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Version: 04/2006

Diaproph Med
Diagnostics Prophylaxis Medicine



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Diaproph Med

Diagnostics Prophylaxis Medicine

DIA-IgG-IgM-Trep

Enzyme immunoassay for the detection of IgG and IgM classes antibodies against Treponema

192 test

Product code: T-2306

INTENDED USE

EXAMPLE

Enzyme immunoassay is intended for the detection of IgG and IgM classe antibodies to *Treponema pallidum* in human serum and plasma.

INTRODUCTION

Syphilis is an infectious disease caused by *Treponema pallidum*, which is characterized skin-lesion, and also mucous, viscera, bones and nervous system injuries. Syphilis is characterized by months of clinical disease followed by years of latency with the potential for relapse to debilitating or lethal late disease if left untreated. The infection is mainly acquired via sexual transmission, but there is possibility to be infected through a placental transmission (congenital syphilis), in private life (contact syphilis) and with infected blood.

The actual tendency of the prevention of syphilis prevalence is a determination infectious patients among human population. For a diagnostics of syphilis laboratory assays for the detection of anti-*T.pallidum* specific antibodies are used. Those tests are designed using cardilepidic antigens or *Treponema pallidum* specific antigens based on enzyme immunoassay approaches.

Today the world diagnostic market offers the variety of ELISA kits for serological syphilis diagnostics. The high sensitivity and specificity of such test kits are stipulated because of using recombinant antigens being analogues of *T. pallidum* proteins (pTp15, pTp17, pTp41 and pTp47), which are not homology with antigens of non pathogenic spirochetes.

General description

DIA-IgG-IgM-Trep is a kit that includes the following main components: *The microplate strips* (a solid phase) – polystyrene plate coated with recombinant proteins (Tpp15, Tpp17, Tpp41 and Tpp47) – analogues of *Treponema pallidum* antigens; the *conjugate concentrate* is a mixture of monoclonal antibodies to human IgG and IgM conjugated with a horseradish peroxidase; the *positive control* is purified human immu-

noglobulines specific to *Treponema pallidum*; the *negative control* is inactivated human serum not containing antibodies to *Treponema pallidum*, HCV, HIV and hepatitis B surface antigen (HBsAg); the *washing solution concentrate* is a phosphate saline buffer with a detergent; the *specimen and conjugates diluents* are phosphate saline buffers with a detergent, casein fraction of milk, block components, dyestuff and preservatives; the *substrate buffer* is a citrate phosphate buffer with a hydrogen peroxide; the *chromogen* is 3,3',5,5'-tetramethylbenzidine (TMB) solution; the *stop-reagent* is a solution of sulphuric acid.

The appearance of components: the *microplate strips* – the plate composing of 6 strips (16 wells each); the *conjugate concentrate* – a red opalescent liquid; *positive and negative controls* are slightly straw opalescent liquid; the *washing solution concentrate* is colourless opalescent liquid, a segregation and formation of crystalline sediment in the solution is allowed that can be dissolved during heating; the *specimen diluent* is a violet opalescent liquid; the *conjugate diluent* is a red opalescent liquid; the *substrate buffer*, *TMB solution* and *stop-reagent* are transparent colourless liquids.

The kit is intended for 192 determinations including controls. Also separate strips can be used for 12 performances of enzyme immunoassay (12 x 16).

PRINCIPLE OF PROCEDURE

The principle of DIA-IgG-IgM-Trep assay is based on solid-phase indirect ELISA.

When investigated specimens of human plasma or serum are placed into wells, antibodies of IgG and IgM types specific to *T. pallidum* if they are present in specimen are bound to recombinant antigens on the solid phase forming antigen-antibodies complexes. Formed complexes are detected using the specific conjugate.

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

The peroxidase reaction is stopped by adding the stop-reagent then determine the optical density of serum specimens at 450/620 nm.

STORAGE CONDITIONS AND TRANSPORTATION

The kit must be stored and transported at 2-8°C. Freezing of the kit is not allowed. The shelf life of the kit is 14 months.

KIT REAGENTS

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

No	Reagents	Presentation
1	Washing solution concentrate (46x)	3 bottles × 25 ml
2	Microplate strips	2 plates (12 x 16)
3	Specimen diluent	1 bottle × 20 ml
4	Conjugate diluent	1 bottle × 26 ml
5	Substrate buffer	1 bottle × 14 ml
6	TMB solution	1 bottle × 14 ml
7	Positive control	1 vial × 0.6 ml
8	Negative control	1 vial × 0.9 ml

Test run is valid if \overline{NC} is not higher than 0.100 OU and \overline{PC} is not lower than 0.6 OU.

If one of three values of NC is higher than 0.100 or more than twice exceed \overline{NC} , this value is to be excluded and \overline{NC} is recalculated considering remaining values of NC.

- Calculate **Cut-off** value.

$$\text{Cut-off} = \overline{NC} + 0.12$$

- 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.

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

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

Specimens shown 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.

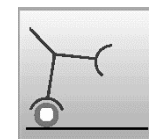
All repeatedly reactive results should be confirmed with an appropriate method.

LIMITATION OF THE PROCEDURE

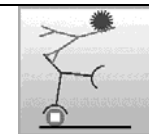
When testing in DIA-IgG-IgM-Trep as highly sensitive immunoassay, a potential non-specific reactions must be taken into account. Therefore, the specificity of repeatable positive specimens must be verified using complementary test methods.

