ELISA-VIDITEST anti-HSV 1+2 IgG

ODZ-169

Instruction manual

PRODUCER: VIDIA spol. s r.o., Nad Safinou II 365, Vestec, 252 42 Jesenice, Czech Republic, Tel.: +420 261 090 565, Web: www.vidia.cz, E-mail: info@vidia.cz

1. TITLE
ELISA-VIDITEST anti-HSV 1+2 IgG - ELISA kit for the detection of IgG antibodies to herpes simplex virus type 1 and 2 in human serum/plasma.

2. INTENDED USE
ELISA-VIDITEST anti HSV 1+2 IgG is intended for in vitro diagnosis of HSV type 1 or 2 associated diseases, i.e. herpes labialis, herpes genitalis, herpesvirus gingivostomatitis and keratoconjunctivitis. The diagnostic kit can be also utilized for differential diagnosis of neuroinfections, infections of eye and skin and exanthematous diseases.
The test does not differentiate HSV-1 and HSV-2 types.
For additional serological testing we recommend determination of IgM and IgA anti-HSV antibodies (ELISA-VIDITEST anti-HSV 1+2 IgM, ELISA-VIDITEST anti-HSV 1+2 IgA), or determination of intrathecal production of anti-HSV antibodies in cerebrospinal fluid (ELISA-VIDITEST anti-HSV 1+2 IgG (CSF)).

3. TEST PRINCIPLE
ELISA-VIDITEST anti HSV 1+2 IgG is a solid-phase immunoanalytical test. The polystyrene strips are coated with a mixture of antigens, which bear immunodominant epitopes of HSV1 and HSV2. Anti-HSV 1+2 antibodies from serum samples bind to the immobilized antigens. The other serum antibodies do not bind and are washed away, those that formed complexes with the antigens are later on recognized by animal anti-human IgG antibodies labeled with horseradish peroxidase. The presence of the labeled antibodies is revealed by an enzymatic reaction with a chromogenic substrate. Negative sera do not react and the mild change in color, if present, may be attributed to the reaction background.

4. KIT COMPONENTS
ELISA break-away strips coated with specific antigens 1 microplate
1.3 mL Standard 1, r.t.u. 1) STANDARD 1 1 vial
1.3 mL Standard 2, r.t.u. STANDARD 2 1 vial
1.3 mL Negative control serum CONTROL 1 vial
13 mL Anti-human IgG antibodies labeled with horseradish peroxidase (Px-conjugate) r.t.u. CONJ 1 vial
125 mL Wash buffer concentrate, 10x concentrated WASH 10x 1 vial
100 mL Dilution buffer (DB), r.t.u. **DIL** 1 vial
13 mL Chromogenic substrate (TMB substrate), r.t.u. **TMB-O** 1 vial
15 mL Stop solution, r.t.u. **STOP** 1 vial
Sealable pouch for unused strips
Instruction manual
Quality control certificate
1) r.t.u., ready to use

| Chromogenic substrate (TMB-O) is compatible and interchangeable between ELISA-VIDITEST kits which contain TMB-O and not with other Chromogenic substrates (TMB, TMB-BF). |

5. MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED
   a. Distilled or deionised water for diluting of the Wash buffer concentrate.
   b. Appropriate equipment for pipetting, liquid dispensing and washing.
   c. Spectrophotometer/colorimeter (microplate reader – wavelength 450 nm).

6. PREPARATION OF REAGENTS AND SAMPLES
   a. Allow all kit components to reach room temperature.
   b. Mix samples (serum/plasma), Standard 1 and Standard 2, Negative control in order to ensure homogeneity and mix all solutions 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). Do not dilute the standards they are ready to use.
   d. Prepare Wash buffer by diluting the Wash buffer concentrate 10x with an appropriate volume of distilled or deionised water (e.g. 100 mL of the concentrated Wash buffer + 900 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 till the crystals dissolve. Diluted Wash buffer is stable for one week if stored at +2 to +10°C.
   e. Do not dilute Px-conjugate, TMB substrate and Stop solution, they are ready to use.

