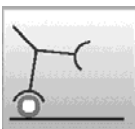
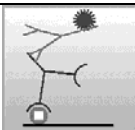


## OVERVIEW OF PROCEDURE

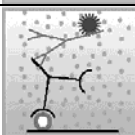
- Wash once with washing solution
- Dispense 80 µl of specimen diluent and 20 µl both control and investigated sera in wells
- Incubate for 60 min at 37°C
- Wash 4 times with washing solution



- 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-Chlamydia

**Enzyme immunoassay for the detection  
of IgG and IgA classes antibodies  
to the Chlamydia trachomatis**

96 tests

Product code: T-0907C

## INTENDED USE

Enzyme immunoassay kit is intended for the detection of antibodies of IgG and/or IgA types to Chlamydia trachomatis in human serum and plasma.

## EXAMPLE FORM

## INTRODUCTION

An infection caused by Chlamydia trachomatis is sexually transmissible disease and in approximately 70% case is subclinical infection, which can lead to female sterility, spontaneous abortion, still dead birth, male sterility and impotence.

Today approaches in the diagnostics of Chlamydia trachomatis infection include a combination of not less than two methods – direct and indirect. The first ones allow detecting of infectious agent. The indirect methods are based on serological investigations of human serum or plasma (detecting of antibodies). The latter allow avoiding false-positive results on the one hand and determine a stage and course of disease.

While immune response IgA antibodies appear in 10-14 day after infection. The level of such antibodies is going down in case of successful treatment and going up when reinfected. The high titre of IgA is evidence of chronic infection. IgG antibodies can be detected beginning from third week of infection. Low titres of latter antibodies can be presented in human serum during many years and indicate about undergone disease. In order to give final diagnosis IgA and IgG antibodies are to be simultaneously detected. Among all serological methods ELISA technique has the most sensitivity and specificity.

## PRINCIPLE OF PROCEDURE

The main components of the assay are a solid phase (microelisa stripplate) and an immunoenzyme conjugate. The solid phase is coated with Major Outer Membrane Protein of Chlamydia trachomatis. The conjugate (anti-IgG and/or anti-IgA) monoclonal antibodies conjugated with a horseradish peroxidase.

When investigated specimens of human plasma or serum are placed into wells, specific antibodies to Chlamydia trachomatis presented in specimen bind to recombinant antigens

on solid phase forming antigen-antibodies complexes. Formed complexes are detected with the specific conjugates (anti-IgA and/or anti-IgG). The substrate buffer (hydrogen peroxide) and TMB solution are added to wells after washing non-bound components. The solution is coloured in case of presence of peroxidase conjugate in complexes.

To stop the colour reaction the stop-reagent is to be added 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 12 months.

## KIT REAGENTS

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

No	Reagents	Presentation
1	<b>Stock washing solution (№1)</b> (concentrate 45x) Phosphate buffer, containing 2.2% Triton X100.	2 bottles 2 × 25 ml
2	<b>Microelisa strips</b> 12 strips per plate each with 8 wells coated with Major Outer Membrane Protein of Chlamydia trachomatis.	1 plate
3	<b>Solution for sera dilution (№3)</b> Phosphate buffer, containing skimmed powdered milk. Preservatives: 0.01% thimerosal.	1 bottle 1 × 15 ml
4	<b>Solution for conjugate dilution (№4)</b> Phosphate buffer, containing skimmed powdered milk. Preservatives: 0.01% thimerosal.	1 bottle 1 × 15 ml
5	<b>Substrate buffer</b> Citrate-phosphate buffer, containing 0.016% hydrogen peroxide.	1 bottle 1 × 8 ml
6	<b>Chromogen TMB</b> Solution containing 0.03% 3,3',5,5'-tetramethylbenzidine.	1 bottle 1 × 8 ml
7	<b>Positive control IgG</b> Human serum containing IgG antibodies to MOMP of Chlamydia trachomatis. Preservatives: 0.1 % sodium azide.	1 vial 1 × 0.3 ml
8	<b>Positive control IgA</b> Human serum containing IgA antibodies to MOMP of Chlamydia trachomatis. Preservatives: 0.1 % sodium azide.	1 vial 1 × 0.3 ml
9	<b>Negative control (C-)</b> Heating inactivated human serum nonreactive for hepatitis B surface antigen (HBsAg) and antibodies to HIV and HCV, MOMP of Chl. trachomatis. Preservatives: 0.1% sodium azide.	1 vial 1 × 1.0 ml
10	<b>Conjugate concentrate anti-IgG</b> Monoclonal antibodies against human IgG bound to a horseradish peroxidase (HRP). Preservatives: 0.01% thimerosal.	1 vial 1 × 1.5 ml
11	<b>Conjugate concentrate anti-IgA</b> Monoclonal antibodies against human IgA bound to a	1 vial 1 × 1.5 ml

