Vitrotest®

Vitrotest HSV1/2-IgG

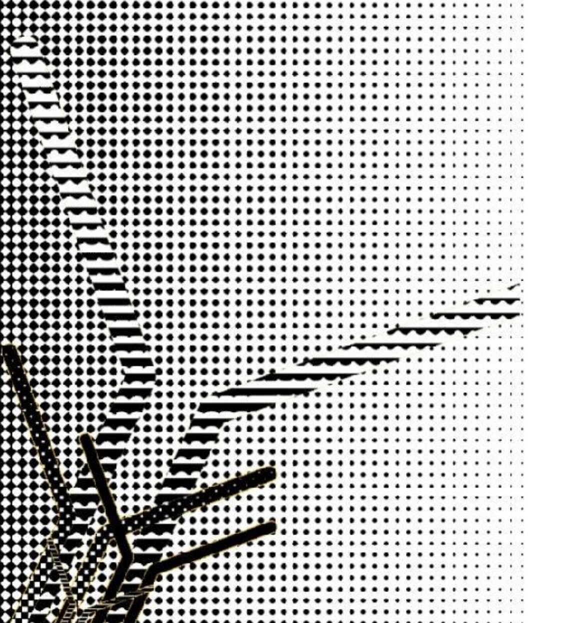
ELISA test kit for qualitative and semi-quantitative determination of IgG antibodies to herpes simplex virus types 1 and 2

Instruction for use

1. Intended use
2. Clinical value
3. Principle of the test
4. Materials and equipment
5. Reservations and safety precautions
6. Storage and stability
7. Specimen collection
8. Dilution of samples and reagent preparation
9. Assay procedure
10. Calculation and interpretation of the results
11. Performance characteristics
12. Limits of the test

Reference sources

Legend



IVD For in vitro diagnostic use

REF TK007

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

«Vitrotest HSV1/2-IgG»
ELISA test kit for qualitative and semi-quantitative determination of IgG class antibodies to herpes simplex virus types 1 and 2

1. Intended use

ELISA test-kit «Vitrotest HSV1/2-IgG» is intended for qualitative and semi-quantitative determination of IgG class antibodies to herpes simplex virus types 1 and 2 (HSV 1/2) 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

Herpes simplex virus (herpes infection) is a chronic relapsing infection caused by herpes simplex virus types 1 and 2. Disease occurs in localized forms with vesicular rashes on the skin and mucous membranes, as well as generalized forms with lesions of many organs.

Upon infection with herpes simplex virus types 1 and 2 is going consistent synthesis of antibodies classes IgM, IgG and IgA. Specific immunoglobulin IgM appear after the tenth day after infection, their level increases within two weeks. 7-10 days after the appearance of specific IgM appear immunoglobulin IgG to HSV that are present in the body for life.

The evidence of primary HSV infection is the detection of specific IgM and/or fourfold increase of the level of specific IgG antibodies in paired sera taken from patients at intervals of 14-20 days. Relapse of herpes usually develops on the background of sufficiently high levels of IgG antibodies or their increasing, as evidenced of the continued stimulation of the immune system of patient. Presence of antiherpetic IgG in low titer does not prove the absence of an active herpes infection.

3. Principle of the test

Principle of the test of «Vitrotest HSV1/2-IgG» kit is based on an indirect enzyme immunoassay technique.

The solid phase is made of strip microplate coated with the mixture of inactivated antigens of herpes simplex virus types 1 and 2. During incubation of samples in wells of ELISA plate specific to HSV 1/2 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.

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 antigens of herpes simplex virus types 1 and 2.

Positive control – 1 vial containing 0,3 ml solution of specific immunoglobulins (pink).

Negative control – 1 vial containing 0,5 ml negative human serum (yellow).

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 Tr100 (20x) – 1 bottle containing 50 ml 20-fold concentrated phosphate buffer with Triton X100 (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.

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 Tr100 (20X), 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 controls of «Vitrotest HSV1/2-IgG» are negative for anti-HCV, anti-HIV1/2, anti-*T.pallidum* antibodies and HBsAg. Nevertheless, all controls 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 HSV1/2-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 controls

Pre-dilute samples and controls by 10 times 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 controls. 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.

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 four wells for controls). Wells with controls 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 controls and unknown samples into the wells in the following order: A1 – pre-diluted 1:10 positive control, B1, C1 and D1 – pre-diluted 1:10 negative control, E1 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:

Calculate the mean optical density (OD) of negative control

$$OD\ NC_{mean} = (OD\ NC1 + OD\ NC2 + OD\ NC3)/3.$$

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

– OD of the positive control is not lower than 1,2 optical unit (OU),

– OD of negative controls should be lower or equal to 0,15 OU;

– OD of every negative control differs no more than twice from the mean value of negative control, that is

$$OD\ NC_{mean} \times 0{,}5 \leq OD\ NCn \leq OD\ NCmean \times 2{,}0.$$

If one of the negative controls does not respect this norm, disregard and recalculate the mean using remaining values.

