ELISA-VIDITEST anti-CMV IgG (CSF)
Cat. No.: ODZ-102

Instruction manual

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1. TITLE
ELISA-VIDITEST anti-CMV IgG (CSF): ELISA kit for the detection of IgG antibodies to human
cytomegalovirus in serum and cerebrospinal fluid.

2. INTENDED USE
ELISA-VIDITEST anti-CMV (CSF) is intended for in vitro diagnosis of CMV-associated diseases, i.e.
infectious mononucleosis, CMV syndrome, hepatitis in infants, interstitial pneumonitis or other forms of
active CMV infection in immunocompromised patients, including their neurological complications
(encephalitis, neuritis, syndrome Guillain-Barré). It may also contribute to differential diagnosis of
autoimmune or neurological diseases.

For serological examination of CMV, this assay should be supplemented with CMV-specific IgM antibody
detection, eventually with the determination of CMV-specific IgA and CMV IgG avidity.

3. TEST PRINCIPLE:
ELISA-VIDITEST anti CMV IgG (CSF) assay is a solid-phase immunoanalytical test. The polystyrene
strips are coated with a mixture of antigens which bear immunodominant epitopes of CMV. Anti-CMV
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 recognised by animal
anti-human IgG antibodies labelled with horseradish peroxidase. The presence of the labelled antibodies
is revealed by an enzymatic reaction with a chromogenic substrate. Negative sera do not react and
the mild change in colour, if present, may be attributed to the reaction background.

Detection of intrathecal production of antibodies (detection of specific IgG antibodies in cerebrospinal
fluid produced intrathecally) is necessary for the assessment of the antibody response within the central
nervous system. It requires parallel measurements of specific IgG antibodies in serum and in
cerebrospinal fluid sample taken from the patient at the same time and the determination of total albumin
and total IgG in both samples. For calculation of the specific antibody production Reiber equation is used
(see 8.2 – processing of the results).

4. KIT COMPONENTS
ELISA 8-well break-away strips coated with specific antigen 
1 microplate
STRIPS Ag
1.3 mL STANDARD A 7 AU (Arteficial units), r.t.u.*
1 vial
1.3 mL STANDARD B 30 AU, r.t.u.
1 vial
1.3 mL STANDARD C 50 AU, r.t.u.
1 vial
1.3 mL STANDARD D 130 AU, r.t.u.
1 vial
1.3 mL STANDARD E 1000 AU, r.t.u.
1 vial
13 mL Anti-human IgG antibodies labelled with horseradish peroxidase, r.t.u.
(Px-conjugate) CONJ 1 vial
125 mL Wash buffer, 10x concentrated WASH 10x 1 vial
125 mL Dilution buffer, r.t.u. DIL 1 vial
13 mL Chromogenic substrate (TMB substrate), r.t.u. TMB-O 1 vial
13 mL Stop solution, r.t.u. STOP 1 vial
Sealable pouch for unused strips
Instruction manual
Quality Control Certificate
* 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 WITH THE KIT
   1. Distilled or deionized water for diluting of washing solution.
   2. Appropriate equipment for pipetting, solution dispensing and washing.

6. PREPARATION OF REAGENTS AND SAMPLES
   a. Allow samples and all kit components to reach room temperature.
   b. Vortex samples and Standards in order to ensure homogeneity and mix the solutions well prior use.
   c. **Dilute serum samples 1:100 (101x) with Dilution buffer** and mix (e.g. 5 µL of serum sample + 500 µL of Dilution buffer). For evaluation of the intrathecal production test two dilutions of serum samples are recommended: 101x and 404x. Dilution 404x prepare by 4x diluting the 101x diluted serum sample (e.g. 150 µL of Dilution buffer + 50 µL of serum sample diluted 101x). **Dilute cerebrospinal fluid samples 2x in Dilution buffer** (e.g. 100 µL of cerebrospinal fluid sample + 100 µL of Dilution buffer). Do not dilute Standards, they are ready to use.
   d. Prepare Wash buffer by diluting the Wash buffer concentrate 10 times 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. 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

