Vitrotest®

Vitrotest TOXO-IgG

ELISA test kit for quantitative determination of IgG antibodies to *Toxoplasma gondii*

Instruction for use

IVD	For in vitro diagnostic use
REF	TK001

ELISA test for the diagnosis of infectious diseases TS (ТУ.У 24.4 – 36555928 – 001:2011)

«Vitrotest TOXO-IgG»
ELISA test kit for quantitative determination of IgG class antibodies to *Toxoplasma gondii*

1. Intended use

ELISA test-kit «Vitrotest TOXO-IgG» is intended for quantitative determination of IgG class antibodies to *Toxoplasma gondii* in human serum or plasma.

The test kit may be used both for the ELISA using automatic pipettes and standard equipment and for setting with the open-system immunoenzymatic automated analyzer.

2. Clinical value

Toxoplasmosis is a parasitic disease, agent of which is an intracellular parasite *Toxoplasma gondii*. *T.gondii* infection rarely leads to clinically significant toxoplasmosis. In case of illness toxoplasmosis occurs latent or chronically. The problem of timely detection of the disease in pregnant women and in patients with suppressed immune systems is of particular importance. The consequences of maternal primary infection during pregnancy in 30-50% of cases lead to abortion or birth of a child with physical and mental disabilities.

At toxoplasmosis specific antibodies of IgM class to *T.gondii* appear on 2nd week of infection, specific IgG antibodies appear later and reach a high concentration at 7-10th week; further high level of IgG keeps or years or reduced to minimum. Increase of more than half titers of IgG class antibodies in serum obtained 2-4 weeks after the initial examination, may indicate either the primary infection or reactivation of chronic toxoplasmosis.

Given the serious consequences of primary toxoplasmosis for pregnancy raises the question of differentiation of primary infection from reinfection or reactivation of toxoplasmosis. Simultaneous detection of specific antibodies of class IgM and IgG does not allow accurate diagnosis of primary toxoplasmosis because specific IgM class antibodies can be detected in human serum for 2 years after infection, as well as reinfection and reactivation disease. In such cases, determination of avidity index of specific IgG antibodies by enzyme immunoassay helps distinguish primary and paste infection. This definition is based on the properties of the immune system change low-avidity synthesis of antibodies during primary infection on the synthesis of high-avidity antibodies at past infection.

A single definition even high titers of IgG antibodies can not be considered evidence of disease.

3. Principle of the test

Principle of the test of «Vitrotest TOXO-IgG» kit is based on an indirect enzyme immunoassay technique.

The solid phase is made of strip microplate coated with the purified antigens of *Toxoplasma gondii*. During incubation of samples in wells of ELISA plate specific to *T.gondii* antibodies are bound to the antigen on the solid phase. After washing out unbound components anti-specific conjugate of anti-IgG monoclonal antibodies with horseradish peroxidase added to the wells, it binds to immune complexes in the solid phase. Unbound components are washed out. The immune complex formed in the wells are visualized by adding chromogen solution of 3,3',5,5'- tetramethylbenzidine (TMB). As a result of the reaction solution in wells where immune complexes were formed would be painted in blue. The reaction is stopped by adding stop reagent, blue colored wells become yellow. The result of the analysis is determined by spectrophotometric reading at 450/620 nm.

Internal calibrators of the test-kit «Vitrotest TOXO-IgG» are standardized with The 3d International Standard Anti-Toxoplasma serum, Human (NIBSC, WHO).

4. Materials and equipment

4.1 Contents of the kit

ELISA plate – 12 strips of 8 wells (with the possibility of separation of the wells) with immobilized antigen of *Toxoplasma gondii*.

Calibrator C0 – 1 vial containing 0,2 ml negative human serum (yellow).

Calibrator C25 – 1 vial containing 0,2 ml solution of specific to *Toxoplasma gondii* human IgG with concentration 25 IU/ml in phosphate buffer with stabilizers and preservatives (green).

