ELISA-VIDITEST anti-Chlamydia trachomatis IgM
Instruction manual


1. TITLE:
ELISA-VIDITEST anti-Chlamydia trachomatis IgM – Elisa kit for the detection of IgM antibodies against Chlamydia trachomatis in human serum.

2. INTENDED USE:
ELISA-VIDITEST anti-Chlamydia trachomatis IgM kit is intended for the detection of anti-Chlamydia trachomatis antibodies in human serum. The laboratory diagnostic procedures in C. trachomatis infections involve the direct pathogen detection (an isolation of the pathogen, direct antigen detection in cervical, vaginal and urethral swabs or nucleic acid amplification tests from urine – PCR, LCR) and serology tests that reveal specific IgG, IgA and IgM antibodies.

Primary chlamydial infection is characterized by the predominant IgM response after 2 to 4 weeks from infection and the delayed IgG and IgA response after 6 to 8 weeks. After the acute C. trachomatis infection IgM antibodies usually decrease and become undetectable in 2 to 6 months. IgG antibody titres decrease slowly, whereas IgA antibodies tend to disappear rapidly. When primary chlamydia infection is suspected, the detection of IgM is highly diagnostic. However, in recurrent or chronic infections the prevalence of IgM is low and therefore the absence of IgM does not necessarily exclude an on-going infection. In reinfections, IgG and IgA levels rise quickly. IgA antibodies have shown to be a reliable immunological marker of primary, chronic and recurrent infections. These antibodies usually decline rapidly to baseline levels following treatment and eradication of the chlamydia infection, however may persist for several months. The persistence of the elevated IgA antibody titres is generally considered as the sign of chronic infection.

3. TEST PRINCIPLE:
ELISA-VIDITEST anti-Chlamydia trachomatis IgM is a solid-phase immunoanalytical test. The purified, homogeneous antigen is fixed to each well of the microtiterstrips. Specific antibodies present in the patient’s sample are bound during the first incubation step. After removing unbound material by washing, the presence of specific antibodies is detected using anti-human IgM conjugate during the second incubation. The unbound peroxidase conjugate is then removed and TMB substrate is added, resulting in the development of a blue colour. The enzyme reaction is terminated by the addition of a stop solution. The intensity of the colour is proportional to the concentration of antibodies in the sample.

4. KIT COMPONENTS:
ELISA break-away strips coated with specific antigen 1 microtitre plate
1.2 mL Negative control r.t.u. 1 vial
1.2 mL Positive control r.t.u. 1 vial
1.2 mL Cut-off control r.t.u. 1 vial
12 mL Peroxidase conjugate (anti-IgM/Px) r.t.u. 1 vial
80 mL Wash buffer 25x concentrated 1 vial
100 mL Dilution buffer r.t.u. 1 vial
13 mL Chromogenic substrate (TMB) r.t.u. 1 vial
15 mL Stop solution r.t.u. 1 vial
Cover foil
bag with zipper + desiccant
Instruction manual
Certificate of quality
1) r.t.u., ready to use

5. MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED:

Distilled or deionised water, test tubes for sample dilution, timer, micropipettes, multipipettes 10-1000 µl, graduated cylinder, ELISA washer or multichannel pipette, ELISA reader (450 nm/ reference wavelength 630/620 nm), paper towels, pipette tips.

6. PREPARATION OF REAGENTS AND SAMPLES
a. Allow all kit components to reach room temperature.
b. Vortex samples, the Controls and Peroxidase conjugate in order to ensure homogeneity and mix all solution well prior use.
c. Dilute serum samples 1:100 in Sample diluent and mix (e.g. 5 µL of serum sample + 500 µL of Sample diluent).
d. Prepare Wash buffer by diluting the Wash buffer concentrate 25 times with an appropriate volume of distilled or deionised water (e.g. 40 mL of the concentrated Wash buffer + 960 mL of distilled water). If there are crystals of salt present in the concentrated Wash buffer, warm up the vial to +32 to +37°C in a water bath. Diluted Wash buffer is stable for 4 weeks if stored at +2 to +8°C.
e. Do not dilute the Controls, Peroxidase conjugate, Chromogenic substrate and Stop solution, they are ready to use.

