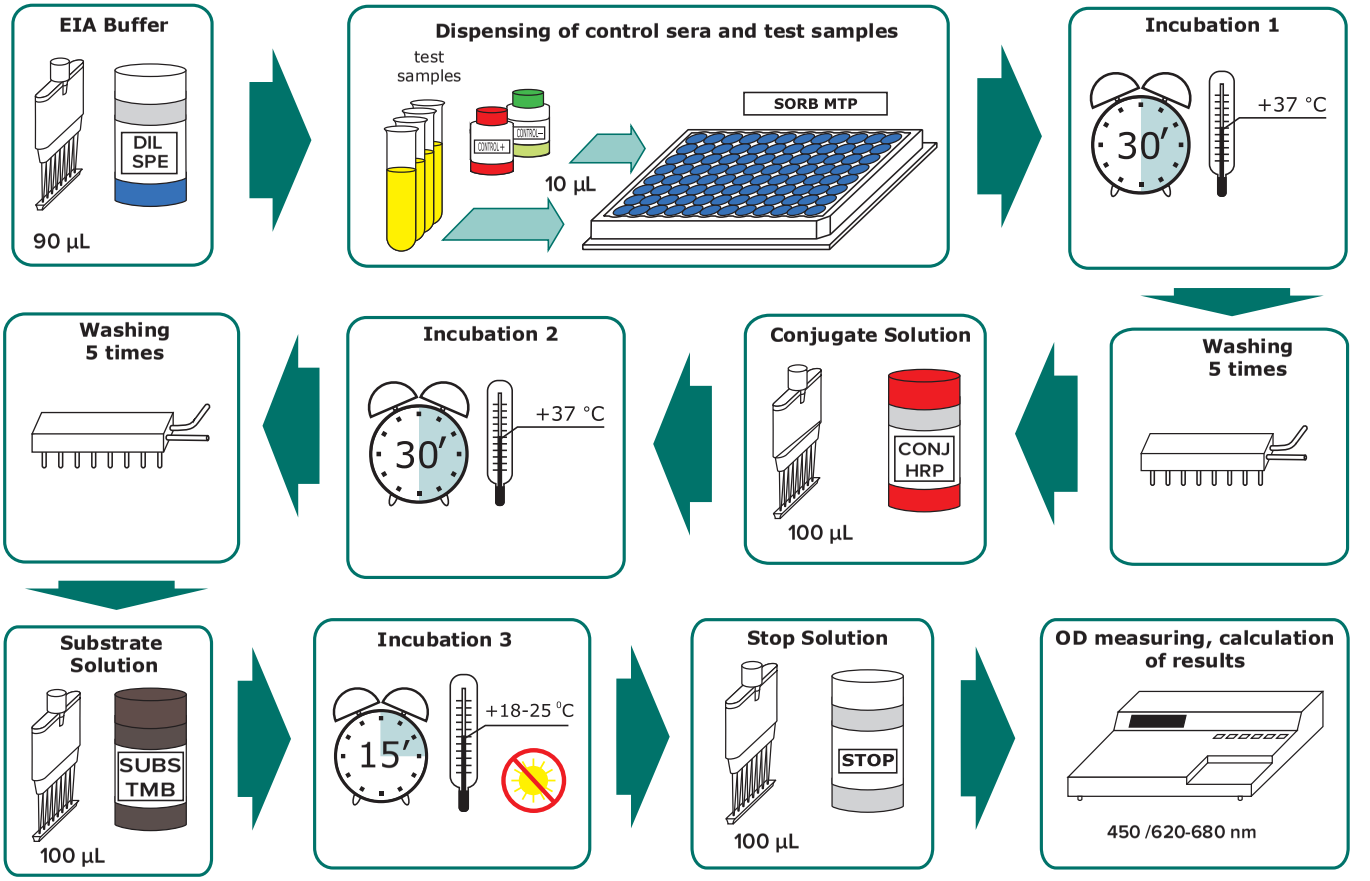
ASSAY PROCEDURE



During performing several independent series of tests, Positive and Negative Control Serum should be used **each time**.

CONTENT

Instruction for use
A solid-phase enzyme immunoassay kit for the qualitative detection of IgM antibodies to *Borelia burgdorferi sensu lato* in human serum or plasma

Borelia burgdorferi IgM EIA

1. INTENDED USE

ELISA reagent kit Borelia burgdorferi IgM EIA is a solid-phase enzyme immunoassay for the qualitative detection of IgM antibodies to *Borelia burgdorferi sensu lato* in human serum or plasma.

The field of application is clinical laboratory diagnostics.

2. GENERAL INFORMATION

Borrelia burgdorferi sensu lato - is a group of borreliosis or Lyme disease pathogens, a common infection, the main host and vector of which is the ixodid tick. The disease is transmitted only through a tick bite.

In the early stages of borreliosis, fatigue, chills and headaches may be observed, and later more serious symptoms may occur, such as joint pain, meningitis, numbness in the extremities, facial nerve paralysis, memory disorders, and eye and heart damage. After the spirochete penetrates the skin, a creeping erythema occurs, and after several days or weeks, it reaches many organs by haematogenous or lymphatic means. In general, the incubation period is from 3 to 45 days.

Early diagnosis of the disease is based on clinical and epidemiological data. The diagnosis is confirmed by laboratory, usually by serological methods - the detection of specific antibodies to *Borrelia burgdorferi* in the blood.

IgM antibodies appear in the blood first, a few days after infection, but can be detected by laboratory tests in 2-3 weeks. After about 6 weeks, the concentration of antibodies reaches a maximum and then gradually decreases. IgG antibodies begin to be detected 4-6 weeks after infection and the maximum amount of IgG antibodies is synthesised 2-3 months after the onset of early symptoms of the disease. Then their number gradually decreases, but they remain in the body for several years.