7.1 Procedure for quantitative antibody determination in serum:
   a. Allow the antigen-coated strips to reach room temperature before opening sealed the aluminium 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. Pipette Standards and samples according to the pipetting scheme (Fig. 1). Start with filling the first well with 100 µL of Dilution buffer (DIL) to estimate the reaction background. Fill the next wells with 100 µL of Standard A, B, C, D and E. Fill the remaining wells with 100 µL of serum samples (S1, S2, S3,...). It is sufficient to apply samples as singlets, however, if you wish to minimize laboratory error apply the samples in doublets. We recommend to include positive reference serum sample (in-house internal control) into each run to follow the sequence, variability and accuracy of calibration.
   c. **Incubate for 60 (+/- 5) minutes 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 4x with 250 µL/well of Wash buffer. Avoid cross-contamination between wells!
   e. Add 100 µL of Px-conjugate r.t.u. into each well.
   f. **Incubate for 60 (+/-5) minutes at room temperature.**
   g. Aspirate and wash 4x with 250 µL/well of Wash buffer. Tap the plate on an adsorbent paper.
   h. Dispense 100 µL of the TMB substrate into each well; pipette in a regular rhythm or use an appropriate dispensing instrument. **Incubate for 10 (+/-5 sec.) minutes at room temperature.**
The time measurement must be started right at the beginning of TMB dispensing. Cover the strips and keep them in the dark during the enzymatic reaction.

i. 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.

j. Read the absorbance at 450 nm with a microplate reader within 20 minutes. It is recommended to use reference reading at 620 - 690 nm.

7.2 Procedure for serum and cerebrospinal fluid samples (detection of intrathecal antibodies):

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. Pipette Standards and samples according to the pipetting scheme (Fig 2). Start with filling the first well with 100 μl of Dilution buffer (DIL) to estimate the reaction background. Fill the next wells with 100 μl of Standard A, B, C, D and E. Fill the remaining wells with 100 μl of serum samples (S1, S2, S3, ...) and cerebrospinal fluid samples. We recommend testing each serum sample in two different dilutions: 1:100 and 1:400 and each cerebrospinal fluid sample in one dilution 2x. If you wish to minimize laboratory error apply the samples in doublets. We recommend to include positive reference serum sample (in-house internal control) into each run to follow the sequence, variability and accuracy of calibration.

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 absorbent paper to remove the remaining drops.

e. Add 100 μL of Px-conjugate r.t.u. 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 into each well.

i. Incubate for 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. Read the absorbance at 450 nm with a microplate reader within 20 minutes. It is recommended to use a reference reading at 620 - 690 nm.

Figure 1: Pipetting scheme

<table>
<thead>
<tr>
<th>QUANTITATIVE ANTIBODY DETERMINATION IN SERUM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
</tr>
<tr>
<td>A</td>
</tr>
<tr>
<td>B</td>
</tr>
<tr>
<td>C</td>
</tr>
<tr>
<td>D</td>
</tr>
<tr>
<td>E</td>
</tr>
<tr>
<td>F</td>
</tr>
</tbody>
</table>
Figure 2: Pipetting scheme

### MEASUREMENT OF INTRATHECAL ANTIBODY PRODUCTION (SERUM AND CSF SAMPLES)

<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>S1 (1:400)</td>
<td></td>
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<td></td>
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<tr>
<td>B</td>
<td>ST A</td>
<td>S2 (1:400)</td>
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<tr>
<td>C</td>
<td>ST B</td>
<td>CSF1 (1:2)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>ST C</td>
<td>CSF2 (1:2)</td>
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<td>E</td>
<td>ST D</td>
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<td>F</td>
<td>ST E</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>S1 (1:100)</td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>S2 (1:100)</td>
<td></td>
<td></td>
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</tbody>
</table>

### 8. PROCESSING OF RESULTS

Regardless of the method chosen, begin the processing with subtraction of the absorbance of the DIL well (background absorbance) from the absorbances in all other wells.

#### 8.1. Quantitative antibody determination in serum samples

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 in each Standard (A-E) is mentioned in Paragraph 4 – Kit components.
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 softwares for the standard curve fitting and for the calculation of the unknowns, e.g. Winliana, KimQ. For better fitting, the polynomic (four-parameter) function is the most convenient.

