ELISA-VIDITEST anti-JCV IgG

ODZ-262

Instruction manual

PRODUCER: VIDIA spol. s r.o., Nad Safinou II 365, 252 50 Vestec, Czech Republic, Tel.: +420 261 090 565, Web: www.vidia.cz

1. TITLE
ELISA-VIDITEST anti-JCV IgG – ELISA kit for the detection of species-specific IgG antibodies against polyomavirus JC (JCV) in human serum and plasma.

2. INTENDED USE
The kit is intended for laboratory diagnostics of diseases caused by or associated with JCV (e.g. progressive multifocal leukoencephalitis (PML) in immunodeficient patients) and for monitoring the of PML risk in patients treated with some types of immunomodulatory biological drugs, e.g., in patients receiving natalizumab.

3. TEST PRINCIPLE
ELISA-VIDITEST anti-JCV IgG is a solid-phase immunoanalytical test. The surface of the wells is coated with recombinant species-specific JCV antigen. If present in the serum samples, respective antibodies bind to the immobilized antigen. In the next step, bound antibodies react with anti-human IgG antibodies labeled with horseradish peroxidase. The amount of bound labeled antibodies is determined by colorimetric enzymatic reaction. 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 recombinant antigens STRIPS |Ag| STRIPS Ag
1 x 12 strips
1.3 mL Standard A=negative control serum, r.t.u. ST A/NC
1 vial
1.3 mL Standard B, r.t.u. ST B
1 vial
1.3 mL Standard C, r.t.u. ST C
1 vial
1.3 mL Standard D=Calibrator, r.t.u. ST D/CAL
1 vial
1.3 mL Standard E=Positive control serum, r.t.u. STE/PC
1 vial
13 mL Anti-human IgG antibodies labelled with horseradish peroxidase (anti-IgG Px-conjugate), r.t.u. CONJ
1 vial
55 mL Wash buffer concentrate, 10x concentrated WASH 10x
1 vial
60 mL Dilution buffer, r.t.u. DIL
1 vial
13 mL Chromogenic substrate (TMB-O substrate), r.t.u. TMB-O
1 vial
13 mL Stop solution, r.t.u. STOP
1 vial
Instruction manual
Quality control certificate
1) r.t.u. ready to use
2) The antibody concentration for each Standard is mentioned in enclosed Quality control certificate (AU/ml (Arteficial units/ml)

Notice: Control sera may be colorless to yellowish or blue due to the use of different diluents.
5. MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

Distilled or deionised water for diluting of the Wash buffer concentrate, appropriate equipment for pipetting, liquid dispensing and washing, spectrophotometer/colorimeter/microplate reader – wavelength 450 nm (620-690 nm reference filter recommended, not required).

6. PREPARATION OF REAGENTS AND SAMPLES

a. Allow all kit components to reach room temperature.

b. Mix samples, Standards and conjugate in order to ensure homogeneity and mix all solution well prior use. Dilute serum samples 1:100 (101x) 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.

c. Prepare Wash buffer by diluting the Wash buffer concentrate 10 times with an appropriate volume of distilled or deionised water (e.g. 50 mL of the concentrated Wash buffer + 450 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 one month if stored at room temperature.

d. Do not dilute Px-conjugate, TMB-O substrate and Stop solution, they are ready to use.

7. ASSAY PROCEDURE

Manufacturer will not be held responsible for results if manual is not followed exactly.

7.1 ASSAY PROCEDURE FOR QUALITATIVE/SEMIQUANTITATIVE ANTIBODY DETERMINATION IN SERUM (PLASMA)

a. Allow the microwell strips sealed inside the aluminum 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. Fill the wells with 100 µL of Standards and diluted serum samples in the following manner: Start with filling the first well with 100 µL of negative control serum ST A/NC. Fill two wells with 100 µL/well of Standard D ST D/CAL (serves as calibrator), next well with Positive control serum ST E/PC and then pipette the serum samples (S1, S2,…) (see Tab. 1). It is sufficient to apply the serum samples in one well. If you wish to minimize the laboratory error apply the ST D/CAL in three wells and the samples and control sera in two well. We recommend including a positive reference serum sample (your in-house internal control) into each run to follow the sequence, variability and accuracy of calibration. Incubate 60 minutes (±5 min) at room temperature.

c. 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.

d. Mix well the bottle with Px-conjugate CONJ and add 100 µL of anti-IgG Px-conjugate r.t.u. into each well. Incubate 60 minutes (±5 min) at room temperature.