3. TEST PRINCIPLE

The detection of IgM antibodies to *Borrelia burgdorferi* is based on the indirect enzyme immunoassay principle. On the inner surface of the microplate wells are immobilized recombinant *Borrelia burgdorferi* antigen. The analysis procedure includes three stages of incubation:

- during the first stage specific to *Borrelia burgdorferi* antibodies from the specimen are bound onto the microwell surface;
- during the second stage horseradish peroxidase-conjugated specific monoclonal anti-IgM antibodies bind to the antigen-antibody complexes, fixed in the formed at the previous stage complexes;
- during the third stage, the complexes formed due to the reaction with the chromogen 3,3',5,5'-tetramethylbenzidine are visualized.

After stopping the reaction with a stop solution, the intensity of the color of the microwells is measured. The optical density (OD) in the microwell is directly related to the concentration of the measured IgM antibodies to *Borrelia burgdorferi* in test specimen.

4. KIT COMPONENTS

Code of component	Symbol	Name	Volume	Qty, pcs.	Description
P118MZ	SORB MTP	**Microplate**	-	1	96-well polystyrene strip microplate coated with recombinant antigen of *Borrelia burgdorferi*, ready to use
CN118MZ	CONTROL -	**Negative Control Serum K-**	0.5 mL	1	Solution based on human serum, free of IgM antibodies to *Borrelia burgdorferi*, with preservative, ready to use (yellow liquid)
CP118MZ	CONTROL +	**Positive Control Serum K+**	0.2 mL	1	Solution based on human serum, containing of IgM antibodies to *Borrelia burgdorferi*, with preservative, ready to use (red liquid)
T118MZ	CONJ HRP	**Conjugate Solution**	12 mL	1	Solution of monocnoclonal antibodies to IgM conjugated to the horseradish peroxidase, ready to use (red liquid)
SP118MZ	DIL SPE	**EIA Buffer**	12 mL	1	Buffer solution with detergent and preservative, ready to use (purple liquid)
R055Z	SUBS TMB	**Substrate Solution**	12 mL	1	Tetramethylbenzidine (TMB) substrate solution, ready to use (colourless liquid)
S008Z	BUF WASH 26X	**26x Concentrate Washing Solution**	30 mL	1	Buffer solution with detergent, 26x concentrate (colourless liquid)
R050Z	STOP	**Stop Solution**	12 mL	1	5.0% solution of sulphuric acid, ready to use (colourless liquid)
The kit also includes instruction for use, quality control data sheet and plate sealing tape (2 pcs).					

5. EQUIPMENT AND MATERIAL REQUIRED BUT NOT PROVIDED

– microplate photometer with 450/620-680 nm wavelength;
– dry thermostat for +37°C±1°C;
– automatic plate washer (optional);
– micropipettes with variable volume, range volume 5-1000 µL;
– graduated cylinder of 1000 mL capacity;
– distilled or deionized water;
– timer;
– vortex mixer;
– disposable gloves;
– absorbent paper.

6. WARNING AND PRECAUTIONS

In order to prevent incorrect results, strictly follow the recommended order and duration of the analysis procedure.

6.1. The kit is for *in vitro* diagnostic use only. For professional laboratory use.

6.2. Follow the rules mentioned below during the kit using:

- do not use kit beyond expire date;
- do not use the kit if its packaging is damaged;
- in order to avoid contamination, use new tips to pipette samples and reagents;
- use only verified equipment;
- close each vial with its own cap, after using the reagent;
- do not use components of other kits or reagents of other manufacturers;
- do not let wells dry after completing the rinsing step; immediately proceed to the next stage;
- avoid bubbles when adding reagents.

ATTENTION! The TMB substrate solution is light sensitive. Avoid prolonged exposure of the component to light.

6.3. Some kit components, such as stop solution, substrate solution, and washing solution, may cause toxic or irritant effects. If they get on the skin or mucosa, the affected area should be washed with plenty of running water.

6.4. All human products, including patient samples, should be considered potentially infectious. Handling and disposal should be in accordance with the procedures defined by an appropriate national biohazard safety guidelines or regulations.

6.5. The positive and negative control serum included in the kit are negative for antibodies to HIV 1,2, hepatitis C virus and HBsAg, but the reagents should be considered as potentially infectious material and handled carefully.

6.6. Specimens must not contain any azide compounds, as they inhibit activity of peroxidase.

6.7. Wear protective gloves, protective clothing, eye protection, face protection.

6.8. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.

6.9. Safety Data Sheet for this product is available upon request directly from XEMA LLC.

6.10. Serious incidents related to the kit must be reported to the manufacturer, Authorized Representative, and to the Competent Authority of the EU member state(s) where the incident has occurred.

7. SPECIMEN COLLECTION, TRANSPORTATION AND STORAGE OF SAMPLES

7.1. Blood sampling should be carried out from the cubital vein with a disposable needle using a vacuum blood sampling system. Serum or plasma specimens should be clearly labeled and identified. Serum must be separated from the clot as early as possible to avoid hemolysis of red blood cells. If there are any visible particles in the sample, they should be removed by centrifugation at 3000-5000 rpm for 20 minutes at room temperature or by filtration.

Don't use samples with high lipidemia, hemolysis as they may give false test results.

7.2. Specimen should be stored at +2…+8°C up to 3 days. Specimen held for a longer time, should be placed in a freezer at -15°C or below, avoid no more than three cycles of thawing-freezing samples.

