

Performance characteristics of the test

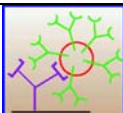
The studies of the sensitivity of the test kit «DIA-IgM-SYPH» were carried on sera samples from patients with syphilis at the different clinical stages: lues I – 55 samples, lues II – 87 samples, latent period – 23 samples. Antibodies type IgM are mostly appeared at stages of lues I and lues II and practically absent during investigation of sera from patients at latent period disease in other words antibodies type Ig M are detected at acute stages of syphilis.

The application opportunity of test kit «DIA-IgM-SYPH» for diagnostics of congenital syphilis was examined on the group of samples from new-born children infected *Treponema pallidum*. Specific antibodies type IgM were revealed in all 17 samples of the given group.

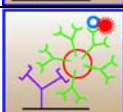
Specificity studies of the test kit «DIA-IgM-SYPH» were conducted using 543 samples of blood donors and it was revealed 2 specimens as false positive.

OVERVIEW OF PROCEDURE

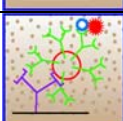
- Dispense 90 µl of specimen diluent and 10 µl both control and investigated sera in wells
- Incubate for 30 min at 37°C
- Wash 4 times with washing solution



- Dispense 100 µl of conjugate solution in wells
- Incubate for 30 min at 37°C
- Wash 6 times with washing solution



- Dispense 100 µl TMB substrate in wells
- Incubate for 30 min at room temperature (colouring)
- Stop the reaction by adding 100 µl stop-reagent
- Read the optical density at 450/620 nm



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Diaproph Med
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DIA-IgM-SYPH

**Enzyme immunoassay
for the detection of specific IgM
antibodies to *Treponema pallidum***

96 tests

Product code: T-1007

INTENDED USE

Enzyme immunoassay kit is an in vitro diagnostics test for the detection specific IgM antibodies to *Treponema pallidum* in human serum and plasma.

DIA-IgM-SYPH can be used for:

1. Detecting the primary antibodies response during infection with *T. pallidum*.
2. Confirmation of active disease by distinguished between IgG and IgM without the need for fraction of sera.
3. Confirmation of infection neonates as IgM cannot cross the transplacental barrier by passive transfer.

EXAMPLE FORM

INTRODUCTION

The syphilis is the prevalent infectious disease caused by *Treponema pallidum*, which is characterized skin-lesion, and also mucous, viscera, bones and nervous system injuries. The disease has a wavylike nature with period changes from exacerbation to latent one during its growing progressively worse. The infection is mainly occurred via sexual transmission, but it is possible a placental transmission (congenital syphilis), during contacts in conditions of life (contact syphilis) and with infected blood.

The actual tendency of the prevention of syphilis spread is to find out infectious patients among population. For the syphilis diagnostics it is used laboratory tests detecting *Treponema pallidum* specific antibodies. Such tests are based on using cardilepidic antigens or *Treponema pallidum* specific antigens. [2, 3, 6]

Today in the world diagnostic market offers the variety of ELISA test kits for serological syphilis diagnostics. The high sensitivity and specificity of such test kits are ensured because of using in its design recombinant antigens of *T. pallidum* (pTp15, pTp17, pTp41 и pTp47), which are not homology with antigens of non pathogenic spirochetes. [1, 6, 7]

During studies of antibody arising in human organism of infectious patients it was found out that antibodies type IgM appear first in blood, which is already detected at second week after infection and reach the maximum concentration in blood at 6-9 weeks. IgG antibodies appear at 4 week after infection. [7] Taking into consideration that the concentration of specific antibodies IgM naturally decreases with the course of time so the titer increasing is the subsidiary evidence of the disease relapse or reinfection. [6, 7]

For congenital syphilis diagnostics it is necessary to use test kits that detects anti-Treponema IgM antibodies in new-born children serum, as circulatory mother's antibodies type IgG in child blood have no diagnostic value. [2, 3, 4, 5, 6, 7]

PRINCIPLE OF PROCEDURE

DIA-IgM-SYPH is the IgM capture immunoenzymatic test intended for the detection of human IgM antibodies specific to T.pallidum.