10.2. Calculation of the results.
Calculate cut-off by adding value 0,2 to the mean NC, that is

$$Cut\ off = OD\ NCmean + 0,2$$

Calculate the index of positivity (IP) for each sample

$$IP = \frac{ODsample}{Cut\ off}$$

10.3. Interpretation of the results
The samples with IP above 1,1 are considered **positive (IP > 1,1)**.
The samples with IP below 0,9 are considered **negative (IP < 0,9)**.
The samples with IP **within 0,9-1,1** are considered **indeterminate (0,9≤ IP ≤1,1)**. It is recommended to retest such samples in duplicate. If the results are again within indeterminate, it is necessary to test sera obtained after 2-4 weeks. If you get undefined results assume that the serum is negative.
Using the index of positivity allows semi-quantitative comparative analysis of specific antibodies in paired serum. IP within 1,1–7,0 is proportional to the amount of specific antibodies. This allows the study of paired sera obtained from patients at intervals of 2–4 weeks. If the IP sample is above 7,0 for the correct evaluation of specific antibodies level is recommended to reanalysis of the sample pre-diluted in 10 times with sample diluent, in determining the index of positivity in this case should multiply the resulting value of IP by 10.
This method of interpretation of the test results allows determining the level of specific antibodies in dynamics.

11. Performance characteristics

11.1. Specificity and sensitivity
Specificity and sensitivity of the test «Vitrotest HSV1/2-IgG» were evaluated using commercial panel «Anti-Herpes Simplex Virus Type 1 & 2 Mixed Titer Performance Panel PTH 201» production "SeraCare Life Sciences" (USA), which consists of 25 characterized serum and plasma samples, 21 of which contain IgG antibodies to HSV1/2 and four sera do not contain anti-HSV1/2 specific antibodies. In the test «Vitrotest HSV1/2-IgG» all positive and negative samples were determined correctly according to passport data.
In the study of specificity of the test «Vitrotest HSV1/2-IgG» using 47 sera negative for antibodies to herpes simplex virus first and second type all 47 samples were found negative.
In comparative studies of the test «Vitrotest HSV1/2-IgG» and another commercial test with CE marking were analyzed 94 sera containing IgG antibodies to HSV1/2 all of these samples were found positive in both test -system.

11.2. Accuracy
Intra assay reproducibility
Coefficient of variation (CV) was calculated in 32 repetitions of two sera with different level of specific antibodies on two series of test kits.

Serum №	OD mean	CV_1, %	CV_2, %
198	0,417	4,7	5,2
311	1,358	3,9	3,4

Inter assay reproducibility
Coefficient of variation (CV) for two sera with different level of specific antibodies was calculated for three days in three ELISA performance, in eight repetitions for each analysis.

Serum №	OD mean	CV, %
198	0,475	7,4
311	1,311	4,8

12. Limits of the test

A positive result in the test «Vitrotest HSV1/2-IgG» is an evidence of presence in a patient of specific IgG antibodies to herpes simplex virus first and/or second type, which are produced by the body when infected with HSV1 or HSV2.
For correct diagnosis of herpes infection is recommended to study the presence of IgG antibodies in paired sera obtained at intervals of blood sampling at least two weeks, and to test sera for the presence of specific antibodies of class IgM, for example, in a test «Vitrotest HSV1/2-IgM».
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:

Symbol	Meaning
IVD	For in vitro diagnostic use
LOT	Batch code
REF	Catalogue number
	Production date
	Expiry date
	Storage temperature limitation
Σ	Meant for <n> tests
	Protect from direct solar radiation
!	Attention! Consult instruction for use
	Manufacturer and its address
i	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 HSV1/2-IgG»

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

Prepare washing solution, dilute 20x concentrate washing solution *Tr100* with distilled water 1:20 (1+19). For example, 4 ml of solution + 76 ml of water

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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 controls. *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 controls and patient samples into the wells :
A1 – positive control, B1, C1, D1 – negative control,
E1 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

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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

Calculate the cut-off of the assay «Vitrotest HSV1/2-IgG»:

$$Cut\text{-}off = OD\ NC_{mean} + 0,2$$

Calculate the index of positivity (IP) for patient samples: $IP = \frac{OD\ of\ patient\ sample}{cut\ off}$

Interpret the results according to the table:

IP value	Result
$IP_{sample} > 1,1$	**positive**
$0,9 \leq IP_{sample} \leq 1,1$	**indeterminate**
$IP_{sample} < 0,9$	**negative**