7.3. For the transportation of samples, it is recommended to use triple packaging. The primary package is the labeled tube containing the sample. Secondary packaging is a polyethylene bag that is hermetically closed with a zip-lock. The outer packaging is a heat-insulating container, while the secondary packaging is placed in the outer packaging for transportation in the center of the thermal container. Frozen refrigerants are placed on the bottom, along the side walls of the thermal container, and cover the samples with them.

8. TRANSPORTATION AND STORAGE TERMS OF KIT, WASTE DISPOSAL

Information about the singularity storage conditions, transportation of the kit, and disposal of waste should be taken into account by all persons who participate in these processes.

8.1. Transportation

The Borrelia burgdorferi IgM EIA kit should be transported in the manufacturer's packaging at +2...+8°C. Single transportation at the temperature up to 25ºC for 5 days is acceptable.

8.2. Storage

The Borrelia burgdorferi IgM EIA kit should be stored in the manufacturer's packaging at +2...+8°C. Do not freeze.

The kit contains reagents sufficient for 96 determinations including Positive and Negative Control Serum.

Once opened test-kit is stable for 2 months when stored properly as intended by manufacturer at 2-8°C.

In case of partial use of the kit, the components should be stored in the following way:

- the remaining strips should be immediately resealed in the bag along with the silica gel, closed with the zip-lock, and stored at +2...+8°C within 2 months;
- EIA Buffer, Substrate Solution, Stop Solution, and Washing Solution concentrate after opening the vial, can be stored tightly closed at +2...+8°C until the kit's shelf life;
- Conjugate Solution, Positive and Negative Control Serum after opening the vial, can be stored tightly closed at +2...+8°C within 2 months;
- diluted Washing Solution can be stored at room temperature (+18…+25°C) for up to 5 days or at +2…+8°C for up to 14 days.

Kits that were stored in violation of the storage condition cannot be used.

8.3. Disposal

Expired kit components, used reagents and materials, as well as residual samples must be inactivated and disposed of in accordance with legal requirements.

9. REAGENTS PREPARATION

9.1. All reagents (including microstrips) and test samples should be allowed to reach room temperature (+18…+25 °C) for at least 30 minutes before use.

9.2. Microplate preparation

Open the package with the microplate and install the required number of strips into the frame. The remaining strips should be immediately resealed in the bag along with the silica gel and closed with the zip-lock to prevent moisture from affecting the plate's strips.

9.3. Washing solution preparation

Add the contents of the 30 mL washing solution concentrate vial to 750 mL of distilled or deionized water and mix thoroughly. In case of partial use of the kit, take the necessary amount of washing solution concentrate and dilute it 26 times with distilled or deionized water.

The spending of the components in case of partial use of the kit is given in the table:

Quantity of strips	1	2	3	4	5	6	7	8	9	10	11	12
Volume of the washing solution concentrate, mL	2.5	5	7.5	10	12.5	15	17.5	20	22.5	25	27.5	30
Volume of water, mL	62.5	125	187.5	250	312.5	375	437.5	500	562.5	625	687.5	750

10. ASSAY PROCEDURE

10.1. Put the desired number of strips into the frame based on the number of test samples and 4 wells for Positive and Negative Control Serum (1 well for Positive Control (CP) and 3 wells for Negative Control Serum (CN)).

10.2. Dispense **90 µL of EIA Buffer** to all wells.

10.3. Dispense **10 µL of Positive and Negative Control Serum as well as 10 µL of test serum/plasma samples** (SAMP) to the wells of the microplate according to the scheme below. The introduction of Positive and Negative Control Serum and test samples should be carried out within 5 minutes to ensure equal incubation time for the first and last samples.

NOTE: during performing several independent series of tests, Positive and Negative Control Serum should be used each time.

Scheme of introduction of samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	CP	SAMP5	SAMP13	SAMP21								
B	CN	SAMP6	SAMP14	SAMP22								
C	CN	SAMP7	SAMP15	SAMP23								
D	CN	SAMP8	SAMP16									
E	SAMP1	SAMP9	SAMP17									
F	SAMP2	SAMP10	SAMP18									
G	SAMP3	SAMP11	SAMP19									
H	SAMP4	SAMP12	SAMP20									

10.4. Carefully mix the contents of the microplate in a circular motion on a horizontal surface, cover strips with a plate sealing tape and incubate for **30 minutes at +37°C**.

10.5. At the end of the incubation period, remove and discard the plate cover. Aspirate and wash each well **5 times** using an automatic washer or an 8-channel dispenser. For each washing, add 300 µL of Washing Solution (see 9.3) to all wells, then remove the liquid by aspiration or decantation. The residual volume of the Washing Solution after each aspiration or decantation should be no more than 5 µL. After washing, carefully remove the remaining liquid from the wells on the absorbent paper. For the automatic washer/analyzer, the Washing Solution volume can be increased to 350 µL.

10.6. Add **100 µL of Conjugate Solution** to all wells.

10.7. Cover strips with a plate sealing tape and incubate for **30 minutes at +37°C**.

10.8. At the end of the incubation period, aspirate and wash each well **5 times** as described in 10.5.

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$$\mathbf{meanOD(CN) = (OD1(CN) + OD2(CN) + OD3(CN))/3}$$

11.3. Calculate the Cut Off value by adding to the mean OD value of the Negative Control Serum the coeficient 0.3.

$$\mathbf{Cut\ off = meanOD(CN) + 0.3}$$

11.4. Calculate Positivity Index (PI) for each sample by dividing the OD of the sample by Cut off value:

$$\mathbf{PI = ODsample/Cut\ off}$$

12. INTERPRETATION OF THE RESULTS

If PI value > 1.1 the result is **POSITIVE**,
If PI value is between 0.9 and 1.1 the result is **EQUIVOCAL**,
If PI value < 0.9 the result is **NEGATIVE**.