DIA-IgM-SYPH is based on using solid phase (microelisa stripplate) coated with monoclonal antibodies to μ -chain of human IgM antibodies and conjugate (a mixture of the recombinant proteins pTp 17 and pTp 47– analogues of T. pallidum antigens bound to a horseradish peroxidase).

When investigated specimens of human plasma or serum are placed into wells, human IgM presented in specimen bind to specific antibodies onto the solid phase forming anti-IgM-IgM complexes. Formed complexes are detected using the specific conjugate.

The substrate buffer (hydrogen peroxide) and TMB solution is added to wells after washing non-bound components. Solution is coloured in case of presence of peroxidase conjugate in complexes.

To stop the colour reaction it is added stop-reagent and then determined the absorbance at 450/620 nm.

STORAGE CONDITIONS AND TRANSPORTATION

The kit must be stored and transported at 2-8°C. The kit must not be frozen. Shelf life of the kit is 9 months.

KIT REAGENTS

For *in vitro* diagnostic use.

Each kit contains:

Label	Reagents	Presentation
R1	Microplate 6 strips per plate each with 16 wells coated with monoclonal antibodies to μ -chain of IgM.	1 plate
R2	Washing solution concentrate (46x) Phosphate buffer, containing 2.2% Triton X100.	2 bottles 2 × 25 ml
R3	Specimen diluent Phosphate buffer, containing skimmed powdered milk. Preservatives: 0.01% thimerosal, 0.1% sodium azide.	1 bottle 1 × 15 ml
R4	Positive control Human serum containing IgM antibodies to Treponema pallidum. Inactivated by treating with β -propiolacton. Preservatives: 0.1% sodium azide.	1 vial 1 × 0.2 ml

- Cover the plate with adhesive film and incubate at 37°C for 30 minutes.
- Aspirate the contents of all wells and wash the plate with **washing solution** 6 times (according the section *Wash procedure*). If necessary, dry the plate by slight tapping upside-down on absorbent paper.
- Pipette into each well 100 μ l of the **TMB substrate**.
- Cover the plate with adhesive film and incubate at 18-30°C for 30 minutes in the dark.
- Add into each well 100 μ l of **stop-reagent** to stop colour reaction (maintain the same pipetting sequence and rate used for TMB substrate addition).
- Read the absorbance at 450/620 nm using a dual wavelength microplate reader within 2 minutes after stopping the reaction.

As an exception, absorbance may be measured at 450 nm (single wavelength) against a blank well; for that purpose to include an empty well in the run.

Results

Calculation of the results

NC – absorbance of the negative control

PC – absorbance of the positive control

\overline{NC} – mean absorbance of the negative control

\overline{PC} – mean absorbance of the positive control

- Calculate the mean absorbance of the negative control.

Test run is valid if \overline{NC} is not higher than 0.100.

Exclude any NC, which is higher than 0.100 or if it is more than twice exceed the \overline{NC} , and recalculate \overline{NC} of the remaining controls.

- Calculate the mean absorbance of the positive control.

Test run is valid if \overline{PC} is not lower than 0.600.

- Calculate **Cut-off** value.

$$\text{Cut-off} = \overline{NC} + 0.30$$

- Determine the grey zone.

Grey zone is the zone with sample absorbance within the range

$$\text{Cut-off} - 10\% \leq \text{OD} \leq \text{Cut-off}.$$

Interpretation of the results

The result is evaluated as **nonreactive** if the specimen absorbance is below the grey zone.

The result is evaluated as **reactive** if the specimen absorbance is equal or greater than the cut-off.

Specimens with absorbance values of the grey zone range are considered **indeterminate** and should be retested with caution in duplicate.

Specimens that show an initially reactive or indeterminate result should be retested in two or more wells:

- specimens reactive in one or more wells are considered as reactive ones;
- specimens nonreactive in two or more wells are considered as nonreactive ones.