Calibrator C50 – 1 vial containing 0,2 ml solution of specific to *Toxoplasma gondii* human IgG with concentration 50 IU/ml in phosphate buffer with stabilizers and preservatives (orange).

Calibrator C100 – 1 vial containing 0,2 ml solution of specific to *Toxoplasma gondii* human IgG with concentration 100 IU/ml in phosphate buffer with stabilizers and preservatives (pink).

Calibrator C200 – 1 vial containing 0,2 ml solution of specific to *Toxoplasma gondii* human IgG with concentration 200 IU/ml in phosphate buffer with stabilizers and preservatives (violet).

Sample pre-diluent – 1 bottle containing 12 ml buffer with detergent and preservatives (brown-green).

Sample diluent – 1 bottle containing 12 ml buffer with detergent and preservatives (yellow).

Conjugate solution – 1 bottle containing 12 ml buffer solution of monoclonal antibodies to human IgG conjugated with horseradish peroxidase, with stabilizers and preservatives (violet). Ready to use.

Washing solution Tw20 (20x) – 1 bottle containing 50 ml 20-fold concentrated phosphate buffer with Tween 20 (colourless).

TMB Solution – 1 bottle containing 12 ml of TMB solution and hydrogen peroxide, with stabilizers and preservatives (colourless).

Stop-reagent – 1 vial containing 12 ml of 0,5M sulphuric acid solution (colourless).

Plate for pre-dilution of serum – 12 strips of 8 wells.

Adhesive film – 2 sheets of adhesive film for covering the plates during incubation.

Sera identification plan – 1 sheet of paper for noting the schemes of samples distribution.

Form for calibrating graphic – 1 sheet for constructing of calibration graph.

Instruction for use – one copy of user manual.

4.2 Additional reagents, materials and equipment

In order to conduct the analysis, the following additional reagents, materials and equipment are required:

– deionized or distilled water;

– filter paper;

– graduated cylinders of 10, 200 and 1000 ml capacity;

– disposable gloves;

– hydrogen peroxide solution 6%;

– disposable glassware for preparing the reagents (bottles and trough);

– timer;

– mono- and multi-channel automatic adjustable pipettes capable of delivering volumes of 20, 200 and 1000 microliters and tips for them;

– thermostat for 37 °C;

– container for solid waste;

– container for liquid waste;

– [1]automatic or semi-automatic washer;

– [2]mono- or multi-channel reader for microplates at 450/620-695 nm.

[1,2] Please, consult us about the adaptation of your equipment.

5. Reservations and safety precautions

5.1. Reservations:

– do not use expired reagents;

– do not use in the analysis and do not mix components of different lots and components of test kits with different nosology;

– do not use reagents of other manufacturers in composition with the Vitrotest® sets;

- Note: possible to use washing solution Tw20 (20X), Sample pre-diluent, TMB solution and Stop-reagent with other series that are different from those attached to the test kit. These reagents are used in other test systems of Ramintek IPC. Please consult us for details.

– close reagent vials after use only with their appropriate caps;

– strictly follow to the washing procedure requirements described in this instruction;

– control the filling and full aspiration of the solution in the wells;

– use a new distribution tip for each serum and reagent;

– protect kit reagents from straight sun rays;

– TMB solution must be colourless or light blue before its use. If solution is dark blue or yellow, it cannot be used. Avoid any contact of the TMB solution with metals or metal's ions. Use glassware thoroughly washed and rinsed with deionized water.

– use only disposable pipette tips for adding TMB-substrate into plate's wells;

– never use the same glassware for conjugate solution and chromogen.

5.2. Safety precautions:

– all reagents included in the kit are intended for "in vitro" diagnostic use;

– wear disposable gloves when perform analysis;

– do not pipette by mouth;

– the calibrators of «Vitrotest TOXO-IgG» are negative for anti-HCV, anti-HIV1/2, anti-*T.pallidum* antibodies and HBsAg. Nevertheless, all calibrators and sera should still be regarded and handled as potentially infectious;

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

– the solid waste must be inactivated with autoclaving at 121°C for 1 hour;

– do not autoclave the solutions that contain sodium azide or sodium hypochlorite;

– avoid spilling of TMB-solution and Stop-reagent and any contact of these solutions with the skin or mucosa;

– in case of spilling of solutions, that do not contain acid, e.g. sera, rinse the surface with hydrogen 6% solution, then dry with filter paper.