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13.1.2. Analytical specificity

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D												
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F												
G												
H												

LOT _____ DATE _____

	Manufacturer
IVD	*In vitro* diagnistic medical device
REF	Catalogue number
	Use-by date
LOT	Batch code
	Temperature limit
	Contains sufficient for <n> tests
	Caution
	Consult instructions for use
	Conformity Marking with technical regulations in Ukraine
EC REP	Authorized representative in the European Community/European Union
CE	CE Conformity Marking

For any issues related to operation of the kit and technical support, please contact by telefon number

+38 044 294-69-78

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Vitrotest®
ELISA kits & components

ТОВ «ВІТРОТЕСТ БІОРЕАГЕНТ"
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Ідентифікаційний код: 42149820
ІПН: 421498226554

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ref. № 187a/24
19.11.2024

STATEMENT

We, **Vitrotest Bioreagent LLC** having a registered office at M.Boychuka 18b, of.56, Kyiv 01103 Ukraine assign SRL Sanmedico, having a registered office at A. Corobceanu street 7A, apt. 9, Chişinău MD-2012, Moldova, as authorized representative in Republic of 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.

Ihor Nikolaienko, Ph.D. _____
Director, Vitrotest Bioreagent LLC

UkrMedCert

8O047
Сертифікація
систем менеджменту

CERTIFICATE

on compliance of Quality Management System

Registration Date:
July 29, 2024
No. UA.SM.211-21
Expiry Date: July 28, 2027
First edition: July 29, 2021

THIS IS TO CERTIFY THAT
QUALITY MANAGEMENT SYSTEM CONCERNING

The Design and Development, Manufacture and Distribution of ELISA test kits for in vitro diagnostics

was implemented by: Vitrotest Bioreagent LLC
at the legal address: Boychuka Myhaila str. 18B, of. 56, Kyiv, 01103, Ukraine
production site address: Kurortna str. 11, Kyiv, 04075, Ukraine

meets the requirements of the standard DSTU EN ISO 13485:2018 (EN ISO 13485:2016, IDT; ISO 13485:2016, IDT).

Compliance control of the certified quality management system with the requirements of the specified standard is carried out through supervision, the frequency and procedures of which are regulated by the procedures of the conformity assessment body.

The conformity assessment body UKRMEDCERT LLC, address: Drahomanova str., 1-A, of. 2, Kyiv, 02059, Ukraine, phone: +38-067-595-02-30, https://ukrmedcert.org.ua

Head of CAB **Tetiana SUKHENKO**

«УКРМЕДСЕРТ» Ідентифікаційний код 34646777

The validity of a certificate of compliance can be verified in the online Register https://ukrmedcert.org.ua or by phone +38-067-595-02-30.
The original version of this Certificate is issued in Ukrainian.

ИНСТРУКЦИЯ ПО ПРИМЕНЕНИЮ

Vitrotest® Lamblia-IgM

Иммуноферментная тест-система для выявления антител класса IgM к *Giardia lamblia (intestinalis)*

TK031
96 анализов

IVD

1. НАЗНАЧЕНИЕ

Иммуноферментная тест-система Vitrotest® Lamblia-IgM предназначена для выявления антител класса IgM к *Giardia lamblia (intestinalis)* в сыворотке или плазме крови человека.

Тест-набор может быть применен как для проведения иммуноферментного анализа (ИФА) с использованием автоматических пипеток и стандартного оборудования, так и для постановки на автоматическом иммуноферментном анализаторе открытого типа.

2. КЛИНИЧЕСКОЕ ЗНАЧЕНИЕ

Giardia lamblia (intestinalis) вызывает лямблиоз (гиардиаз) - паразитарную инвазию, которая протекает в виде латентного паразитоносительства и манифестных формах (нарушение функций кишечника). Заболевания лямблиозом зафиксировано на всех 5 континентах и в большинстве стран мира. Уровни инфицирования варьируют от <1 до 50 %. Во многих развивающихся странах, с отсутствующими базовыми санитарными условиями, инфицирование *Giardia* в возрасте 2 лет является почти стопроцентным. В противоположность этому, в развитых странах инфицированность лямблией составляет лишь 3-7 %. Заболевания распространены среди всех возрастных групп, однако основной контингент составляют дети дошкольного возраста.

Основной путь передачи *G. lamblia* - фекально-оральный. Паразит имеет простой, двухстадийный жизненный цикл. После проглатывания хозяином цист, из последних в двенадцатиперстной кишке появляются трофозоиты, которые прикрепляются к слизистой оболочке тонкого кишечника.

Трофозоиты существуют только на поверхности слизистой оболочки верхнего отдела тонкой кишки. Поэтому лямблии механически блокируют слизистую оболочку и нарушают пристеночное пищеварение и двигательную активность тонкой кишки. Лямблии вызывают ухудшение всасывания жиров, углеводов, витаминов С и В12 и обусловливают вторичную бактериальную инфекцию. Симптомами лямблиоза могут быть: диарея, усталость, отек, апатия, потеря массы тела, снижение аппетита, бледность, мышечные подергивания. Со стороны желудочно-кишечного тракта лямблиоз проявляется главным образом в виде энтероколита с катаральными проявлениями.

Многочисленные факты свидетельствуют о роли гуморального иммунного ответа в элиминации *G.lamblia*. Как было показано на модели с экспериментальным инфицированием людей, уровень антител класса IgM значительно возрастал на 14-21 день после инфицирования и постепенно снижался после терапии. В противоположность этому, уровни антител класса IgG оставались повышенными после успешного лечения. Динамика уровня IgA была подобна таковой IgM.