e. Aspirate and wash four times with 250 µL/well of Wash buffer. (see c.)

f. Dispense 100 µL of TMB-O substrate into each well. Incubate for 10 minutes (+/-30 seconds) at room temperature in dark. The time measurement must be started at the beginning of TMB-O dispensing. Follow this rule and keep the time interval. Pipette fast in regular rhythm or use appropriate dispensing device. Cover the strips with an aluminum foil or keep them in the dark during the incubation with TMB-O substrate.

g. Stop the reaction by adding 100 µL of Stop solution STOP. Use the same pipetting rhythm as with the TMB-O substrate to ensure the same reaction time in all wells. Check that no air-bubbles are present inside the well otherwise tap gently the microplate to remove them.
h. Measure the absorbance at 450 nm with a microplate reader **within 10 minutes** after termination of the reaction. It is recommended to use a reference reading at 620-690 nm.

Table 1: Pipetting scheme for semiquantitative analysis

<table>
<thead>
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<th>9</th>
<th>10</th>
<th>11</th>
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<tbody>
<tr>
<td>a</td>
<td>ST A/NC</td>
<td>S5</td>
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<td>b</td>
<td>ST D/CAL</td>
<td>S...</td>
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<td>d</td>
<td>ST E/PC</td>
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<td>S1</td>
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<tr>
<td>f</td>
<td>S2</td>
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<td>g</td>
<td>S3</td>
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<td></td>
</tr>
<tr>
<td>h</td>
<td>S4</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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</tbody>
</table>

7.2 ASSAY PROCEDURE FOR QUANTITATIVE ANTIBODY DETERMINATION IN SERUM (PLASMA)

a. Allow the antigen-coated strips to reach room temperature before opening sealed the aluminum bag in order to prevent water condensation within the wells. Withdraw the adequate number of strips and put the remaining strips in the sealable pouch and keep the desiccant inside.

b. Fill the wells with 100 µL of Standard A, B, C, D, E (ST A/NC, ST B, ST C, ST D/CAL, ST E/PC). Fill the remaining wells with 100 µL of diluted serum samples (S1, S2, S3,...) (see Tab. 2). If you wish to minimize laboratory error apply the standards and samples in doublets. We recommend including positive reference serum sample (your in-house internal control) into each run to follow the sequence, variability and accuracy of calibration. Incubate for **60 (±- 5) minutes** at room temperature.

c. Aspirate the liquid from the wells into a collecting bottle containing appropriate disinfectant (see Safety Precautions). Wash and aspirate the wells 4x with 250 µL/well of Wash buffer. Avoid cross-contamination between wells. Invert the plate and tap it on an adsorbent paper to remove the remaining drops.

d. Mix well the bottle with anti-IgG Px-conjugate r.t.u. **CONJ** and add 100 µL of anti-IgG Px-conjugate into each well. **Incubate for 60 (±-5) minutes** at room temperature.

e. Aspirate and wash 4x with 250 µL/well of Wash buffer. Aspirate and tap the plate.

f. Dispense 100 µL of the **TMB-O** substrate into each well. Incubate for **10 minutes (±-5 seconds)** at room temperature in dark. Measurement must be started at the beginning of dispensing. Follow this rule and keep the time interval. Pipette fast in regular rhythm or use appropriate dispensing device. Cover the strips with an aluminum foil or keep them in the dark during the incubation.

g. Stop the reaction by adding 100 µL of Stop solution **STOP**. Use the same pipetting rhythm as with the TMB-O substrate to ensure the same reaction time in all wells. Check that no air-bubbles are present inside the well otherwise tap gently the microplate to remove them.

h. Measure the absorbance at 450 nm with a microplate reader **within 10 minutes** after termination of the reaction. It is recommended to use a reference reading at 620-690 nm.
8. TEST EVALUATION.

8.1 Qualitative interpretation

1. Compute the absorbance mean of Standard D \( \text{ST D/CAL} \). If you applied calibrator into 3 wells and if any of the three values falls out of the range more than 20% of the mean absorbance then exclude the deviating well from the calculation and compute a the mean using the values from the other two wells.
2. Compute the cut-off value by multiplying the Standard D mean with a Correction factor. The Correction factor value for the particular Lot is written in enclosed Quality control certificate.
3. Samples with absorbances lower than 90% of the cut-off value are considered negative and samples with absorbances higher than 110% of the cut-off value are considered positive.