6. Storage and stability

Reagents of the kit are stable up to the expiry date on the label, when stored at 2-8 °C.

Transport the test-kit at 2-8 ºC. Disposable transportation at temperature not higher than 20ºC during two days is allowed.

7. Specimen collection

The serum or plasma samples should be stored at 2-8 ºC not more then 3 days after collection. It is possible to store them longer, but frozen (-20 to-70 ºC). Before use frozen samples, wait for 30 minutes for the reagents to stabilize at room temperature. Mix thawed samples well to homogeneity. Avoid repeated freezing/thawing. Samples containing aggregates must be clarified by centrifugation. Do not use samples with contaminated, hyperlipeamic and hyperhaemolysed sera.

8. Dilution of samples and reagent preparation

It is very important to bring all reagents of the «Vitrotest TOXO-IgG» kit to room temperature (18-25°C) for 30 minutes before use.

8.1. ELISA plate preparation

Before opening the ELISA plate, allow it to stabilize at room temperature for 30 minutes to avoid water condensation inside the wells. Open the vacuum bag and remove necessary amount of wells. Immediately after removal of wells, the remaining strips should be **resealed with zip-lock and stored at 2-8 °C**. Microplate in thus packed bag is stable for 3 month.

8.2. Washing solution

The vial contains 50 ml of a concentrated 20X buffer with detergent. Dilute the washing solution 1:20 (1+19) with distilled or deionised water, then mix. For example: for 4 ml of concentrate – 76 ml of distilled water is enough for one strip. Crystals in the solution disappear by warming up to 37°C for 15-20 min.

The diluted washing solution can be stored at 2-8°C not more than 7 days.

8.3. Pre-dilution of samples and calibrators

Pre-dilute samples and calibrators by 10-fold with sample pre-diluent. For this purpose, in the required number of wells of plate for pre-dilution of sera (comes in a set) dispense 90 µl of sample pre-diluent and add 10 µl of sera and calibrators. Gently pipette the mix in wells, avoiding foaming. After addition of serum color of the solution in wells changes from brown-green to blue.

The procedure of dilution of samples and controls should be carried out immediately prior to analysis.

Sera with an expected concentration of specific antibodies above calibrator K200 (200 IU/ml) are recommended to investigate in two dilutions 1/100 and 1/1000. To prepare 1/1000 dilution, add 10 µl of dilution to 990 µl of sample pre-diluent.

9. Assay procedure

9.1. Take out from the protective packing the support frame and the necessary number of wells (the number of investigated samples and five wells for calibrators). Wells with calibrators must be included in each analysis.

9.2. Complete the sera identification plan.

9.3. Prepare washing solution (refer to point 8.2).

9.4. Conduct pre-dilution of sera (refer to point 8.3).

9.5. Dispense 90 µl of sample diluent in each well.

9.6 Dispense 10 µl of pre-diluted calibrators and unknown samples into the wells in the following order: A1, B1, C1, D1, E1 – pre-diluted 1:10 calibrators K200, K100, K50, K25, K0, respectively, F1 and rest wells – pre-diluted 1:10 unknown samples. Thus, the final dilution of serum in wells of ELISA plate should be 1: 100. Gently pipette the mix in wells, avoiding foaming – color of the solution in wells changes from yellow to green.

9.7. Cover strips with an adhesive film and incubate for 30 min at 37 °C.

9.8. After completing the incubation remove the adhesive film carefully and wash the wells five times using the automatic washer or 8-channel pipette in the following order:

– aspirate the content of wells strips into a liquid waste container;

– fill the strip wells with a minimum of 300 microliters of washing solution for each well (respect the soak time of a minimum of 30 seconds);

– aspirate the solution of all wells, the residual volume of solution after aspiration must be lower than 5 microliters;

– repeat the washing step four more times;

– after the last aspiration blot the microplate by turning it upside down on absorbent paper.