Диагностика лямблиоза традиционно базируется на клинической истории, симптомах, наличии цист в фекальных пробах или трофозоитов в материале, полученном из тонкого кишечника при дуоденальной аспирации или дуоденальной биопсии. Альтернативными методами является выявление антигена *G.lamblia* в фекалиях и определение уровня специфических антител класса IgM против *Giardia* в сыворотке пациента. Серологическое тестирование рассматривается как полезное дополнение в диагностике гиардиаза. По литературным данным присутствие специфических антител класса IgM коррелирует с острым процессом и может быть использовано в эпидемиологических исследованиях лямблиоза.

3. ПРИНЦИП АНАЛИЗА

Выявление специфических к *G. lamblia* антител класса IgM в тест-системе Vitrotest® Lamblia-IgM базируется на принципе «непрямого» твердофазного ИФА при двухэтапной инкубации. В лунках планшета засорбированы очищенные антигены *G. lamblia*. Во время первого этапа инкубации исследуемых образцов в лунках ИФА-планшета происходит связывание, при условии присутствия в образцах, специфических к *G. lamblia* антител с антигенами на твердой фазе. Лунки отмываются для удаления несвязанных компонентов, остаются только специфические комплексы антиген-антитело. После этого добавляется конъюгат антивидовых анти-IgM моноклональных антител с пероксидазой хрена, которые связываются с иммунными комплексами на твердой фазе. Несвязанные компоненты удаляются во время отмывания. Комплексы антиген-антитело выявляются путем добавления раствора хромогена 3,3',5,5'-тетраметилбензидина (ТМБ) с перекисью водорода. После 15 min инкубации реакция останавливается при добавлении стоп-реагента. Оптическая плотность (ОП) в лунках определяется на спектрофотометре при длине волны 450/620-695 nm. Интенсивность желтой окраски пропорциональна количеству антител в образце.

4. МАТЕРИАЛЫ И ОБОРУДОВАНИЕ

4.1. Состав набора

ELISA STRIPS	1x96 лунок	**ИФА-планшет** В каждой лунке планшета засорбированы очищенные антигены *G. lamblia.* Лунки можно отделять. 12 стрипов по 8 лунок.
CONTROL +	1x0,5 ml	**Положительный контроль** Раствор специфических моноклональных антител с консервантом (розовый).
CONTROL –	1x0,5 ml	**Отрицательный контроль** Отрицательная сыворотка крови человека с консервантом (желтый).
SAMPLE DILUENT	1x12 ml	**Раствор для разведения образцов** Буферный раствор с детергентом и консервантом (фиолетовый).
CONJUGATE SOLUTION	1x12 ml	**Раствор конъюгата** Буферный раствор моноклональных антител к IgM человека, конъюгированных с пероксидазой хрена, со стабилизаторами и консервантом (зелёный), готовый к использованию.
TMB SOLUTION	1x12 ml	**Раствор ТМБ** Раствор ТМБ, H_2O_2, стабилизатор, консервант (бесцветный), готовый к использованию.
WASH TWEEN 20X	1x50 ml	**Раствор для промывания Tw20 (20x)** 20-ти кратный концентрат фосфатного буфера с Твином-20 и NaCl (бесцветный).
STOP SOLUTION	1x12 ml	**Стоп-реагент** Раствор 0,5 mol/l H_2SO4 (бесцветный), готовый к использованию.

Клейкая пленка (2), бланк внесения проб (1), инструкция по применению и сертификат качества.

4.2. Дополнительные реактивы, материалы и оборудование

- Автоматические пипетки переменного объема на 10-1000 µl и наконечники к ним;
- спектрофотометр (ридер) для микропланшетов на 450/620-695 nm;
- мерная лабораторная посуда (10-1000 ml);
- деионизированная или дистиллированная вода;
- термостат на 37 °C;
- автоматический или полуавтоматический промыватель планшетов (вошер);
- контейнеры для отходов потенциально зараженного материала;
- таймер;
- фильтровальная бумага;
- одноразовые перчатки;
- дезинфицирующие средства;
- защитная одежда.

5. МЕРЫ ПРЕДОСТОРОЖНОСТИ

5.1. Предостережения

Соблюдение времени инкубации и температуры является чрезвычайно важным для корректного результата ИФА.

- не использовать компоненты тест-системы по окончанию срока годности;
- не использовать при анализе и не смешивайте компоненты разных серий, компоненты из тест-систем различных нозологий или реагенты других производителей в сочетании с наборами Vitrotest®;

Примечание: допускается использование WASH TWEEN 20X, TMB SOLUTION *и* STOP SOLUTION *других серий.*

- после использования реагента закрывать каждый флакон своей крышкой;
- во время промывания контролировать наполнение и полную аспирацию раствора из лунок;
- каждый раз использовать новый наконечник пипетки для внесения образцов или реагентов;
- избегать попадания прямых солнечных лучей на реагенты тест-системы;

– TMB SOLUTION должен быть бесцветным перед использованием. Если раствор окрашен в синий или желтый цвет его нельзя использовать. Избегать контакта TMB SOLUTION с металлами или ионами металлов. Для работы используйте только чистую, тщательно вымытую дистиллированной водой посуду;
– ни в коем случае не использовать одну и ту же посуду для CONJUGATE SOLUTION и TMB SOLUTION.

Производитель не несет ответственности за любые некорректные результаты и неблагоприятные случаи, возникшие вследствие нарушений вышеуказанных предостережений. Производитель не несет ответственности за визуальный учет результатов анализа (без использования спектрофотометра).

