ELISA-VIDITEST anti-Chlamydia pneumoniae IgM

ODZ-184

Instruction manual


1. TITLE
ELISA-VIDITEST anti-Chlamydia pneumoniae IgM – ELISA kit for the qualitative determination of IgM antibodies to Chlamydia pneumoniae in human serum and plasma.

2. INTENDED USE
ELISA-VIDITEST anti-Chlamydia pneumoniae IgM kit is intended for the detection of anti-Chlamydia pneumoniae IgM antibodies in human serum and plasma.

The laboratory diagnostic procedures in C. pneumoniae infections involve an isolation of the pathogen from cell culture, direct antigen detection, nucleic acid amplification tests and serology tests. Difficulties in sample collection and inaccessibility of culture and non-culture methods have made serology the method of choice. Besides the microimmuno-fluorescence method (MIF), antibodies to C. pneumoniae can also be detected by an enzyme immunoassay.

Primary chlamydial infection is characterized by the predominant IgM response within 2 to 4 weeks and the delayed IgG and IgA response within 6 to 8 weeks. After the acute C. pneumoniae 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. The persistence of the elevated IgA antibody titres is generally considered as the sign of chronic infection.

3. TEST PRINCIPLE
ELISA-VIDITEST anti-Chlamydia pneumoniae 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 the 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 addition of the stop solution. The intensity of the yellow colour thus developed is proportional to the concentration of antibodies in the sample.
4. KIT COMPONENTS

ELISA break-away strips coated with the specific antigen STRIP Ag 12 pieces
1.2 mL Negative control r.t.u. 1) CONTROL - 1 vial
1.2 mL Positive control r.t.u. CONTROL + 1 vial
1.2 mL Cut-off control r.t.u. CUTOFF 1 vial
12 mL Peroxidase conjugate (anti-IgM/Px) r.t.u. CONJ 1 vial
7.5 mL RF sorbent r.t.u. RF SORB 1 vial
80 mL Wash buffer 25x concentrated WASH 25x 1 vial
100 mL Dilution buffer r.t.u. DIL 1 vial
13 mL Chromogenic substrate (TMB substrate) r.t.u. TMB 1 vial
15 mL Stop solution r.t.u. STOP 1 vial
Cover membrane
Bag with zipper + desiccant
Instruction manual
Certificate of quality
1) r.t.u., ready to use

Wash buffer 25x conc., stop solution r.t.u., dilution buffer r.t.u. and chromogenic substrate TMB r.t.u. are intended for ELISA-VIDITEST anti-Chlamydia trachomatis and pneumoniae kits only and they are not interchangeable with another ELISA-VIDITEST kits produced by VIDIA spol. s r.o.

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, thermal incubator (37 °C) with high relative humidity.

6. PREPARATION OF REAGENTS AND SAMPLES

a. Allow all kit components to reach room temperature.
b. Vortex samples, Controls and Peroxidase conjugate in order to ensure homogeneity and mix all solution well prior use.
c. Dilute serum samples 1:50 in Dilution buffer and mix (e.g. 10 µL of serum sample + 500 µL of Dilution buffer). Then add RF sorbent. RF sorbent contains anti-human IgG antibodies to saturate IgG and rheumatoid factor. Diluted serum sample (1:50) mix 1:1 with RF sorbent (e.g. 75 µL of diluted serum sample + 75 µL of RF sorbent). The final serum sample dilution is 1:100. Incubate for 30 minutes at room temperature or incubate over night at +2 °C to +8 °C.
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. Controls should not be pre-treted with RF sorbent.
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 Sample diluent, Controls and serum samples to the wells according to the pipetting scheme in Figure 1: Fill the first well with Dilution buffer (DIL) to determine the reaction background. Fill the next two wells with Cut-off control (CUTOFF). The next wells fill with Negative control (CONTROL-) and Positive control (CONTROL+). 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 a laboratory error, apply controls and samples as doublets. Cover the strips with an adhesive seal and incubate 60 minutes (+/- 5 min) at 37 °C (+/- 1 °C).

Note: In order to take into consideration the pipetting time, it is recommended to repeat the Cut-off control well every 4 strips (or after a pipetting time ≥ 5 min) and evaluate the following wells with the corresponding Cut-off control values for the calculation of the new cut-off value.

c. Aspirate the liquid from 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 with a soaking time approx. 30 seconds. 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.

d. Add 100 µL Peroxidase conjugate r.t.u. into each well. Cover the strips with an adhesive seal and incubate 30 minutes (+/- 2 min) at 37 °C (+/- 1 °C).

e. Aspirate and wash four times with 300 µl/well of Wash buffer as in section c above.

f. Dispense 100 µl of TMB into each well. Incubate 15 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.

g. Stop the reaction by adding 100 µL of Stop solution. Use the same pipetting rhythm as with the TMB to ensure the same reaction time in all wells. Tap gently the microplate for a few times to ensure complete mixing of the reagents.

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

Note: Do not allow the wells to dry out between incubations. Comply the test with the given incubation temperatures and times.