7. ASSAY PROCEDURE:
a. Allow the vacuum-closed aluminium bag with strips to reach room temperature. Withdraw an adequate number of strips and put the unused strips into the provided bag and seal it carefully with the desiccant kept inside.
b. Pipette 100 µL of Dilution buffer, Controls and serum samples to the wells according to the pipetting scheme in Figure 1 (page 3): fill the first well with Dilution buffer (DB) to determine the reaction background. Fill the next two wells with Cut-off control (COC). The remaining wells fill with diluted serum samples (S1...). It is satisfactory to apply one serum into one well (S1, S2, S3...). However, if you want to minimize laboratory error, apply controls and samples as doublets. In order to take into consideration the pipetting time, it is recommended to repeat the COC well every 4 strips (or after a pipetting time >=5 min) and evaluate the following wells with the corresponding COC values for the calculation of the new cut-off value. Cover the wells with the provided membrane.
c. Incubate 30 minutes (+/- 2 min.) at room temperature.
d. Aspirate the liquid from the wells into a collecting bottle containing appropriate disinfectant (see Safety Precautions). Wash and aspirate the wells four times with 300 µl/well of Wash buffer. Avoid cross-contamination between wells!
  If some liquid remains in the wells, invert the plate and tap it on an adsorbent paper to remove the last remaining drops.
e. Add 100 µL Peroxidase conjugate r.t.u. into each well. Incubate 30 minutes (+/- 2 min) at room temperature.
f. Aspirate and wash four times with 300 µl/well of Wash buffer.
g. Dispense 100 µl of TMB substrate into each well. Incubate for 10 minutes (+/- 30 seconds) at room temperature. The time measurement must be started at the beginning of TMB dispensing. Cover the strips with an aluminium foil or keep them in the dark during the incubation with TMB substrate.
h. Stop the reaction by adding 100 µL of Stop solution. Use the same pipetting rhythm as with the TMB substrate to ensure the same reaction time in all wells. Tap gently the microplate for a few times to ensure complete mixing of the reagents.

i. Read the absorbance at **450 nm with a microplate reader within 10 minutes**. It is recommended to use reference reading at 630 (620) nm.

**Fig.1.: Pippeting scheme**

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<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>DB</td>
<td>S4</td>
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<td>b</td>
<td>COC</td>
<td>S..</td>
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<td>c</td>
<td>COC</td>
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<td>S29</td>
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<td>NC</td>
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<tr>
<td>e</td>
<td>PC</td>
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<tr>
<td>h</td>
<td>S3</td>
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<td>S27</td>
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</tbody>
</table>

**8. PROCESSING OF RESULTS:**

Begin the processing of results with subtraction of the background absorbance (absorbance of the DB well) from the absorbances of all other wells.

**8.1 Processing of results for Qualitative interpretation**

1. Compute the mean absorbance of the two wells of Cut-off control (COC). If you applied COC after every 4 strips, use the particular COC value for the corresponding samples.
2. Compute the **Cut-off value**. The Cut-off value is calculated from the absorbance of the Negative control and the absorbance of the Cut-off control.

   \[
   \text{Cut-off value} = \text{OD CC} + \text{OD NC}
   \]

3. Define the Cut-off range:

   \[
   \text{Cut-off range} = \text{Cut-off value} \pm 10\%
   \]

   - sample OD value < Cut-off value – 10% **NEGATIVE RESULT**
   - sample OD value > Cut-off value + 10% **POSITIVE RESULT**

   The result is equivocal if OD value sample ≥ Cut-off value – 10% and ≤ Cut-off value + 10 %. Equivocal results should be retested. If the result is again indifferent then it is recommended to use an alternative testing method or to obtain another sample from the patient, usually withdrawn 1-2 weeks later.
8.2. Semiquantitative evaluation

Determine the Positivity Index for each serum sample as follows:
1. Compute the Cut-off value (see previous paragraph 8.1)
2. Compute the Positivity Index according to the following formula:

\[
\text{Sample Positivity Index} = \frac{\text{OD value of sample}}{\text{Cut-off value}}
\]