5.2. Меры безопасности

– все реагенты набора предназначены только для *in vitro* диагностики и могут использоваться только квалифицированным персоналом;
– постановку анализа проводить только в одноразовых перчатках и защитных очках;
– не допускается принимать пищу, пить, курить или пользоваться косметикой в комнате выполнения теста;
– не пипетировать растворы ртом;
– положительный контроль не содержит компонентов человеческого происхождения;
– отрицательный контроль тест-системы Vitrotest® Lamblia-IgM протестирован и найден отрицательным на антитела к ВИЧ½, ВГС и Treponema pallidum и HBsAg, однако работать с ним и исследуемыми образцами следует как с потенциально опасным инфекционным материалом;
– некоторые компоненты тест-системы содержат низкие концентрации вредных веществ и могут вызвать раздражение кожи и слизистых оболочек. При попадании TMB SOLUTION, STOP SOLUTION и CONJUGATE SOLUTION на слизистые оболочки и кожу необходимо немедленно промыть пораженное место большим количеством воды;
– в случае разбрызгивания растворов, не содержащих кислоту, например, сывороток, обработать поверхность дезинфицирующим средством, а затем вытереть насухо фильтровальной бумагой. В другом случае кислоту сначала необходимо нейтрализовать раствором бикарбоната натрия, а затем вытереть поверхность, как описано выше.

5.3. Утилизация отходов

– жидкие отходы следует инактивировать, например, раствором перекиси водорода в конечной концентрации 6 % в течение 3 h при комнатной температуре, или гипохлоритом натрия в конечной концентрации 5 % в течение 30 min, или другими разрешенными дезинфицирующими средствами;
– твердые отходы следует инактивировать путем автоклавирования при температуре 121 °C в течение 1 h;
– не автоклавировать растворы, содержащие азид натрия или гипохлорит натрия;
– удаление инактивированных отходов проводить в соответствии с действующим национальным законодательством;
– удаление остальных компонентов тест-систем после использования проводить согласно GLP (good laboratory practice) и действующего национального законодательства в сфере обращения с отходами.

6. ХРАНЕНИЕ И СТАБИЛЬНОСТЬ

Реагенты тест-системы стабильны в течение срока годности, указанного на этикетке, если их хранить при 2-8 °C. Не допускается замораживание тест-системы. Транспортировать набор при температуре 2-8 °C. Допускается однократная транспортировка при температуре не выше 23 °C в течение двух суток.

После вскрытия первичной упаковки компоненты тест-системы являются стабильными в течение 3 месяцев, кроме тех, которые указаны в п. 8 настоящей Инструкции.

7. ПОДГОТОВКА ОБРАЗЦОВ

Образцы сыворотки или плазмы (EDTA, литий-гепарин, фторид калия) крови хранить при температуре 2-8 °C не более 3 суток после забора. Для более длительного хранения образцы хранить в морозильной камере при температуре от -20 до -70 °C. Замороженные образцы перед использованием необходимо разморозить и выдержать при комнатной температуре в течение 30 min. Не использовать прогретые образцы. После размораживания образцы следует перемешать для достижения однородности. Избегать повторного замораживания-оттаивания исследуемых образцов. В случае помутнения сыворотки (или плазмы) освободить образец от нерастворимых включений центрифугированием при 3000 оборотов/min в течение 10-15 min. Не использовать образцы сывороток (или плазмы) с выраженной липидемией, гемолизом, а также бактериальным прорастанием. На результаты анализа не влияет присутствие в образце билирубина в концентрации до 0,21 mg/ml (361,8 µmol/l), гемоглобина в концентрации до 10 mg/ml и триглицеридов в концентрации до 10 mg/ml (11,3 mmol/l).

8. ПОДГОТОВКА РЕАГЕНТОВ

Очень важно выдержать все реагенты тест-системы при комнатной температуре (18-25 °C) в течение 30 min перед использованием!

8.1. Подготовка ИФА-планшета

ELISA STRIPS упакован под вакуумом с влагопоглотителем.

Для предупреждения конденсации воды в лунках необходимо открывать ELISA STRIPS только после выдерживания 30 min при комнатной температуре. Потом раскрыть вакуумную упаковку, отделить необходимое количество лунок, а остальные сразу же тщательно упаковать с влагопоглотителем и *хранить плотно закрытыми на замок (zip-lock)* при температуре 2-8 °C. Хранение таким образом упакованного планшета обеспечивает его стабильность в течение 3 месяцев.

8.2. Приготовление раствора для промывания

Для приготовления раствора для промывания развести концентрат WASH TWEEN 20X 1:20 (1+19) дистиллированной или деионизированной водой, затем перемешать. Например, 4 ml концентрата + 76 ml воды, что достаточно для 8 лунок. В случае наличия кристаллов в концентрате раствора для промывания, прогреть флакон при 37 °C до полного растворения кристаллов (15-20 min). Разведенный раствор можно хранить при температуре 2-8 °C не более 7 суток.

9. ПРОЦЕДУРА АНАЛИЗА

9.1. Подготовить необходимое количество лунок ELISA STRIPS для анализа (количество исследуемых образцов и четыре лунки для контролей), вставить их в рамку ИФА-планшета. Лунки с контролями обязательно включать в каждую постановку анализа.

9.2. Заполнить бланк внесения проб.

9.3. Приготовить раствор для промывания согласно пункта 8.2.

9.4. Внести во все лунки планшета по 90 µl SAMPLE DILUENT.