Fig. 1: Pipetting scheme

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>DIL</td>
<td>S4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b</td>
<td>CUTOFF</td>
<td>S...</td>
<td>S28</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c</td>
<td>CUTOFF</td>
<td></td>
<td></td>
<td>S29</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d</td>
<td>CONTROL-</td>
<td></td>
<td></td>
<td>S...</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>e</td>
<td>CONTROL+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>f</td>
<td>S1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>g</td>
<td>S2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>S27</td>
<td></td>
</tr>
<tr>
<td>h</td>
<td>S3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>S2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
8. PROCESSING OF RESULTS

Begin the processing of results with subtraction of the background absorbance (absorbance of the DIL 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 (CUTOFF). If you applied Cut-off control after every 4 strips, use the particular Cut-off control value for the consecutive 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.

\[
\text{Cut-off value} = \text{OD (CONTROL -)} + \text{OD (CUTOFF)}
\]

3. Define the Cut-off range: \( \text{Cut-off range} = \text{Cut-off value} \pm 10\% \)
   - sample OD value < Cut-off value – 10\% \hspace{0.5cm} \text{NEGATIVE RESULT}
   - sample OD value > Cut-off value + 10\% \hspace{0.5cm} \text{POSITIVE RESULT}

The result is equivocal in range:

\[
\text{Cut-off value} – 10\% \leq \text{sample OD value} \leq \text{Cut-off value} + 10\%
\]

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. Following the confirmation of the equivocal result the monitoring of the patient’s antibodies is recommended in order to exclude unspecific reactions or cross-reactivity, which may also cause equivocal results.

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:

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

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. Following the confirmation of the equivocal result the monitoring of the patient’s antibodies is recommended in order to exclude unspecific reactions or cross-reactivity, which may also cause equivocal results.
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.

<table>
<thead>
<tr>
<th>Antibodies against ( C. pneumoniae )</th>
<th>Interpretation</th>
<th>Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>- - -</td>
<td>No specific antibodies detectable</td>
<td>No indication of infection. In suspicion of the acute infection a repeat of testing within 10 to 14 days is recommended. In case of justified clinical suspicion direct antigen detection in sputum or aspirate should be performed.</td>
</tr>
<tr>
<td>- + +</td>
<td>Possibility of an early stage of infection or solitary, persisting IgA</td>
<td>Retest IgG, IgM a IgA after 10 to 14 days to determine seroconversion.</td>
</tr>
<tr>
<td>- - +</td>
<td>-</td>
<td>Monitoring of IgG, IgM and IgA antibodies (sample collection within 10-14 days).</td>
</tr>
<tr>
<td>+ + +</td>
<td>Acute or recent infection possible</td>
<td>Monitoring of IgG, IgM and IgA antibodies to determine seroconversion.</td>
</tr>
<tr>
<td>+ - +</td>
<td>Reinfection or chronic infection possible</td>
<td>-</td>
</tr>
<tr>
<td>- + -</td>
<td>Recent infection possible</td>
<td>Monitoring of IgG, IgM and IgA antibodies to determine seroconversion.</td>
</tr>
<tr>
<td>+ + -</td>
<td>Indication of a past infection; Reinfection or chronic infection might also be possible</td>
<td>In case of clinical suspicion control tests of IgG and IgA antibodies within 10 to 14 days are recommended.</td>
</tr>
</tbody>
</table>

(Note to table: – Negative, + Positive)

10. VALIDITY, SPECIFICITY AND SENSITIVITY OF THE TEST

ELISA-VIDITEST anti-\( Chlamydia pneumoniae \) IgM kit is intended for the detection of anti-\( Chlamydia pneumoniae \) IgM antibodies in human serum and plasma. Suitable specimens are serum or plasma (heparinised) samples obtained by standard laboratory techniques. The samples should not be heat-inactivated since non-specific results may occur. Results on tests using CSF are not available.

10.1. Validity of the test

All controls/calibrators should be carried out with every test run. The test is valid if:

a. The background of the reaction (the absorbance of the Dilution buffer) is less than 0.100.

b. OD values of Negative control (CONTROL -) should be in range that is written in enclosed Quality control certificate.

c. The mean of Cut-off control (CUTOFF) absorbances should be in range that is written in enclosed Quality control certificate.

d. Index value of Positive control should should be in range that is written in enclosed Quality control certificate.

If the criteria are invalid retesting is required.
10.2. Precision and reproducibility of the test
Intra-assay reproducibility was determined by testing samples of different levels of antibody reactivity for at least 22 times in one test run. The coefficient of variation (CV) of the reactive IgM samples was < 10%.
Inter-assay reproducibility was determined by testing samples of different levels of antibody reactivity in 10 different test runs. The CV of the reactive IgM samples was < 10%.

10.3. Diagnostic sensitivity and specificity of the test
Diagnostic sensitivity of the test is 96.8% and diagnostic specificity is 81.8%. Evaluation was performed on 135 serum samples tested with another commercially available diagnostic test (ELISA/MIF).
For the calculation of the diagnostic sensitivity and specificity, the equivocal results were interpreted as positive. The results refer to the groups of samples investigated.