OVERVIEW OF PROCEDURE

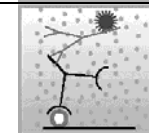
- Wash once with washing solution
- Dispense 80 µl of specimen diluent and 20 µl both controls and investigated samples in wells
- Incubate for 60 min at 37°C (forming complex antigen-antibody)
- Wash 4 times with washing solution



- Dispense 100 µl of conjugate solution in wells
- Incubate for 30 min at 37°C (forming complex antigen-antibody with conjugate)
- 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



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

Follow this procedure in each washing:

- aspirate wells contents completely;
- then fill wells completely with washing solution (not less than 350 µl per well) avoiding buffer overflow from one well to the 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 80 µl of the **specimen diluent** into each well.
- Distribute in the wells as follows:
 - wells A1, B1 : 20 µl of **positive control**.
 - wells C1, D1, E1 : 20 µl of **negative control**.
 - the rest wells : 20 µl of investigated **specimens**.

Carefully repipette mixture in wells. (During repipetting solution turns its colour).

- Cover the plate with an adhesive film and incubate at 37°C for 60 minutes.
- Aspirate the contents of all wells and wash the plate with the **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.
- Cover the plate with an adhesive film and incubate at 37°C for 30 minutes.
- Aspirate the contents of all wells and wash the plate with the **washing solution** 6 times (according the section *Wash procedure*). If necessary, dry the plate by slight tapping upside-down on absorbent paper.
- Pipette 100 µl of the **TMB substrate** into each well.
- Cover the plate with an adhesive film and incubate at 18-25°C for 30 minutes in dark.
- Add 100 µl of **stop-reagent** into each well 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.

As an exception, absorbance may be measured at 450 nm (single wavelength) against a blank well; for that 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

Validation criteria

- Calculate \overline{NC} and \overline{PC} .

9	Conjugate concentrate (51x)	1 vial × 0.6 ml
10	Stop-reagent	1 bottle × 25 ml
11	Adhesive film	6 items

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

- DIA-IgG-IgM-Trep kit is only intended for professional application and not to be subject to free sale in chemist's shop system. Performance of DIA-IgG-IgM-Trep can be only realised in specially equipped diagnostic laboratories for ELISA that has appropriate permission (accreditation) of national state bodies/authorities. Special trained and skilled personnel is allowed to conduct assay performance.
- 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 β-propiolacton. The absence of residual infectious capability of the positive control is confirmed by the validation. HOWEVER, ALL SAMPLES, CONTROLS AND MATERIALS USED FOR THE TEST PERFORMANCE MUST BE TREATED AND HANDLED AS BEING POTENTIALLY INFECTIOUS AND APPROPRIATE SAFETY PRECAUTIONS MUST BE TAKEN. The negative control has been tested and found non-reactive for hepatitis B surface antigen (HBsAg), antibodies to T.pallidum, HCV and HIV (HIV-1, HIV-2), however for the purpose of additional protection treated with heating.
- Any equipment directly in contact with specimens and reagents as well as the washing solution be considered as contaminated products and treated to good laboratory practice.

- 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 materials and products prior to utilization or discarding must be decontaminated following one of below mentioned methods:
 - 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 T.pallidum, 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 can be stored at 2-8°C but no longer than 72 hours. If necessary those specimens may be frozen (not more than five 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, hyperlipidemiae (high lipaemic) or bacterial contamination of human serum or plasma cannot be used in ELISA procedure of DIA-IgG-IgM-Trep. Negative samples with high level of bilirubin may lead to unreliable results and should be considered as risk of false positive results.

DIA-IgG-IgM-Trep is intended for testing of serum or plasma specimens obtained from blood preparations containing citrate, heparin and EDTA as anticoagulants. And others blood preparations such as purified globulins, albumins, blood factors etc. cannot be as samples for testing in DIA-IgG-IgM-Trep.

ASSAY PROCEDURE

Reagents and specimens should reach a room temperature (18-25°C) before the assay performance and can remain at room temperature during testing. After use reagents should be returned to 2-8°C.

Reagents preparation (for 16 wells)

Microplate strips

Open the pack and remove the strip plate. Return unused strips in the pack. Reseal the pack and store at 2-8 °C.

The strips are stable for 30 days 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 the one bottle is intensively shaken. 6 ml of the concentrate is taken from the bottle and dissolved in 270 ml of the distilled or deionised water.

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

Conjugate solution

Dilute 400 µl of the **Conjugate concentrate** with 2.0 ml of the **Conjugate diluent** in a clean vial. Mix well avoiding foaming.

Conjugate solution is to be prepared before use.

TMB substrate

In order to prepare **TMB substrate**, dilute 1.0 ml of the **TMB solution** with 1.0 ml of the **Substrate buffer** in a clean vial (1:1). Mix well. TMB substrate must be colourless before use.

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

The solution is to be prepared before use.

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.
- The temperature in room where performing analysis should be in the range 18-25°C.
- Reagents and samples should be at room temperature before testing begins. Return the reagents to 2-8 °C after use.
- 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 must be performed strictly according to the instructions, as insufficient plate washing leads to incorrect results.