9.5. Внести в лунки по 10 µl контролей и исследуемых образцов: в лунку A1 – CONTROL +, в лунки B1, C1 та D1 – CONTROL –, в остальные лунки - исследуемые образцы. Осторожно пипетировать смесь в лунках, не допуская пенообразования. При внесении образцов происходит изменение цвета раствора в лунках с фиолетового на синий.
Отбор, внесение и пипетирование CONTROL + *проводить с особою тщательностью.*

9.6. Заклеить стрипы клейкой пленкой и инкубировать в течение 30 min при температуре 37 °C.

9.7. По окончании инкубации осторожно снять клейкую пленку и промыть лунки пять раз с использованием автоматического промывателя или 8-канальной пипетки следующим образом:
- удалить содержимое лунок в контейнер для жидких отходов;
- наполнить лунки не менее чем по 300 µl раствора для промывания, оставить не менее чем на 30 s;
- аспирировать раствор из лунок, остаточный объем раствора после аспирации на всех этапах промывания должен составлять не более 5 µl;
- повторить процедуру промывания еще четыре раза;
- после последней аспирации избавиться от лишней влаги, постукивая планшетом по фильтровальной бумаге.

9.8. В лунки внести по 100 µl CONJUGATE SOLUTION. Стрипы накрыть новой клейкой пленкой и инкубировать в течение 30 min при 37 °C.

9.9. По окончании инкубации осторожно снять клейкую пленку и промыть лунки пять раз, как описано в пункте 9.7.

9.10. Не касаясь дна и стенок лунок планшета, внести по 100 µl TMB SOLUTION в лунки.

9.11. Инкубировать стрипы в течение 15 min в темном месте при комнатной температуре 18-25 °C. Не использовать клейкую пленку на данном этапе.

9.12. Для остановки ферментативной реакции внести в лунки по 100 µl STOP SOLUTION придерживаясь той же последовательности, что и при внесении TMB SOLUTION.

9.13. Измерить на ридере ОП в каждой лунке при длине волны 450/620-695 nm в течение 5 min после остановки реакции. До проведения измерения необходимо убедиться в чистоте наружной поверхности дна лунок и отсутствии пузырьков.

Учет результатов анализа можно проводить в одноволновом режиме при длине волны 450 nm, в этом случае следует оставить лунку для установления бланка (в такую лунку вносить только TMB SOLUTION *и* STOP SOLUTION*).*

10. УЧЕТ РЕЗУЛЬТАТОВ И ИХ ИНТЕРПРЕТАЦИЯ

10.1. Учет результатов анализа

Рассчитать среднее значение ОП отрицательного контроля (N_c), уровень граничного значения (Cut off - CO) и индекс позитивности образца (IP_{sample}),

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

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

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

где OD_{sample} – $ОП_{образца}$

10.2. Достоверность результатов анализа

Данные теста считаются достоверными, если они отвечают следующим требованиям:

CONTROL +	ОП ≥ 1,200
CONTROL –	ОП ≤ 0,150
CONTROL –	Nc × 0,5 ≤ Ncn ≤ Nc × 2,0, где Ncn - каждое n-значение ОП отрицательного контроля (Nc1, Nc2, Nc3)

Если одно из значений ОП отрицательного контроля выходит за пределы указанного выше интервала, его отбрасывают и рассчитывают среднее Nc по остальным значениям ОП отрицательного контроля. Если более одного из значений ОП отрицательного контроля не отвечают указанным требованиям, то тест считается некорректным и требует повторного анализа.

10.3. Интерпретация результатов

$IP_{sample} > 1{,}1$	ПОЛОЖИТЕЛЬНЫЙ
$0{,}9 \leq IP_{sample} \leq 1{,}1$	НЕОПРЕДЕЛЕННЫЙ*
$IP_{sample} < 0{,}9$	ОТРИЦАТЕЛЬНЫЙ

* Неопределенные образцы рекомендуется исследовать повторно. Если результаты снова будут в пределах неопределенных, следует произвести забор нового образца.

11. ДИАГНОСТИЧЕСКИЕ ХАРАКТЕРИСТИКИ ТЕСТА

11.1. Специфичность и чувствительность

Для оценки чувствительности проанализировано 23 образца сывороток крови детей, предварительно охарактеризованные в другой коммерческой тест-системе как содержащие антитела класса IgM к *G. lamblia*. В тест-системе Vitrotest® Lamblia-IgM из 23 образцов в 21 обнаружены антитела класса IgM к *G. lamblia*. Также в сравнительных исследованиях тест-системы Vitrotest® Lamblia-IgM с другой коммерческой тест-системой был проанализирован 231 образец сывороток крови доноров. При этом относительная специфичность составила 97,4%.

11.2. Точность

Повторяемость результатов в пределах одной постановки анализа (Intra assay repeatability)

Коэффициент вариации (CV) для трех сывороток с разным уровнем специфических антител оценивали в 24 повторах на одной серии тест-системы.

№ сыворотки	$ОП_{ср}$	$IP_{ср}$	CV, %
18S	0,524	1,79	5,2
14S	0,821	2,80	5,5
3S	1,608	5,49	4,1

Воспроизводимость результатов между различными постановками анализа (Inter assay reproducibility)

Коэффициент вариации (CV) для трех сывороток с разным уровнем специфических антител оценивали в течение четырех дней в четырех постановках анализа, по 8 повторов в каждом анализе.

№ сыворотки	$ОП_{ср}$	$IP_{ср}$	CV, %
18S	0,548	1,88	5,2
14S	0,794	2,72	5,6
3S	1,632	5,59	3,7

12. ОГРАНИЧЕНИЯ АНАЛИЗА

Окончательный диагноз не может быть установлен только на основании результатов серологического теста, следует учитывать клинические проявления заболевания и данные лабораторных исследований (такие как результаты обнаружения цист в фекальных пробах или трофозоитов в дуоденальном содержимом; результаты обнаружения антигена *G.lamblia* в фекалиях).