10.4. Interaction
Crossreactions between C. pneumoniae and other members of the Chlamydiaceae family (C. trachomatis 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 rejected in individual cases.
Lipaemic, hemolytic or icteric samples should only be tested with reservations although in our experience they have no influence on results.
Microbial contaminated specimen may cause interferences.

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.
Microtiterplates are coated with inactive antigen. However, normal laboratory precaution should be maintained when handling with infectious material.
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.

c. 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. The solutions are not interchangeable with another ELISA-VIDITEST kits produced by VIDIA spol. s r.o.

d. Avoid microbial contamination of serum samples and kit reagents. Avoid cross-contamination of reagents.

e. Peroxidase conjugate and Sample diluent are conserved with 0,049% Thiomersal.

f. Controls and RF sorbent are conserved with 0,095% sodium azide.

g. Avoid contact of the TMB with oxidizing agents or metal surfaces.

h. Follow the assay procedure indicated in the Instruction manual. Variations in the test results are usually due to:

* Insufficient mixing and prewarming to room temperature 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

<table>
<thead>
<tr>
<th>Error</th>
<th>Possible causes</th>
</tr>
</thead>
<tbody>
<tr>
<td>No colorimetric reaction</td>
<td>No peroxidase conjugate dispensed. Contamination of peroxidase conjugate (possibly with control sera during pipetting) may cause an inactivation.</td>
</tr>
<tr>
<td>Generally too high reaction</td>
<td>Incorrect peroxidase conjugate (i.e. not from original test kit). Incubation time too long or incubation temperature too high. Water quality for washing solution insufficient (low grade of deionisation).</td>
</tr>
<tr>
<td>Generally too weak reaction</td>
<td>Incorrect peroxidase conjugate (i.e. not from original test kit). Incubation time too short or incubation temperature too low.</td>
</tr>
<tr>
<td>Blank too high</td>
<td>Incorrect pipetting of sample diluent. Contaminated reagens. Reagents expired. Exceeding of incubation time and temperature. External contamination of the microtiterstrips bottom (clean carefully!).</td>
</tr>
<tr>
<td>False positive / negative samples</td>
<td>Incorrect dilution of samples. Lipaemic, hemolytic or icteric samples. Microbial contaminated specimen.</td>
</tr>
<tr>
<td>Unexplainable outliers</td>
<td>Contamination of pipettes, tips or containers, e.g. with metal (iron, copper etc.). Insufficient washing.</td>
</tr>
<tr>
<td>High variation (within a test)</td>
<td>Reagent (including microtiterstrips) not pre-warmed to room temperature prior to use. Washer is not washing correctly.</td>
</tr>
<tr>
<td>High variation (from test to test)</td>
<td>Incubation conditions not constant (time, temperature). Controls and samples are not carried out at same time (same intervals) - check pipetting order. Person related variation. Strips dried out after washing.</td>
</tr>
</tbody>
</table>

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. The expiration date is indicated at the ELISA kit label and at all reagent labels.

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. Seal all bottles properly after use in order to avoid bacterial contamination. Store at +2 °C to +8 °C.
d. Unused diluted washing buffer is stable for 4 weeks when stored at +2 °C to +8 °C.
e. 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.
f. Do not store diluted samples, use them immediately.
g. Kits are shipped in cooling bags, the transport time of 72 hours have no influence on expiration. If you find damage at any part of the kit, please inform the manufacturer immediately.
14. FLOW CHART

<table>
<thead>
<tr>
<th>Step 1</th>
<th>Prepare reagents and samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Step 2</td>
<td>Dispense 100 μL/well controls and samples</td>
</tr>
<tr>
<td></td>
<td>Incubate 60 minutes in incubator at 37 °C</td>
</tr>
<tr>
<td></td>
<td>Wash 4 times (300 μL/well, soaking time 30 sec.), aspirate</td>
</tr>
<tr>
<td>Step 3</td>
<td>Dispense 100 μL/well of peroxidase conjugate</td>
</tr>
<tr>
<td></td>
<td>Incubate 30 minutes in incubator at 37 °C</td>
</tr>
<tr>
<td></td>
<td>Wash 4 times (300 μL/well, soaking time 30 sec.), aspirate</td>
</tr>
<tr>
<td>Step 4</td>
<td>Dispense 100 μL/well of chromogenic substrate (TMB)</td>
</tr>
<tr>
<td></td>
<td>Incubate 15 minutes in dark at room temperature</td>
</tr>
<tr>
<td>Step 5</td>
<td>Dispense 100 μL/well of stop solution</td>
</tr>
<tr>
<td>Step 6</td>
<td>Read the absorbance at 450 nm within 10 minutes</td>
</tr>
</tbody>
</table>

General references:


Kazár, J.: Chlamýdiové pneumónie; Antibiotiká a rezistencia 2, 5, 2006; 9-12.

Date of the last revision of this manual: 05/2014