9.9. Dispense 100 µl of conjugate solution per well. Cover strips with an adhesive film, incubate for 30 min at 37°C.

9.10. After completing the incubation remove the adhesive film carefully and wash the wells five times as described above (refer to point 9.8).

9.11. TMB-solution is ready to use TMB-substrate solution with hydrogen peroxide. TMB-solution should be colorless, protect TMB-substrate solution from straight sun rays. To add TMB-solution only new tips must be used: carefully select a TMB-solution from the vial and without touching the bottom and walls of the hole plate, add 100 µl TMB solution per well.

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

9.13. Add 100 µl of stop-reagent in each well. Respect the same distribution sequence than for the TMB-substrate solution.

9.14. Read the optical density of every strip well in dual wavelength reading at 450/620 nm, within the 5 minutes of stopping the reaction. Pay attention to the cleanness of the wells bottom outside.

Measurement in the single-wave procedure at 450 nm is possible. Reserve blank well to adjust spectrophotometer in such analysis. Only TMB-substrate and stop-reagent must be added in blank well.

10. Calculation and interpretation of the results

10.1. Test validity conditions:

In order for an assay to be considered valid, the following criteria must be met:

– optical density (OD) of Calibrator C0 is not higher than 0,15 optical unit (OU),

– OD of Calibrator C25 is not lower than 0,2 OU,

– OD of Calibrator C50 exceeds OD of C25 at least 1,5 times, ie OD C50 ≥ OD K20 x 1,5,

– OD of Calibrator C100 exceeds OD of C50 at least 1,3 times, ie OD C100 ≥ OD C50 x 1,3,

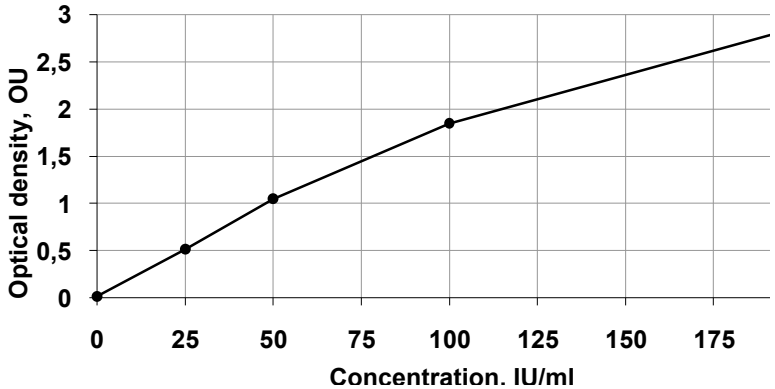
– OD of Calibrator C200 is not lower than 1,5 OU.

10.2. Calculation of the results.

To obtain quantitative results of determination the concentration of specific IgG antibodies in IU/ml construct a calibration graph: on the axis OY set aside five calibrators OD values K0, K25, K50, K100 and K200, and the OX axis set aside corresponding concentrations - 0, 25, 50, 100, 200 IU/ml, respectively.

Using the calibration graph determine the concentration (IU/ml) of specific antibodies in the samples, which corresponds to the obtained OD.

Example of calibration graph shown in Figure.



Note:
Do not use this graph to determine the concentration of specific IgG antibodies in Your analysis.

For the samples investigated in 1/1000 dilution concentration of specific antibodies determined according to schedule should multiply the degree of dilution, ie

final concentration = concentration according to schedule × 1000

For convenience of calculating results can be used computer programs for reading and calculation results of research.