- Reagents and samples should be at room temperature (18-30°C) before testing begins. Return the reagents to 2-8°C after use.
- The temperature in room where performing analysis should be in the range 18-30°C.
- It should accurately dissolve reagents avoiding its contamination.
- Do not perform the test in the presence of reactivity vapours (for example, from sodium hypochlorite, acids, alkalis, or aldehydes) or dust because the enzymatic activity of the conjugate may be affected.
- Use glass vessels thoroughly washed and rinsed with deionized water or use disposable ones.
- Do not allow drying contents of wells on all stages of procedure.
- Enzyme reaction is sensitive to metal ions, so avoid contacting with metal elements.
- TMB substrate (substrate buffer + TMB solution) is to be colourless. Appearance of colouring after dilution is evidence of unavailability for using and solution is to be replaced. The solution is to be prepared in clean plastic ware or clean glassware. The reagent is to be kept in dark.
- Prevent the direct light to fall on the working surface during ELISA procedure.
- Use a new tip for brining specimens in wells.
- Never use the same trough for distribution conjugate and TMB substrate.
- Check the pipettes and other equipment for accuracy and correct operation.
- Do not change the assay procedure.

Wash procedure

Washing must be performed strictly according to the instructions, as insufficient plate washing leads to incorrect results.

Use automatic washer*, as recommended; in case of its absence or faulty work – use multi-channel pipette for washing.

Follow this procedure in each washing:

- aspirate the wells contents completely into a waste flask;
- then fill the wells completely with washing solution (not less than 350 µl per well) avoiding overflow of buffer from one well to another;
- aspirate completely.

Make sure that no fluid remains on the top and the bottom of the strips and stripholder after the last aspiration (e. g. by blotting with absorbent tissue).

* Contact our company for further information on the different types of washers validated by our technical services.

Test procedure

- Fit the stripholder with required number of **strips**.
- Pipette 90 µl of the **specimen diluent** into each well.
- Distribute in the wells as follows:
 - wells A1: 10 µl of **positive control**.
 - wells B1, C1: 10 µl of **negative control**.
 - the rest wells : 10 µl of **specimens**.
- Cover the plate with adhesive film and incubate at 37°C for 30 minutes.
- Aspirate the contents of all wells and wash the plate with **washing solution** 4 times (according the section *Wash procedure*). If necessary, dry the plate by slight tapping upside-down on absorbent paper.
- Pipette 100 µl of the **conjugate solution** into each well.

R5	Negative control Heating inactivated human serum nonreactive for hepatitis B surface antigen (HBsAg) and antibodies to Treponema pallidum, HIV and HCV. Preservatives: 0.1% sodium azide.	1 vial 1 × 0.35 ml
R6	Conjugate concentrate A mixture of the recombinant proteins pTp 17 and pTp 47 bound to a horseradish peroxidase. Preservatives: 0.01% thimerosal.	1 vial 1 × 1.5 ml
R7	Conjugate diluent Phosphate buffer, containing skimmed powdered milk. Preservatives: 0.1% 2-methyl-4-isothiazolin-3-one.	1 bottle 1 × 15 ml
R8	TMB solution Solution containing 0.03% 3,3',5,5'-tetramethylbenzidine.	1 bottle 1 × 8 ml
R9	Substrate buffer Citrate-phosphate buffer, containing 0.016% hydrogen peroxide.	1 bottle 1 × 8 ml
R10	Stop-reagent 0.5 M sulphuric acid solution.	1 bottle 1 × 15 ml
	Adhesive film	3 items

ADDITIONAL MATERIALS AND INSTRUMENTS REQUIRED

- distilled or deionized water;
- disposable gloves;
- disposable V-shaped troughs;
- container or vial for reagent preparation (glass, or plastic);
- graduated cylinder (1000 ml);
- absorbent paper;
- sodium hypochlorite solution or other accepted disinfectant;
- sodium bicarbonate;
- ethanol, 70°;
- automatic single-channel pipettes (e.g. 5-40, 20-200, 200-1000 µl) with disposable tips;
- automatic multi-channel pipettes (50-300 µl) with disposable tips;
- incubator, 37±1°C;
- microwell wash system*;
- microwell reader* (with filters for 450/620 nm);
- biohazard waste containers for potentially contaminated materials.

* Contact our company for further information on the equipment validated by our technical services.

