Vitrotest® Vitrotest Chlamydia-IgM

ELISA test kit for qualitative determination of IgM antibodies to *Chlamydia trachomatis*

Instruction for use

IVD	For in vitro diagnostic use
REF	TK036

«Vitrotest Chlamydia-IgM»
ELISA test kit for qualitative determination of IgM class antibodies to *Chlamydia trachomatis*

1. Intended use

ELISA test-kit «Vitrotest Chlamydia-IgM» is intended for qualitative determination of IgM class antibodies to *Chlamydia trachomatis* 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

Serological methods of diagnosis of chlamydial infections (including ELISA) allow determining the nature and stage of the disease, especially for chronic disease of many months and years. To this end, with ELISA is able to define specific antibodies of classes IgM, IgA and IgG, which gradually are synthesized and accumulate in serum and in human biological secretions.

The first since moment of infection appear specific antibodies of IgM class to *Chlamydia trachomatis*. They appear in the first weeks after infection and are an indicator of primary infection of *Chlamydia trachomatis*. Specific antibodies to *Chlamydia trachomatis* of IgA class are present both in serum and in secretory forms - as a measure of acute infection and manifestation of chronic form of the disease. In serum IgA class antibodies appear in 10-14 days after onset, a little earlier than antibodies of IgG class, but at lower concentrations. They can be detected in early disease in the discharge from the genital organs. High concentrations of this class of antibodies may indicate chronic infection. Specific IgA antibodies have a half life of 5-7 days; they can be used to monitor the effectiveness of treatment. Reduction of these antibodies in 2-3 times indicates successful treatment.

IgG class antibodies appear from the third week after onset. Their presence reflects an overall picture of the immune response due to acute, chronic or past infection. In the latter case, IgG can be detected at a low level for many years.

3. Principle of the test

Principle of the test of «Vitrotest Chlamydia-IgM» kit is based on «IgM-capture» solid phase immunoassay technique.

The solid phase is made of strip microplate coated with the monoclonal antibodies specific for human IgM. During incubation of samples in wells of ELISA plate IgM immunoglobulins are bound with monoclonal antibodies on the solid phase. After washing out unbound components the conjugate solution of recombinant antigen containing species-specific fragment of Major Outer Membrane Protein of Chlamydia with horseradish peroxidase added to the wells, and binds to specific immunoglobulin IgM 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 monoclonal antibodies specific for human IgM.

Positive control – 1 vial containing 0,3 ml solution of human immunoglobulin IgM gonjugated with monoclonal antibodies specific for horseradish peroxidase (pink).

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

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

Sample diluent – 1 bottle containing 20 ml buffer with skim milk extract, detergent and preservative (violet).

Conjugate diluent – 1 bottle containing 14 ml buffer solution with detergent and preservative (yellow).

Conjugate (11X) – 1 vial containing 1,4 ml of 11-fold concentrated conjugate of recombinant protein of *Chlamydia trachomatis* with horseradish peroxidase in buffer with stabilizers (blue).

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 0,5 M sulphuric acid solution (colourless).

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 Tw20 (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 it's using. If solution is dark blue or yellow, it can't 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;
– control samples of «Vitrotest Chlamydia-IgM» are nonreactive for HBsAg and antibodies to HIV ½, HCV, *Treponema pallidum*. Nevertheless, all controls and sera should still be regarded and handled as potentially infectious;
– the liquid waste must be inactivated with, for example, 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. Reagent preparation

It is very important to bring all reagents of the «Vitrotest Chlamydia-IgM» 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. Conjugate solution

To prepare conjugate working solution:

Dilute conjugate concentrate 11x (blue) in a clean vial to 1:11 with conjugate diluent (yellow). Solution becomes green. Example: 100 µl of conjugate concentrate add to 1000 µl of conjugate diluent, it is enough for 8 wells.

Conjugate working solution is stable for one day at 2-8°C.

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

9.5. Distribute 10 µl of controls and unknown samples into the wells in the following order: A1 – positive control, B1, C1 and D1 – negative control, E1 and rest wells – unknown samples. Gently pipette the mix in wells, avoiding foaming. After addition of serum color of the solution in wells changes from violet to blue.

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

9.7. 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.

9.8. Prepare conjugate working solution (refer to point 8.3).

9.9. Distribute 100 µl of conjugate solution per well. Cover strips with an adhesive film, incubate for 30 min **at room temperature 18-25°C**.

9.10. After completing the incubation remove the adhesive film carefully and wash the wells five times as described above (refer to point 9.7).

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 1,5 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

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

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 0,30 to the mean NC, that is

$$Cut\ off = OD\ NCmean + 0,3.$$

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 does not contain specific antibodies of IgM class to *Chlamydia trachomatis*.

Interpretation of results of determination of IgG, IgA and IgM classes antibodies specific to *Chlamydia trachomatis*

Specific antibodies to *Chlamydia trachomatis*			Interpretation of result
IgG	IgA	IgM	
Absent	Absent	Absent	sample does not contain specific antibodies, or their concentration is below the sensitivity of analysis
Absent	Present	Present	Probable early stage of infection
Present	Absent	Absent	Probable past infection
Present	Present	Present	Acute infection
Present	Present	Absent	Acute or chronic infection

11. Performance characteristics
11.1. Specificity and sensitivity
Diagnostic characteristics of the test «Vitrotest Chlamydia-IgM» were tested on samples of blood serum of patients with symptoms of acute urogenital chlamydiosis. In all samples there were found specific to *Chlamydia trachomatis* antibodies of IgA class, 47% of them were detected IgM class antibodies to *Chlamydia trachomatis*. In the study of specificity of the set «Vitrotest Chlamydia-IgM» using 94 sera negative for antibodies to *Chlamydia trachomatis* all 94 samples were found negative.

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 №	IP	CV_1, %	CV_2, %
1293	2,35	4,4	4,2
1215	6,92	4,9	3,2

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

Serum №	IP	CV, %
1293	2,3	6,1
1215	7,1	5,1

12. Limits of the test

A positive result in the test «Vitrotest Chlamydia-IgM» is an evidence of presence in patient of specific IgM antibodies to *Chlamydia trachomatis*, which are produced by the body when infected with the causative agent of genital chlamydiosis.

It should be noted that in case of early chlamydial infection ELISA result may be negative due to the lack of antibodies in the early stages of the disease. In the presence of clinical manifestations of the disease is recommended to retest the patient at least in two weeks. Two or three-fold increase of antibodies level indicates active infection.

For diagnosis should be considered as the 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 Chlamydia-IgM»

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

Dispense 90 µl of sample diluents into the wells

Dispense 10 µl of controls and patient samples into the wells:
A1 – positive control,
B1, C1, D1 – negative control,
E1 and other wells – patient samples
After dispensing of serum the color in well switches from violet to blue

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

Wash wells five times

Dilute conjugate (11×) (blue) by conjugate diluent (yellow) 1:11 (1+10). For example, 100 µl of conjugate + 1000 µl of conjugate diluent (*solution becomes green*)

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

Cover wells with adhesive film, incubate for 30 min at room temperature (18-25°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 Chlamydia-IgM»:
$$Cut\text{-}off = OD\ NC_{mean} + 0,3$$

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

Interpret the results according to the table:

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