10.3. Interpretation of the results.

The results of determination the concentration of specific IgG antibodies in IU/ml are interpreted as follows:

Concentration	*Result*
> 30 IU/ml	**Positive**
20-30 IU/ml	**Indeterminate**
< 20 IU/ml	**Negative**

11. Performance characteristics

11.1. Specificity and sensitivity

Specificity and sensitivity of the test «Vitrotest TOXO-IgG» were evaluated using commercial panel «Anti-Toxoplasma Mixed Titer Performance Panel PTT201» production "SeraCare Life Sciences" (USA), which consists of 25 characterized serum and plasma samples. In the test «Vitrotest TOXO-IgG» all positive and negative samples were determined correctly according to passport data.

In comparative studies of the test «Vitrotest TOXO-IgG» and another commercial test with CE marking among 93 negative and 65 positive for anti-TOXO IgG antibodies sera observed 100% coincidence of positive and negative results.

11.2. Accuracy

Intra assay reproducibility

Coefficient of variation (CV) for calibrators was calculated in 32 repetitions on two series of test kits.

Calibrator	CV_1, %	CV_2, %
C25	5,2	5,8
C50	4,3	3,8
C100	3,1	3,4
C200	3,4	3,2

Inter assay reproducibility

Coefficient of variation (CV) for calibrators was calculated for three days in three ELISA performances, in eight repetitions for each analysis.

Calibrator	CV, %
C25	7,2
C50	5,1
C100	4,8
C200	4,1

12. Limits of the test

A positive result in the test «Vitrotest TOXO-IgG» is an evidence of presence in a patient of specific IgG antibodies to *Toxoplasma gondii*, which are produced by the body when infected with *T.gondii*. The presence of antibodies of this class in infants is not evidence of *T.gondii* infection.

For correct diagnosis of toxoplasmosis is recommended to conduct research of the presence of IgG antibodies in paired sera obtained with interval of blood sampling at least two weeks, and to test sera for the presence of specific antibodies of class IgM, for example, in test kit «Vitrotest TOXO-IgM ultra».

For diagnosis should take into account both results of laboratory tests and clinical manifestations of the disease.

Reference sources

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Legend
Interpretation of notation conventions:

IVD	For in vitro diagnostic use
LOT	Batch code
REF	Catalogue number
	Production date
	Expiry date
	Storage temperature limitation
	Meant for <n> tests
	Protect from direct solar radiation
	Attention! Consult instruction for use
	Manufacturer and its address
	Consult instructions for use

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

Ramintek Innovation-Production Company
03039 Ukraine, Kiev, 7 October 40th Anniversary Av., of. 227 (registered address)
07300 Vishgorod, Kiev region, 19 Sholudenko Str. (factual address)
Tel. +380 44 222-76-72

Scheme of the assay «Vitrotest TOXO-IgG»

Keep reagents at room temperature 18-25°C during 30 minutes

Prepare washing solution, dilute 20x concentrate washing solution *Tw20* with distilled water 1:20 (1+19). For example, 4 ml of solution + 76 ml of water

Complete the sera identification plan

Prepare pre-dilution (1:10) of sera: in wells of plates for pre-dilution add 90 µl of sample pre-diluent (brown-green) and 10 µl of samples or calibrators.
After dispensing of serum the color in well switches from brown-green to blue

Dispense 90 µl of sample diluent (yellow) and add 10 µl of pre-diluted calibrators and patient samples into the wells:
A1 – Calibrator K200, B1 – Calibrator K100,
C1 – Calibrator K50, D1 – Calibrator K25,
E1 – Calibrator K0, F1 and other wells – patient samples

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

Wash wells five times

Dispense 100 µl of conjugate solution (violet) into the wells

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

Wash wells five times

Dispense 100 µl of TMB substrate solution into the wells

Incubate the plate for 30 min in the dark at room temperature (18-25°C)

Add 100 µl of stop-reagent in each well

Read optical density at 450/620 nm

Construct a calibration graph, determine the concentration IU/ml for specific IgG antibodies to *Toxoplasma gondii* in the samples

Interpret the results according to the table

Concentration	*Result*
> 30 IU/ml	**Positive**
20-30 IU/ml	**Indeterminate**
< 20 IU/ml	**Negative**