7. ASSAY PROCEDURE
   a. Allow the microwell strips sealed inside the aluminium bag to reach room temperature. Withdraw an adequate number of strips and put the unused strips into the provided pouch and seal it carefully with the desiccant kept inside.
   b. Pipette Standards and samples according to the pipetting scheme (page 3, fig 1). Fill first well with Dilution buffer (DIL) to determine reaction background. Fill the next two wells with STANDARD 1 (calibrator). Fill the next well with CONTROL - (negative control serum). The remaining wells fill with diluted tested sera (S1...). It is also suitable to apply STANDARD 2 (positive control serum) for the test control. It is satisfactory to apply one serum into one well (S1, S2, S3...). However, if you want to minimize a laboratory error, apply control sera and tested sera as doublets, Standard 1 as triplet.
   c. Incubate 60 minutes (±5 min) at room temperature.
d. Aspirate the liquid from wells into a waste bottle containing an appropriate disinfectant (see Safety Precautions). Wash and aspirate the wells four times with 250 µl/well of Wash buffer. Avoid cross-contamination between wells. If any liquid stays trapped inside the wells, invert the plate and tap it on an adsorbent paper to remove the remaining drops.

e. Mix Px-conjugate r.t.u. well and then add 100 µL of the Px-conjugate into each well.

f. **Incubate 60 minutes (±5 min) at room temperature.**

g. Aspirate and wash four times with 250 µl/well of Wash buffer.

h. Dispense 100 µl of TMB substrate (TMB-O) into each well.

i. **Incubate 10 minutes (±5 seconds) at room temperature.**

   The time measurement must be started at the beginning of TMB dispensing. Keep the strips in the dark during the incubation with TMB substrate.

j. 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 few times to ensure complete mixing of the reagents.

k. Measure the absorbance at **450 nm with a microplate reader within 10 minutes.** It is recommended to use a reference reading at 620-690 nm.

Figure 1: Pipetting scheme

<table>
<thead>
<tr>
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<th>1</th>
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<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>
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<tbody>
<tr>
<td>a</td>
<td>DIL</td>
<td>S5</td>
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<tr>
<td>b</td>
<td>STANDARD 1</td>
<td>S…</td>
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<td>STANDARD 1</td>
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<tr>
<td>d</td>
<td>CONTROL -</td>
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<td>S3</td>
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<td>h</td>
<td>S4</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 DIL well) from the absorbances of all other wells.

8.1 **Processing of results for Qualitative interpretation**

1. Compute the mean absorbance of STANDARD 1. If you applied Standard 1 into 3 wells and if any of the three Standard 1 absorbances falls out of the range +/-20% of the mean absorbance then exclude the deviating well from the calculation and compute a new Standard 1 mean using the values from the other two wells.

2. Compute the cut-off value of the test by multiplication the Standard 1 mean by the Correction factor. **The Correction factor values for the Standard 1 determined for this lot of the kit is stated in the Quality control certificate.**

3. Sera that have absorbance value < 90% cut-off are negative and sera with absorbance value > 110% cut-off are considered to be positive.
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:

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

3. Express a serum reactivity according to Table 1 (Semiquantitative interpretation of results)

<table>
<thead>
<tr>
<th>Positivity index</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 0.90</td>
<td>Negative</td>
</tr>
<tr>
<td>0.90 - 1.10</td>
<td>+/-</td>
</tr>
<tr>
<td>1.11 - 5.0</td>
<td>+</td>
</tr>
<tr>
<td>5.01 - 10.00</td>
<td>++</td>
</tr>
<tr>
<td>10.01 - 15.00</td>
<td>+++</td>
</tr>
<tr>
<td>&gt; 15.00</td>
<td>++++</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.

Example: Absorbance of Standard 1 = 1.652; 1.623
Mean of absorbance of Standard 1 = 1.638
Absorbance of serum sample = 0.600
Correction factor = 0.16
Cut-off = 1.638 x 0.16 = 0.262
Positivity index value = 0.600 / 0.262 = 2.29

9. INTERPRETATION OF THE TEST

IgG anti-HSV antibodies are of anamnestic character, i.e. they persist after primary infection in patient’s serum for the rest of his life. Most of anti-HSV antibodies cross react with both types of HSV virus. Significant increase of IgG anti-HSV antibody titre can result from the virus reactivation, but due to the recurrent character of reactivations it may not be always demonstrable. Serological results can be interpreted only in context with other laboratory tests results and with patient’s clinical findings.

10. CHARACTERISTICS OF THE TEST

10.1. Validity of the test

The test is valid if:

a. The background absorbance (the absorbance of the Dilution buffer (DIL)) is less than 0.100.
b. The Standard 1 absorbance is in the range stated in Quality control certificate for this kit lot.
c. The ratio of CONTROL- absorbance / cut-off is lower than 0.8.
d. OD of standards follows the order:
10.2. Precision of the test
The intraassay variability (within the test) and the interassay variability (between tests) were determined with samples of different absorbance values.

10.2.1. Intraassay variability
The coefficient of intraassay variability is max. 5%. It is measured for each particular Lot at least on 12 parallels of the same microtitration plate.