If OD of investigated sample exceed 2.5 OU, it is recommended investigation of such serum to be done after preliminary dilution 1:10 with the solution for serum dilution (a finite dilution in a well 1:50). In that case result calculation is conducted according to the following:

$$DU = \frac{OD_{\text{serum}} \times 100}{\text{cut-off}}$$

Results are interpreted as follows:

> 10 DU	<b>positive</b>
9 – 10 DU	<b>undetermined</b>
< 9 DU	<b>negative*</b>

## Interpretation of the results

Presence of antibodies to Chlamydia trachomatis		Result interpretation
IgG	IgA	
Negative	Negative	Negative, or the samples contains very low antibody titer *
Negative	Indetermined or positive	Possibility of early phase of infection*
Indetermined or positive	Negative	Past infection
Positive	Positive	Acute or chronic infection

\* In case of early Chlamydia infection, the result can be negative. When clinical presentations are apparent, a performance of repeated testing in 14-21 days is recommended.

## LIMITATION OF THE PROCEDURE

During diagnostics results should be interpreted in conjunction with the patient' s clinical condition and the results of others diagnostics procedures.

- well A1: 20 µl of **positive control IgG or positive control IgA** depending on which antibodies are to be detected in investigated samples.
- wells B1, C1, D1: 20 µl of **negative control (C-)**.
- the rest wells : 20 µl of **specimens**.

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 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** anti-IgG or anti-IgA, depending on which antibodies are to be detected in investigated samples, into each well.
- 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 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 addition).
- 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

#### Validation characteristics of assay

NC – absorbance of the negative control

PC – absorbance of the positive control

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

- Calculate the mean absorbance of the negative control.

Test run is valid if  $\overline{NC}$  is not higher than 0.100 optical units (OU).

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

Test run is valid if PC is not lower than 0.600 OU.

- Calculate **Cut-off** value.

$$\text{Cut-off} = \overline{NC} + 0.120$$

- Determine the grey zone.

Grey zone is the zone with sample absorbance within the range

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

### Evaluation of results in DIA Units

- DIA Units (DU) are recommended for evaluation of antibody titre changing in dynamics:

$$DU = \frac{OD_{\text{serum}} \times 10}{\text{cut-off}}$$

No	Reagents	Presentation
	horseradish peroxidase (HRP). Preservatives: 0.01% thimerosal.	
12	<b>Stop-reagent</b> 0.5 M sulphuric acid solution.	1 bottle 1 × 15 ml
13	<b>Adhesive film</b>	3 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

- 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 positive and negative controls were tested and found nonreactive for hepatitis B surface antigen (HBsAg), antibodies HCV and HIV (HIV-1, HIV-2).
- 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 (18-25°C) before beginning the assay and can remain at room temperature during testing. Return reagents to 2-8°C after use.

### Reagents preparation

#### Microelisa strips

Open the pack and remove the plate. 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 **Stock washing solution (№1)** for the presence of salt crystals. If crystals are seen in the solution, dissolve them by heating at 35-37°C.

Dilute the 4 ml of the **stock washing solution (№1)** with 180 ml of the distilled or de-ionised water, shake intensively to dissolve any crystals that may be present.

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

#### Conjugate solution

Dilute 1 ml of the **solution for conjugate dilution (№4)** with 100 µl of the **concentrate conjugate** (11x) anti-IgG or anti-IgA depending on which antibodies are to be detected in investigated samples with in a clean vial. Mix well avoiding foaming.

Conjugate solution has to be prepared before use.

**Conjugate solution** is stable for 6 hours at 2-8°C.

#### TMB substrate

In order to prepare **TMB substrate**, dilute 0.5 ml of the **chromogen TMB** with 0.5 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 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 (18-25°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 (solution for chromogen preparation + chromogen TMB) 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 80 µl of the **solution for sera dilution (№3)** into each well.
- Distribute in the wells as follows: