ELISA-VIDITEST anti-TOXO IgA

Instruction manual


1. TITLE:
ELISA-VIDITEST anti-TOXO IgA – ELISA kit for the detection of IgA antibodies to Toxoplasma gondii in human serum and plasma.

2. INTENDED USE:
In the diagnosis of toxoplasmosis a test combination is recommended to differentiate between acute and past infection.
The detection of specific **IgG antibodies** is positive 1 or 2 weeks after infection. The IgG titre reaches a maximum after a few weeks, then falls in the course of the following months, generally remaining at a low level lifelong.
The determination of **IgM antibodies** is an important indicator of an acute infection, or congenital toxoplasmosis. As the first immune response to infection the IgM antibodies appear after a few days and disappear generally after 3-5 months. But IgM antibodies can persist for some years after infection so further tests are necessary to clarify the stage of the infection.
The presence of **IgA antibodies** indicates an acute infection. IgA antibodies to Toxoplasma gondii occur 10-30 days after an acute infection, somewhat later than IgM antibodies, and disappear after 3-6 months.

3. TEST PRINCIPLE:
ELISA-VIDITEST anti-TOXO IgA is a solid-phase immunoanalytical test. The purified, homogeneous antigen is fixed to each well of the microtiter strips. 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 IgA 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 colour is proportional to the concentration of the antibodies in the sample.

4. KIT COMPONENTS:
ELISA break-away strips coated with the specific antigen 12 pieces
1.2 mL Negative control (NC) r.t.u. 1 vial
1.2 mL Positive control (PC) r.t.u. 1 vial
1.2 mL Cut-off control (COC) r.t.u. 1 vial
12 mL Peroxidase conjugate (anti-IgA/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 membrane
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, Negative control, Positive control, Cut-off control and Peroxidase conjugate in order to ensure homogeneity and mix all solution well prior use.
   c. Dilute serum samples 1:100 in Dilution buffer and mix (e.g. 5 µL of serum sample + 500 µL of Dilution buffer).
   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 Sample diluent, 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 next wells fill with Negative control (NC) and Positive control (PC). 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 the Cover membrane or cover.
   c. Incubate 30 minutes (+/- 2 min.) at room temperature.
   d. 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. 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 into each well. Incubate 10 minutes (+/- 5 seconds) at room temperature. The time measurement must be started at the beginning of TMB dispensing. Cover the strips with opaque cover or keep them in the dark during the incubation with TMB.
   h. 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.
   i. 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.
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).
2. Compute the Cut-off value. The Cut-off value is calculated from the absorbance of the COC and the absorbance of the Negative control.

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

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 a sample OD value ≥ Cut-off value – 10% and ≤ Cut-off value + 10 %. The samples with equivocal results should be retested. If the result is again indifferent then it is recommended to use an alternative testing method or to obtain new sample from the patient.

8.2. Processing of results for Qualitative interpretation using Positivity index value
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>Interpretation</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 new sample from the patient.

9. RESULT INTERPRETATION:
(Note to table: – Negative, + Positive)

<table>
<thead>
<tr>
<th>Antibodies against T. gondii</th>
<th>Interpretation</th>
<th>Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG IgM IgA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- - -</td>
<td>No specific antibodies detectable.</td>
<td>During pregnancy recheck in the 2nd and 3rd trimester.</td>
</tr>
<tr>
<td>- + +</td>
<td>Possible early phase of infection, re-infection or latent infection may also be possible.</td>
<td>Monitoring of IgG, IgM antibodies (Sample collection within 10-14 days) to determine seroconversion; supplementary tests e.g. IFA, CF, ISAGA, immunoblot, determination of Toxo-specific IgA antibodies and determination of the avidity of the Toxo-specific IgG antibodies.</td>
</tr>
<tr>
<td>- + -</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ + +</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ - -</td>
<td>Possible past infection, probable congenital toxoplasmosis, or re-infection.</td>
<td>By suspect of acute infection-monitoring of IgG- and IgM-antibodies, supplementary tests e.g. IFA, CF, ISAGA, immunoblot, determination of Toxo-specific IgA antibodies and determination of the avidity of the Toxo-specific IgG antibodies.</td>
</tr>
<tr>
<td>+ - +</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

10. VALIDITY, SPECIFICITY AND SENSITIVITY 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 of Cut-off control (COC) absorbances is higher than 0.200.
c. OD values of Negative control (NC) is less than 0.100.
d. Index value of Positive control 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. Intraassay 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>22</td>
<td>0.532</td>
<td>0.029</td>
<td>5.5%</td>
</tr>
<tr>
<td>22</td>
<td>1.278</td>
<td>0.058</td>
<td>4.5%</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>0.529</td>
<td>0.042</td>
<td>0.401 – 0.658</td>
<td>7.9%</td>
</tr>
<tr>
<td>10</td>
<td>1.301</td>
<td>0.097</td>
<td>1.009 – 1.595</td>
<td>7.5%</td>
</tr>
<tr>
<td>10</td>
<td>1.985</td>
<td>0.133</td>
<td>1.583 – 2.387</td>
<td>6.7%</td>
</tr>
</tbody>
</table>

10.3. Diagnostic sensitivity and specificity of the test
Diagnostic sensitivity of the test is 80.4% and diagnostic specificity is 83.3%. Evaluation was performed on 63 serum samples tested with another commercially available diagnostic test (ISAGA).

10.4. Interaction
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-TOXO IgG a 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% Thimerosal.

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:

Step 1. Prepare reagents and samples

↓

Step 2. Dispense 100 μL/well controls and samples

↓
Incubate 30 minutes at room temperature

↓
Wash 4 times (300 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)

↓
Incubate 10 minutes at room temperature

↓

Step 5. Dispense 100 μL/well of Stop solution

↓

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

General references:


Date of the last revision of this manual: 12/2010  
Next recommended revision: 12/2011