8.2 Semiquantitative interpretation

1. Determine the cut-off value as previously in 8.1 (2.)
2. Compute the Index value for each serum sample by dividing the test serum absorbance by cut-off value.
3. Interpret the results according to the following table (Results interpretation):

RESULTS INTERPRETATION

<table>
<thead>
<tr>
<th>Index value</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>&gt; 1.00</td>
<td>Positive*</td>
</tr>
</tbody>
</table>

*on the basis of the Positivity Index value it is possible to estimate semiquantitatively the amount of antibodies in the sample

Example of calculation:

- Standard D absorbances = 0.814; 0.876
- Mean of Standard D = 0.845
- Sample absorbance = 0.800
- Correction factor of Standard D = 0.37
- Cut-off value = 0.845 x 0.37 = 0.313
- Index value = 0.800 / 0.313 = 2.56

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.
8.3 Quantitative antibody determination in serum samples in artificial units (AU/mL)

Compute the sample antibody concentration in artificial units (AU/mL) as follows:

1. Construct the calibration curve by plotting the absorbance of Standards (y-axis – in linear scale) to antibody concentration in artificial units (AU/mL) (x-axis – may have logarithmic scale). The antibody concentration of each Standard is mentioned in Quality control certificate.

2. Find the place where the absorbance of tested samples intersect calibration curve and find the corresponding values on the axis x. It is possible to use various software for the standard curve fitting and for the calculation of the unknown values, e.g. Winliana, KimQ. For better fitting, the polynomic (four-parameter) function is the most convenient.

3. Interpretation of antibody concentration in serum (101x dilution):

<table>
<thead>
<tr>
<th>Concentration (AU/mL)</th>
<th>Evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 20</td>
<td>Negative</td>
</tr>
<tr>
<td>20 - 25</td>
<td>+/-</td>
</tr>
<tr>
<td>&gt; 25</td>
<td>Positive</td>
</tr>
</tbody>
</table>

Note 1: Sample in the range between 20 – 25 AU/ml is equivocal. In such case it is recommended repeating the assay. If the result of the sample is in the grey zone again, use an alternative diagnostic method or initiate taking another blood sample 1-2 weeks later.

Note 2: The quantification is accurate only within the linear part of the calibration curve. If the sample OD is outside the linear interval (OD 0,200-2,000), it is necessary to repeat the test with more diluted serum samples to obtain the precise quantification.

9. RESULTS INTERPRETATION

50-60% of the population is infected with Polyoma JC virus in childhood. Infection occurs without symptoms and passes into a latent stage, which is associated with long-term presence of anamnestic IgG antibodies in the serum. In latently infected people, the virus may be reactivated repeatedly; they may be reinfected with another virus strain. Reactivation/reinfection can be associated with transient viremia or asymptomatic viral shedding in urine. In rare cases in immunodeficient patients, reactivation or reinfection can cause infection of the central nervous system (PML). The development of this disease is influenced by many factors, both viral and host origin, and also by method of treatment. The presence of antibodies against JCV is one of the risk factors in the development of PML in patients treated with natalizumab. Significant increase or a high level of antibodies against JCV in these patients may indicate reinfection, or reactivation of the virus. The recombinant antigen which is used in the test does not cross-react with other human polyomaviruses (Polyoma BK, polyomavirus Merkel cell carcinoma).

10. CHARACTERISTIC OF THE TEST

10.1 Validity of the test
The test is valid if:

The mean absorbance values of standards/ control sera, and the ratio between the absorbance values of ST E/PC / ST D/CAL are in the ranges stated in the Quality control certificate for this kit lot.

10.2 Precision of the test
The intra-assay variability (within the test) and the inter-assay reproducibility (between tests) were determined using samples with different absorbance values. In anti-JCV IgG-positive samples intra-assay and inter-assay variability coefficients (CV) did not exceed 8% and 15% of mean absorbance values, respectively.
10.2.1 Variability (intra-assay)
(n= number of parallel determinations in the same test)

<table>
<thead>
<tr>
<th>n</th>
<th>A</th>
<th>±δ</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>0.947</td>
<td>0.025</td>
<td>2.7%</td>
</tr>
<tr>
<td>8</td>
<td>2.592</td>
<td>0.075</td>
<td>2.9%</td>
</tr>
</tbody>
</table>

10.2.2 Reproducibility (inter-assay)
(n= number of parallel determinations in the same test)

<table>
<thead>
<tr>
<th>n</th>
<th>A</th>
<th>±δ</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>0.640</td>
<td>0.079</td>
<td>12.3%</td>
</tr>
<tr>
<td>4</td>
<td>1.246</td>
<td>0.148</td>
<td>11.9%</td>
</tr>
<tr>
<td>4</td>
<td>2.648</td>
<td>0.110</td>
<td>4.1%</td>
</tr>
</tbody>
</table>

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

10.3 Diagnostic sensitivity and specificity of the test
The diagnostic sensitivity and specificity of the test was performed with a set of serum samples that were characterized by validated tests: a) reference ELISA test in National reference laboratory for papillomaviruses and polyomaviruses in Prague, b) ELISA STRATIFY™ JCV Dx SELECT test in reference laboratory in Copenhagen.