3. Determine the serum reactivity according to the following table:

<table>
<thead>
<tr>
<th>Index value</th>
<th>Evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 0.9</td>
<td>Negative (-)</td>
</tr>
<tr>
<td>0.9 – 1.1</td>
<td>Equivocal (+/-)</td>
</tr>
<tr>
<td>&gt; 1.1</td>
<td>Positive (+)</td>
</tr>
</tbody>
</table>

Note! An indifferent sample reactivity, i.e. interpreted as +/-, requires repetition of the sample testing. If the result is again indifferent then it is recommended to use an alternative testing method or to obtain another sample from the patient, usually withdrawn 1-2 weeks later.

9. RESULT INTERPRETATION:

It is recommended to test serum samples for all three antibody classes (IgG, IgM and IgA) for the optimal result interpretation. In cases of acute chlamydial infections the serology results may be negative despite clinical symptoms and positive antigen detection. If a serological confirmation of a positive antigen result is desired we recommend testing after 10-14 days to find seroconversion.

(Note to table: – Negative, + Positive)

<table>
<thead>
<tr>
<th>Antibodies against C.trachomatis</th>
<th>Interpretation</th>
<th>Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgM</td>
<td>IgA</td>
<td>IgG</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>-</td>
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<td>+</td>
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<tr>
<td>+</td>
<td>+</td>
<td>+</td>
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</tbody>
</table>
10. CHARACTERISTICS OF THE TEST:

10.1. Validity of the test
The test is valid if:
   a. The background of the reaction (the absorbance of the Dilution buffer) is less than 0.100.
   b. The mean Cut-off control (COC) absorbance is higher than 0.200.
   c. OD value of Negative control (NC) is less than 0.100.
   d. Index value of Positive control (PC) should be ≥ 1.5 (OD PC/Cut-off value ≥ 1.5).

10.2. Precision and reproducibility of the test
The intraassay variability (within the test) and the interassay variability (between tests) were performed with samples of variable absorbance values.

10.2.1. Itraassay variability
(n = number of parallels) :

<table>
<thead>
<tr>
<th>n</th>
<th>A</th>
<th>SD</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>0.380</td>
<td>0.028</td>
<td>7.4%</td>
</tr>
<tr>
<td>20</td>
<td>1.315</td>
<td>0.063</td>
<td>4.8%</td>
</tr>
</tbody>
</table>

10.2.2. Interassay variability

<table>
<thead>
<tr>
<th>n</th>
<th>A</th>
<th>SD</th>
<th>min – max</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0.465</td>
<td>0.042</td>
<td>0.421 – 0.552</td>
<td>9.0%</td>
</tr>
<tr>
<td>11</td>
<td>1.558</td>
<td>0.101</td>
<td>1.431 – 1.732</td>
<td>6.5%</td>
</tr>
<tr>
<td>11</td>
<td>2.153</td>
<td>0.141</td>
<td>1.856 – 2.238</td>
<td>6.5%</td>
</tr>
</tbody>
</table>

10.3. Diagnostic sensitivity and specificity of the test
Evaluation was performed on 48 serum samples that were previously tested with another commercially available diagnostic test (ELISA/MIF). The determined diagnostic sensitivity of the test was 100% and diagnostic specificity was 93.3%. For the calculation of the diagnostic sensitivity and specificity, the indefinite results were interpreted as positive.

10.4. Interaction
Crossreactions between C. trachomatis and other members of the Chlamydiaceae family (C. pneumoniae and C. psitacci) may occur due to the similarity of the antigens among these bacteria. In the case of positive or equivocal results it is advisable to exclude the above-mentioned species with an appropriate test (MIF, Western blot).

In addition, cross reactivity with antinuclear antibodies and heterophilic antibodies could not be in individual cases rejected.

Lipaemic, hemolytic or icteric samples should only be tested with reservations although in our experience they have no influence on results.