**Result interpretation:**

<table>
<thead>
<tr>
<th>Concentration (AU/mL)</th>
<th>Evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 13.00</td>
<td>Negative</td>
</tr>
<tr>
<td>13.00 - 16.00</td>
<td>+/-</td>
</tr>
<tr>
<td>16.01 - 35.00</td>
<td>+</td>
</tr>
<tr>
<td>35.01 - 120.00</td>
<td>++</td>
</tr>
<tr>
<td>120.01 - 600.00</td>
<td>+++</td>
</tr>
<tr>
<td>&gt; 600.00</td>
<td>++++</td>
</tr>
</tbody>
</table>

**Note 1:** Sample in the range between 13.00 – 16.00 AU/ml is equivocal. In such case it is recommended to repeat 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, it is necessary to repeat the test with more diluted serum samples to obtain the precise quantification.
8.2 Processing of results for estimation of the intrathecal antibody production

An automatic data processing is possible when purchasing the VIDITAB software available from VIDIA.

1) Calculate the anti-CMV IgG antibody concentrations (AU/mL) for all tested samples by multiplying the AU of the sample subtracted from the calibration curve with the sample dilution. For sera AU x 101 and AU x 404, for cerebrospinal fluid AU x 2.

2) Calculate the Specific antibody quotient as follows:

\[ Q_{spec} = \frac{\text{concentration of IgG anti-CMV (AU/mL) in cerebrospinal fluid}}{\text{concentration of IgG anti-CMV (AU/mL) in serum}} \]

3) Compute the Total antibodies quotient as follows:

\[ Q_{total} = \frac{\text{concentration of total IgG (mg/mL) in cerebrospinal fluid}}{\text{concentration of total IgG (mg/mL) in serum}} \]

4) Compute the Albumine quotient as follows:

\[ Q_{alb} = \frac{\text{concentration of albumin (mg/mL) in cerebrospinal fluid}}{\text{Concentration albumin (mg/mL) in serum}} \]

5) Calculate the Limite quotient \( Q_{lim} \) that shows status of the hematoencephalic barrier

\[ Q_{lim} = 0.93 \times 10^{-6} \sqrt{(Q_{alb})^2 + 6 \times 10^{-6} - 1.7 \times 10^{-3}} \]

6) Compute the Antibody Index AI:

a) If \( Q_{total} \text{ IgG} \ < \ Q_{lim} \), then calculate AI using the formula:

\[ AI = \frac{Q_{spec}}{Q_{total}} \]

b) If \( Q_{total} \text{ IgG} \ > \ Q_{lim} \) compute AI using the formula:

\[ AI = \frac{Q_{spec}}{Q_{lim}} \]

9. INTERPRETATION OF RESULTS

9.1. Interpretation of the anti-CMV antibodies in serum.

IgG anti-CMV antibodies in serum are of anamnestic character: After primary infection they persist in the serum for the rest of life. Significant rise in the IgG anti-CMV concentration in paired serum samples, taken 2-3 weeks apart, can indicate CMV reactivation. Serological findings must be interpreted in the context with the clinical symptoms and with the other laboratory results only.

9.2. Evaluation of intrathecal antibody synthesis

<table>
<thead>
<tr>
<th>AI</th>
<th>Intrathecal antibody synthesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 1.5</td>
<td>intrathecal antibody production not found</td>
</tr>
<tr>
<td>1.5 – 2.0</td>
<td>suspect intrathecal antibody production</td>
</tr>
<tr>
<td>&gt; 2.0</td>
<td>intrathecal antibody production proven</td>
</tr>
</tbody>
</table>
10. VALIDITY OF THE TEST

10.1. Validity
The test is valid when:
- The background of the reaction (absorbance of the Dilution buffer well) is less than 0.100.
- The mean Standard D absorbance should be in range that is written in enclosed Quality control certificate.
- The absorbance of Standards follows this order: ST A < ST B < ST C < ST D < ST E.