Example: (N = number of parallels of the same microtitration plate, SD = standard deviation)

<table>
<thead>
<tr>
<th>N</th>
<th>Mean absorbance</th>
<th>SD</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>0.453</td>
<td>0.013</td>
<td>2.8%</td>
</tr>
<tr>
<td>16</td>
<td>1.079</td>
<td>0.030</td>
<td>2.7%</td>
</tr>
</tbody>
</table>

10.2.2. Interassay variability
The coefficient of interassay variability is max. 15%. It is measured for each particular Lot as comparison of the OD values of the same serum sample in several consecutive tests.

Example: (N = number of an independent examination of the same serum sample, SD = standard deviation):

<table>
<thead>
<tr>
<th>N</th>
<th>Mean Absorbance</th>
<th>SD</th>
<th>Range (min-max)</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0.142</td>
<td>0.021</td>
<td>0.121 – 0.169</td>
<td>14.8%</td>
</tr>
<tr>
<td>10</td>
<td>0.534</td>
<td>0.046</td>
<td>0.474 – 0.626</td>
<td>8.6%</td>
</tr>
<tr>
<td>10</td>
<td>0.864</td>
<td>0.072</td>
<td>0.756 – 0.998</td>
<td>8.3%</td>
</tr>
<tr>
<td>10</td>
<td>2.089</td>
<td>0.115</td>
<td>1.990 – 2.334</td>
<td>5.5%</td>
</tr>
</tbody>
</table>

10.3. Spiking recovery test
Measured values of recovery test for every Lot are between 80-120% of expected values.

10.4. Diagnostic sensitivity and specificity
The diagnostic sensitivity of the test is 98% and the specificity is 97.2%. Evaluation was performed by the comparing the VIDITEST kit with two other commercial ELISA tests and with indirect immunofluorescence test.

<table>
<thead>
<tr>
<th>HSV 1+2 status</th>
<th>Total</th>
<th>Positive</th>
<th>Equivocal</th>
<th>Negative</th>
<th>Specificity: 97 % *</th>
<th>Sensitivity: 98.8%*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>112</td>
<td>3</td>
<td>2</td>
<td>97</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>82</td>
<td>80</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Equivocal results were not taken in account for calculation

10.5. Interference
Haemolytic and lipemic samples have no influence on the test results up to concentration of 50 mg/mL of haemoglobin, 5 mg/mL of bilirubin and 50 mg/mL of triglycerides.
11. SAFETY PRECAUTIONS

All ingredients of the kit are intended for laboratory use only.
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 off according to the appropriate regulations.
Autoclave all reusable materials that were in contact with human samples for 1 hour at 121°C, burn disposable ignitable materials, decontaminate liquid wastes and nonignitable materials with 3% chloramine.
The waste from strip washing disinfect in waste container using appropriate disinfection solution (e.g. Incidur, Incidin, chloramin, ...) in concentrations recommended by the producer.
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, if necessary.
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.

12. HANDLING PRECAUTIONS

Manufacturer guarantees performance of the entire ELISA kit.
Follow the assay procedure indicated in the Instruction manual.
Standards, TMB substrate, Dilution buffer and Px-conjugate contain preservative ProClin 300®.
Wash buffer, chromogenic substrate TMB, Stop solution and Dilution buffer are interchangeable between ELISA-VIDITEST kits unless otherwise stated in the Instruction manual.
Avoid microbial contamination of serum samples and kit reagents.
Avoid cross-contamination of reagents.
Avoid contact of the TMB substrate with oxidizing agents or metal surfaces.
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 Px-conjugate
* Use of identical pipette tip for different solutions

13. STORAGE AND EXPIRATION

Store the kit and the kit reagents at +2 to +10°C, in a dry place and protected from the light.
Store unused strips in the sealable pouch and keep the desiccant inside.
Store undiluted serum samples at +2 to +10°C up to one week. For longer period make aliquots and keep them at -20°C. Avoid repeated thawing and freezing.

Do not store diluted samples. Always prepare fresh.

Kits are shipped in cooling bags, the transport time up to 72 hours have no influence on expiration. If you find damage at any part of the kit, please inform the supplier immediately. 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 of Standards and samples

↓

  Incubate 60 minutes at room temperature

↓

  Wash 4 times (250 µL / well), aspirate

↓

Step 3  Dispense 100 µL / well of Px-conjugate

↓

  Incubate 60 minutes at room temperature

↓

  Wash 4 times (250 µL / well), aspirate

↓

Step 4  Dispense 100 µL / well of TMB substrate

↓

  Incubate 10 minutes in dark at room temperature

↓

Step 5  Dispense 100 µL / well of Stop solution

↓

Step 6  Read the absorbance at 450 / 620-690 nm within 10 min.

References:


Date of the last revision of this manual: 05/2013

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