11. SAFETY PRECAUTIONS
All ingredients of the kit are intended for laboratory use only.

Only qualified and well-trained employees should carry out the assay procedure.

Do not smoke, eat or drink during work. Do not pipette by mouth. Wear disposable gloves while handling reagents or samples and wash your hands thoroughly afterwards. Avoid spilling or producing aerosol.

Controls contain human sera that has been tested negative for HBsAg, anti-HIV-1,2 and anti-HCV. However they should be regarded as contagious and handled and disposed of according to the appropriate regulations.
Autoclave all reusable materials that were in contact with human samples for 1 hour at 121°C, or disinfect with 3% chloramines for 30 minutes. Decontaminate liquid wastes with disinfection solution (Incidure, Incidine, chloramine). Liquid wastes containing acid (Stop solution) should be neutralized in 4% sodium bicarbonate solution.

Handle Stop solution with care. Avoid contact with skin or mucous membranes. In case of contact with skin, rinse immediately with plenty of water and seek medical advice.

Controls containing sodium azide may react with lead and copper plumbing, building up explosive metal acids. Flush with sufficient water when disposing of reagents.

12. HANDLING PRECAUTIONS:
   a. If user modifies the assay procedure mentioned in this Instruction manual then the user has to validate that method and be responsible for its use.
   b. Manufacturer guarantees performance of the entire ELISA kit. Washing solution 25x conc., Stop solution r.t.u. a dilution buffer r.t.u can be used in ELISA-VIDITEST anti-Chlamydia trachomatis a C. pneumoniae IgG, IgM and IgA. The TMB solution r.t.u. is interchangeable only with the same lot on the bottle.
   c. Avoid microbial contamination of serum samples and kit reagents. Avoid cross-contamination of reagents.
   d. Peroxidase conjugate and Sample diluent are conserved with 0.049% Thiomersal.
   e. Controls are conserved with 0.095% sodium azide.
   f. Avoid contact of the TMB with oxidizing agents or metal surfaces.
   g. Follow the assay procedure indicated in the Instruction manual. Variations in the test results are usually due to:
      * Insufficient mixing of reagents and samples
      * Inaccurate pipetting and inadequate incubation times
      * Poor washing technique or spilling the rim of well with sample or Peroxidase conjugate
      * Use of identical pipette tip for different solutions

13. STORAGE AND EXPIRATION:
   a. Store the kit and the kit reagents at +2 to +8°C in a dry place and protected from the light, avoid from freezing
   b. Use only intact vacuum-sealed strips. Store unused strips in the sealable pouch and keep the desiccant inside. These strips are then stable for 4 weeks.
   c. Unused diluted washing buffer is stable for 4 weeks when stored at +2°C to +8°C.
   d. Suitable specimens are serum or plasma (heparinized) samples obtained by standard techniques. The samples should not be heat-inactivated since non-specific results may occur. Store the unused undiluted tested samples in aliquots at -18°C to -28°C. Repeated freezing a thawing is not recommended. If you wish to store serum samples at +2°C to 8°C use them within one week.
   e. Do not store diluted samples, use them immediately.
   f. Kits are shipped in cooling bags, the transport time of 72 hours have no influence on expiration.
   g. If you find damage at any part of the kit, please inform the manufacturer immediately.
   h. Expiration date is indicated at the ELISA kit label and at all reagent labels.
14. FLOW CHART:

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**Step 1.** Prepare reagents and samples

**Step 2.** Dispense 100 μL/well controls and samples

↓

Incubate 30 minutes at room temperature

↓

Wash 4 times (3000 μL/well), aspirate

↓

**Step 3.** Dispense 100 μL/well of peroxidase conjugate

↓

Incubate 30 minutes at room temperature

↓

Wash 4 times (300 μL/well), aspirate

↓

**Step 4.** Dispense 100 μL/well of chromogenic substrate (TMB)

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Incubate 10 minutes at room temperature

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**Step 5.** Dispense 100 μL/well of stop solution

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**Step 6.** Read the absorbance at 450 nm within 10 minutes

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General references:


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Date of the last revision of this manual: 09/2010
Next recommended revision: 09/2011