SAFETY PRECAUTIONS AND WARNINGS

- Use a new tip for pipetting specimens in wells.
- All reagents included in the kit are intended for "in vitro" diagnostic use.
- Wear disposable gloves when handling reagents and samples and thoroughly wash hands after handling them.
- Do not pipette by mouth.
- Human origin material used in the preparation of the negative and positive controls. The positive control has been inactivated by heating and treated with chloroform. The negative control has been tested and found nonreactive for hepatitis B surface antigen (HBsAg), antibodies to T. pallidum, HCV and antibodies to HIV (HIV-1, HIV-2), however for the purpose of additional protection treated with heating.
- Because no known test method can offer complete assurance that infectious agents are absent, handle reagents and patient samples as if capable of transmitting infectious disease.
- Any equipment directly in contact with specimens and reagents as well as the washing solution be considered as contaminated products and treated as such.
- Avoid spilling samples or solution containing samples.
- Spills must be treated with ethyl alcohol 70°. If the contaminating fluid is an acid, spill must be neutralized with sodium bicarbonate and dried with absorbent paper. The materials used for cleaning must be discarded in a contaminated residue container.
- Samples and reagents of human origin, as well as, contaminated material and products must be discarded after decontamination:
 - Either by immersion solid wastes in sodium hypochlorite at a final concentration of 5%, liquid wastes in sodium hypochlorite at a final concentration of 1% during 30 min.
 - Or by autoclaving at 121°C during 2 hours. The best method of inactivating of HIV, HBV, and HCV is an autoclaving.
 - DO NOT PLACE SOLUTIONS CONTAINING SODIUM HYPOCHLORITE IN THE AUTOCLAVE.
- Do not forget neutralize acid solutions before autoclave.
- Avoid any contacts substrate solution, chromogen and stop-reagent with skin and mucous covers.
- The negative and positive controls contain sodium azide as a preservative. Sodium azide may react with laboratory plumbing forming copper or lead azides. Such azides are explosive. To prevent azide build-up, flush the pipes with a huge quantity of water if solutions containing azide are disposed of the sink after inactivation.

SPECIMEN PREPARATION

Serum or plasma specimens are to be stored at 2-8°C during 72 hours. If necessary these specimens may be frozen (not more than two freezing-thawing procedures are allowed) at temperature below -20°C.

All specimens containing aggregates and visible suspended particles are to be clarified by centrifugation.

Specimens with sodium azide, hemolysis, hyperlipidemiae or bacterial contamination may not be used in the ELISA procedure.

ASSAY PROCEDURE

Reagents and specimens should be at room temperature (18-30°C) before beginning the assay and can remain at room temperature during testing. Return reagents to 2-8°C after use.

Reagents preparation (for 8 wells)

Microplate

Open the pack and remove strips. Return unused strips in the pack. Reseal the pack and return to 2-8°C.

The strips are stable for 4 weeks at 2-8°C after opening the pack.

Washing solution

Check **washing solution concentrate** for the presence of salt crystals. If crystals are seen in the solution, dissolve them by heating at 35-37°C.

Dilute the washing solution concentrate 1:45 with distilled or deionised water (see chart below), shake intensively.

Washing solution is stable for 10 days at 2-8°C.

Number of wells	washing solution concentrate	Deionised water
8	4 ml	180 ml

Conjugate solution

Dilute the **conjugate concentrate** with **conjugate diluent** (see chart below) in a clean vial. Mix well avoiding foaming.

Conjugate solution has to be prepared before use.

Conjugate solution is stable for 2 weeks at 2-8°C.

Number of wells	Conjugate concentrate	Conjugate diluent
8	100 µl	1 ml

TMB substrate

To prepare **TMB substrate**, combine the required amount of **TMB solution** in a clean vial in equal parts with **substrate buffer** according to the number of wells being run (see chart below). Mix well. TMB substrate must be colourless before use.

The TMB substrate must be kept away from light and no solutions contact with metals or metal ions is allowed.

The solution has to be prepared before use.

TMB substrate is stable for 2 weeks at room temperature (18-30°C) if kept in the dark.

Number of wells	TMB solution	Substrate buffer
8	1 ml	1 ml

Procedural notes

Authenticity of results depends on correct execution following instructions:

- Reagents should not be used beyond the expiry date shown on the package label.
- Reagents should not be mixed from different lots during performing test.