<table>
<thead>
<tr>
<th>Results of comparative test</th>
<th>negative</th>
<th>equivocal</th>
<th>positive</th>
<th>sum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seronegative</td>
<td>26</td>
<td>1</td>
<td>1</td>
<td>28</td>
</tr>
<tr>
<td>Seropositive</td>
<td>2</td>
<td>1</td>
<td>37</td>
<td>40</td>
</tr>
</tbody>
</table>

The diagnostic sensitivity of the test is 95% and the specificity is 96% (equivocal samples excluded)

10.4 Interference
Hemolytic and lipemic samples have no influence on the test results up to concentration of 50 mg/mL of hemoglobin, 5 mg/mL of bilirubin and 50 mg/mL of triglycerides. However, examination of such samples is not recommended.

10.5 The limit of quantification
The limit of quantification was defined as the lowest measurable concentration which can be distinguished with 95% confidence from zero. This value is 3 AU/mL.

11. SAFETY PRECAUTIONS
a. All ingredients of the kit are intended for laboratory use only.
b. Standards contain human sera that have 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.
c. Autoclave all reusable materials that were in contact with human samples for 0.5 hour at 121°C, burn disposable ignitable materials, decontaminate liquid wastes and non-ignitable materials with 3% chloramine. Liquid wastes containing acid (Stop solution) should be neutralized in 4% sodium bicarbonate solution.
d. 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.
e. 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

a. Manufacturer guarantees performance of the entire ELISA kit.
b. Wash buffer, Chromogenic substrate TMB-O, Stop solution and Dilution buffer are interchangeable between ELISA-VIDITEST kits unless otherwise stated in the instruction manual.
c. Avoid microbial contamination of serum samples and kit reagents.
d. Avoid cross-contamination of reagents when handling, diluting and storing reagents.
e. Controls (Standards), TMB-O substrate, Dilution buffer and anti-IgG Px-conjugate contain preservative ProClin 300®.
f. Avoid contact of the TMB-O substrate 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 Px-conjugate
   • Use of identical pipette tip for different solutions
   • Contamination of pipettes used for application of samples, controls, TMB-O substrate or Px-conjugate (for application of the conjugate it is recommended using a pipette just for this purpose).

13. STORAGE AND EXPIRATION

The ELISA kit should be used within three months after opening.

a. Store the kit and the kit reagents at +2 to +10°C, in a dry place and protected from the light. Under these conditions, the expiration date is indicated at the ELISA kit label and at all reagent labels.
b. 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.
c. Store unused strips in the sealable pouch and keep the desiccant inside.
d. Store undiluted serum samples at +2 to +10°C up to one week. For longer period make aliquots and keep them at -18 to -28°C. Avoid repeated thawing and freezing.
14. USED SYMBOLS

- number of tests
- Conformité Européenne – product meets the requirements of European legislation
- in vitro diagnostics
- standard deviation
- coefficient of variation
- optical density
- manufacturer
- expiration
- Lot of the kit
- storage at +2°C - +10°C
- Celsius degree
- percentage
- number of tested samples
- value of tested sample
- read usage instructions
- catalog number

**Recomended literature:**


15. FLOW CHART

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Step 1</td>
<td>Prepare reagents and samples</td>
</tr>
<tr>
<td>Step 2</td>
<td>Dispense 100 μL/well standards and samples&lt;br&gt;Incubate 60 minutes at room temperature&lt;br&gt;Wash 4 times (250 μL/well), aspirate</td>
</tr>
<tr>
<td>Step 3</td>
<td>Dispense 100 μL/well of Px-conjugate r.t.u.&lt;br&gt;Incubate 60 minutes at room temperature&lt;br&gt;Wash 4 times (250 μL/well), aspirate</td>
</tr>
<tr>
<td>Step 4</td>
<td>Dispense 100 μL/well of TMB-O substrate&lt;br&gt;Incubate 10 minutes 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 / 620-690 nm within 10 minutes</td>
</tr>
</tbody>
</table>

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