Нельзя полностью исключить перекрестные реакции с антителами к антигенам других паразитов, ревматоидным фактором.

Специфические к *Giardia lamblia* антитела могут не проявляться у детей со стойким и продолжительным лямблиозом.

13. ПРОБЛЕМЫ, КОТОРЫЕ МОГУТ ВОЗНИКНУТЬ ПРИ ПРОВЕДЕНИИ ИФА И СПОСОБЫ ИХ УСТРАНЕНИЯ

Возможные причины	*Способы устранения проблем*
Высокий фон в лунках всего планшета	
Загрязненный промыватель	Прочистить головку промывателя и промыть 30 % раствором этилового спирта, затем дистиллированной водой
Низкое качество или загрязненность воды	Использовать очищенную воду с удельным сопротивлением ≥ 10 MΩ·cm.
Использование плохо вымытой посуды	Использовать химически чистую посуду
Использование дезинфицирующих средств, содержащих хлор	Не использовать хлорсодержащие дезинфицирующие средства
Использование загрязненных наконечников	Использовать новые наконечники
Увеличено время инкубации или изменен температурный режим	Придерживаться режима инкубации согласно инструкции по применению
Высокий фон в отдельных рядах	
Повторное внесение раствора ТМБ	Раствор ТМБ вносить один раз
Загрязнение конуса автоматичной пипетки раствором конъюгата	Прочистить пипетку и осторожно набирать раствор
Загрязнен один из каналов промывателя	Прочистить канал промывателя, промыть вошер
Значение ОП положительного контроля ниже установленной границы	
Неправильно приготовлен или не внесен один из реагентов (конъюгат или раствор ТМБ)	Повторно провести ИФА, обратить внимание на приготовление этих реагентов
Сокращено время инкубации на одном их этапов	Проводить инкубацию согласно инструкции по приминению
Интенсивность окрашивания лунок не отвечает полученной ОП	
Смещен оптический луч	Проверить корректность работы ридера

ЛИТЕРАТУРА

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ГРАФИЧЕСКИЕ ОБОЗНАЧЕНИЯ

REF	Номер по каталогу
	Используйте инструкцию по применению
IVD	Медицинское изделие для диагностики in vitro
	Производитель
	Предупреждение
Σ	Достаточно для проведения *<n>* количества исследований
	Ограничение температуры
LOT	Код партии
	Использовать до
	Беречь от прямых солнечных лучей
	Знак соответствия техническим регламентам
DO NOT FREEZE	Не замораживать

ТУ У 24.4-36555928-001:2011
Inst_Lamblia-IgM_TK031_V07_RU
Редакция Инструкции №7 от 22.02.2024

По вопросами и пожеланиями относительно работы изделия обращайтесь к производителю:

ООО «Витротест Биореагент»,
ул. М. Бойчука 18 б, оф. 56, г. Киев, 01103, Украина (юридический адрес)
ул. Курортная, дом. 11, г. Киев, 04075, Украина (местонахождение производства)

тел.: +38 (044) 222-76-72, +38 (097) 222-76-72
e-mail: info@vitrotest.ua, www.vitrotest.ua

Vitrotest® Lamblia-IgM

СХЕМА АНАЛИЗА

Выдержать все реагенты не менее 30 min при 18-25 °C перед использованием

Внести 90µl SAMPLE DILUENT в лунки
(фиолетовый цвет)

Внести 10 µl контролей и образцов в лунки:
A1 – CONTROL +,
B1, C1, D1 – CONTROL –,
E1 и другие лунки – исследуемые образцы
(цвет изменится с фиолетового на синий)

Заклеить стрипы пленкой, инкубировать 30 min при 37 °C

Промыть лунки 5 раз разведённым 1:20 (1+19) раствором для промывания Tw20 по 300 µl в лунку с 30 s замачиванием

Добавить 100 µl CONJUGATE SOLUTION в лунки
(зеленый цвет)

Заклеить стрипы новой клейкой пленкой, инкубировать 30 min при 37 °C

Промыть лунки 5 раз разведённым 1:20 (1+19) раствором для промывания Tw20 по 300 µl в лунку с 30 s замачиванием

Добавить 100 µl TMB SOLUTION в каждую лунку

Инкубировать 15 min в темноте при 18-25 °C без клейкой пленки

Остановить реакцию внесением 100 µl STOP SOLUTION
(цвет меняется с синего на жёлтый)

Определить оптическую плотность (ОП) при 450/620-695 nm

УЧЕТ РЕЗУЛЬТАТОВ

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

CO = Nc + 0,25;

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

Nc - среднее значение ОП для 3 CONTROL –

CO - Cut off, IP- индекс позитивности,

OD_{sample} – ОП$_{образца}$

ИНТЕРПРЕТАЦИЯ РЕЗУЛЬТАТОВ

$IP_{sample} > 1,1$	ПОЛОЖИТЕЛЬНЫЙ
$0,9 \leq IP_{sample} \leq 1,1$	НЕОПРЕДЕЛЕННЫЙ
$IP_{sample} < 0,9$	ОТРИЦАТЕЛЬНЫЙ

Vitrotest Europe Sp. z O.O.
Krakowska str 139-155,
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NIP: 8992881308

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.

Vitrotest Europe Sp. z o.o.
ul. Krakowska 139-155, 50-428 Wrocław
NIP: 8992881308, REGON: 386329301
KRS: 0000844411

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.
ul. Krakowska 139-155, 50-428 Wrocław
NIP: 8992881308, REGON: 386329301
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Galyna Rayevska, Ph.D.
Chief of the Board

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

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

REFERENCE

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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