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, σ = standard deviation)

<table>
<thead>
<tr>
<th>n</th>
<th>Mean value</th>
<th>±σ</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>1.396</td>
<td>0.033</td>
<td>2.4 %</td>
</tr>
<tr>
<td>16</td>
<td>0.340</td>
<td>0.015</td>
<td>4.3 %</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, σ = standard deviation):

<table>
<thead>
<tr>
<th>n</th>
<th>Mean value</th>
<th>±σ</th>
<th>min – max</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>0.237</td>
<td>0.024</td>
<td>0.210 – 0.288</td>
<td>10.1</td>
</tr>
<tr>
<td>8</td>
<td>1.100</td>
<td>0.087</td>
<td>0.998 – 1.280</td>
<td>7.9</td>
</tr>
<tr>
<td>8</td>
<td>1.624</td>
<td>0.086</td>
<td>1.516 – 1.778</td>
<td>5.3</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 specificity and sensitivity
To assess of diagnostic effectivity 544 CMV-negative and 1101 CMV-positive serum samples were tested in double-blinded multilaboratory study. The results were confirmed using independent commercial IVD diagnostic tests. Diagnostic sensitivity was 99.8% and the specificity 100%.

10.4. Interference
Haemolytic, icteric and lipaemic samples showed no influence on results up to the concentration of 50 mg/mL of hemoglobin, 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 of 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.

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.

The waste from strip washing disinfect in waste container using appropriate disinfection solution (e.g. Incidur, Incidin, chloramin,...) in concentrations recommended by the producer.

Do not pipette by mouth. Do not smoke, eat or drink where specimens or kit reagents are handled. Wear disposable gloves while handling kit reagents or specimens and wash your hands thoroughly afterwards. Avoid spilling or producing aerosol.

12. HANDLING PRECAUTIONS

a. Manufacturer guarantees performance of the entire ELISA kit.
b. Follow the assay procedure indicated in the Instruction manual.
c. Wash buffer, chromogenic substrate TMB, Stop solution and Dilution buffer are interchangeable between ELISA-VIDITEST kits unless otherwise stated in the instruction manual.
d. Avoid contamination of serum samples and kit reagents.
e. Avoid cross-contamination of reagents.
g. Avoid microbial contamination of serum samples and kit reagents.
h. Avoid cross-contamination of reagents.
i. Avoid contact of the TMB substrate with oxidizing agents or metal surfaces.
j. 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 DATE

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 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 of 72 hours have no influence on expiration.

If you find damage at any part of the kit, please inform the manufacturer immediately.

Expiration date is indicated at the ELISA kit label and at all reagent labels.
14. **Flow Chart**:

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Step 1</strong></td>
<td>Prepare reagents and samples</td>
</tr>
<tr>
<td>↓</td>
<td></td>
</tr>
<tr>
<td><strong>Step 2</strong></td>
<td>Dispense 100 μL / well of Standards and samples</td>
</tr>
<tr>
<td>↓</td>
<td>Incubate 60 minutes at room temperature</td>
</tr>
<tr>
<td>↓</td>
<td>Wash 4 times (250 μL / well), aspirate</td>
</tr>
<tr>
<td>↓</td>
<td></td>
</tr>
<tr>
<td><strong>Step 3</strong></td>
<td>Dispense 100 μL / well of Px conjugate</td>
</tr>
<tr>
<td>↓</td>
<td>Incubate 60 minutes at room temperature</td>
</tr>
<tr>
<td>↓</td>
<td>Wash 4 times (250 μL / well), aspirate</td>
</tr>
<tr>
<td>↓</td>
<td></td>
</tr>
<tr>
<td><strong>Step 4</strong></td>
<td>Dispense 100 μL / well TMB substrate</td>
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<tr>
<td>↓</td>
<td>Incubate 10 minutes in dark at room temperature</td>
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<td>↓</td>
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<tr>
<td><strong>Step 5</strong></td>
<td>Dispense 100 μL / well of Stop solution</td>
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<td>↓</td>
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</tr>
<tr>
<td><strong>Step 6</strong></td>
<td>Read optical density at 450 nm (ref. 620 – 690 nm) within 20 minutes</td>
</tr>
</tbody>
</table>

**References:**


Revision date: 06/2013