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Diaproph Med
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DIA-SYPH

**The enzyme immunoassay test system for
the detection of antibodies to the syphilis infectious
agent *Treponema pallidum***

192 tests

Product code: T-0606

EXAMPLE

INTENDED USE

Enzyme immunoassay kit is intended for the detection of antibodies to infectious agent – *Treponema pallidum* in human serum and plasma by ELISA.

INTRODUCTION

Syphilis is an 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 there 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 detection of anti-*T.pallidum* specific antibodies. Those tests are designed using cardilepidic antigens or *Treponema pallidum* specific antigens.

Today there is 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 stipulated because of using recombinant antigens of *T. pallidum* (pTp15, pTp17, pTp41 and pTp47), which are not homology with antigens of non pathogenic spirochetes.

PRINCIPLE OF PROCEDURE

DIA-SYPH is an immunoenzyme test kit is based on principle of one-step “sandwich”. There is used a solid phase (microplate strips) coated with a mixture of recombinant proteins (pTp17 and pTp47), which are analogues of *T.pallidum* antigens. The conjugate is presented with the mixture of recombinant proteins conjugated with a horseradish peroxidase.

When investigated specimen of human plasma or serum and conjugate are placed into wells, *T.pallidum*-specific antibodies presented in specimen bind both to recombinant

antigens on solid phase and conjugate antigens forming antigen-antibodies complexes. 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 14 months.

KIT REAGENTS

For *in vitro* diagnostic use. Each kit contains:

No	Reagents	Presentation
1	Microplate strips Microplate wells are coated with recombinant pTp17 and pTp47 antigens.	2 plates
2	Washing solution concentrate Phosphate buffer, containing 2.2 % Triton X100.	3 bottles 3 × 25 ml
3	Positive control Human serum reactive for antibodies to T.pallidum. Inactivated by heating.	1 vial 1 × 0.8 ml
4	Negative control Heating inactivated human serum nonreactive for hepatitis B surface antigen (HBsAg) and antibodies to T.pallidum, HIV and (HCV).	1 vial 1 × 1.8 ml
5	Conjugate concentrate Recombinant T.pallidum antigens bound to a horseradish peroxidase (HRP). Preservatives: 0.1 % MIT.	1 vial 1 × 0.45 ml
6	Conjugate diluent Phosphate buffer, containing powdered milk. Preservatives: 0.01 % thimerosal.	1 bottle 1 × 20 ml
7	TMB solution Solution containing 0.03 % 3,3',5,5'-tetramethylbenzidine.	1 bottle 1 × 14 ml
8	Substrate buffer Citrate-phosphate buffer, containing 0.016 % hydrogen peroxide.	1 bottle 1 × 14 ml
9	Stop-reagent 0.5M sulphuric acid solution.	1 bottle 1 × 25 ml
10	Adhesive film	6 items

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

Specimens with absorbance value lying within 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:

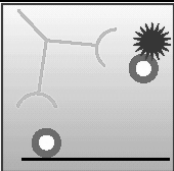
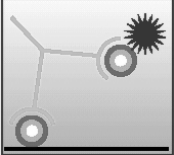
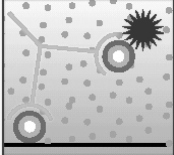
- specimens reactive in one or more wells are considered as reactive ones;
- specimens nonreactive in two or more wells are considered as nonreactive ones.

All repeatedly reactive results should be confirmed with an appropriate method.

LIMITATION OF THE PROCEDURE

All highly sensitive immunoassay systems have a potential for nonspecific reactions therefore the specificity of repeatable reactive specimens should be verified using appropriate test methods.

OVERVIEW OF PROCEDURE

<ul style="list-style-type: none"> • Dispense 60 µl of conjugate solution and 30 µl both control specimens and investigated sera into wells 	
<ul style="list-style-type: none"> • Incubate for 60 min at 37 °C (forming complex antigen-antibody with conjugate) • Wash 8 times with washing solution 	
<ul style="list-style-type: none"> • Dispense 100 µl TMB substrate into 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 	

- Distribute in the wells as follows:
 - wells A1, B1 : 30 µl of **positive control**.
 - wells C1, D1, E1 : 30 µl of **negative control**.
 - the rest wells : 30 µl of **samples**.

Carefully repipette mixture in wells. (During repipetting solution turns its colour).
- Cover the plate with adhesive film and incubate at 37 °C for 60 minutes.
- Aspirate the contents of all wells and wash the plate with **washing solution** 8 times (according the section *Wash procedure*). If necessary, dry the plate by slight tapping upside-down on absorbent paper.
- Pipette into each well 100 µl of the **TMB substrate**.
- Cover the plate with adhesive film and incubate at 18-25 °C for 30 minutes in the dark.
- Add into each well 100 µl of **stop-reagent** to stop colour reaction (maintain the same pipetting sequence and rate used for TMB substrate dispensing).
- Read the absorbance at 450/620 nm using a dual wavelength microplate reader within 5 minutes after stopping the reaction.
Absorbance may be measured at 450 nm (single wavelength) against a blank well; for that purpose 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 than twice exceeded the \overline{NC} , and recalculate \overline{NC} using rest controls.
- Calculate \overline{PC} .
Test run is valid if \overline{PC} is not lower than 0.600.
- Calculate **Cut-off** value.
$$\text{Cut-off} = \overline{NC} + \underline{0.2}$$
- Determine the grey zone.
Grey zone is the zone with sample absorbance within the range
$$\text{Cut-off} - 10 \% \leq OD \leq \text{Cut-off}.$$

Interpretation of the results

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

ADDITIONAL MATERIALS AND INSTRUMENTS REQUIRED

- distilled or deionized water;
 - disposable gloves;
 - disposable V-shaped troughs;
 - vial for reagents 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 dual wavelength 450/620);
 - 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. 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 buffer, TMB solution and stop-reagent with skin and mucous covers.
- The negative control contains 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 (18-25°C) before beginning the assay and can remain at room temperature during testing. After use return reagents to 2-8°C.

Reagents preparation (for 16 wells)

Microplate strips

Open the pack and remove required number of strips. Leave unused strips in the pack. Reseal the pack and return to 2-8 °C.

The plate is 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. The content of one bottle is intensively shaken. Take 6 ml of the **washing solution concentrate** and dissolve in 270 ml of the distilled water then mix. If some crystals are still observed, the solution can be heating (35-37 °C) before use until crystals are to be completely dissolved.

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

Conjugate solution

Take 1.5 ml of the **conjugate concentrate** and place it in the clean vessel then add 30 µl of the **conjugate** (concentrate 51x). Mix well avoiding foaming.

Conjugate solution has to be prepared before use.

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

TMB substrate

To prepare **TMB substrate**, mix 1 ml of **TMB solution** and 1 ml of the **substrate buffer** in a clean vial. The mixture is intensively shaken. TMB substrate is to be colourless before use.

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

The substrate solution is to be prepared before use.

TMB substrate is stable for 2 weeks at room temperature (18-25 °C) if kept in the dark.

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.
- Reagents and samples should be at room temperature (18-25 °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 18-25 °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 solution and TMB substrate.
- Check the pipettes and other equipment for accuracy and correct operation.
- Do not change the assay procedure.

Wash procedure

Washing is to 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;
- allow to soak during 40-60 seconds;
- completely aspirate the solution from wells.

Make sure that no fluid remains on the top and the bottom of the plate 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

- Pipette 60 µl of the **conjugate solution** into each well of the plate.