ELISA-VIDITEST anti-CMV IgM capture
Cat. No. ODZ-162
Instruction manual

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1. TITLE:
ELISA-VIDITEST anti-CMV IgM capture - ELISA kit for the detection of IgM antibodies to human cytomegalovirus (CMV) in serum.

2. INTENDED USE:
The test is intended for the diagnosis of CMV-caused or CMV-associated diseases such as infectious mononucleosis (IM), CMV syndrome or organ-associated CMV infection in immunocompromised patients, acute CMV infection in infants and congenital CMV infection in newborns. In complex with other serological tests (TORCH) it can be used for diagnosis of acute CMV infection in pregnant women.

3. TEST PRINCIPLE:
ELISA-VIDITEST anti-CMV IgM capture is based on the anti-μ-capture principle. Anti-human IgM antibodies are fixed to each well of the microtiter strips. All the IgM class antibodies present in the patient’s sample are bound during the first incubation to the solid-phase antibody. After removing unbound material by washing CMV antigen/Peroxidase conjugate is added. During the second incubation the antigen/conjugate will bind to the CMV-specific IgM antibodies, which have been captured by the anti-human IgM antibodies during the first incubation. Excess conjugate is removed by washing and TMB substrate is added, resulting in the development of a blue colour. The enzyme reaction is terminated by the addition of a 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 anti-human IgM antibody STRIPS, Ab 1 microplate
1.3 mL Negative control serum r.t.u. CONTROL - 1 vial
1.3 mL Calibrator, r.t.u. CAL 1 vial
Lyophilized mixture of CMV specific antigens Ag, LYOF 6 vials
0.15 mL Px-conjugate, anti-GST Px, 101x concentrated CONJ 101x 1 vial
55 mL Wash buffer concentrate, 10x concentrated WASH, 10x 1 vial
100 mL Dilution buffer, r.t.u. DIL 1 vial
13 mL Chromogenic substrate TMB-BF, r.t.u. TMB-BF 1 vial
13 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-BF] is compatible and interchangeable between ELISA-VIDITEST kits which contain TMB-BF and not with other Chromogenic substrates TMB, TMB-O.
5. MATERIAL REQUIRED BUT NOT PROVIDED WITH THE KIT:
Distilled or deionised water for dilution of the Wash buffer concentrate and the lyophilized antigen. Appropriate equipment for pipetting, liquid dispensing and washing, spectrophotometer/colorimeter (microplate reader – wavelength 450 nm).

6. PREPARATION OF REAGENTS AND SAMPLES:

a) Allow all kit components to reach room temperature.

b) Vortex samples and the Controls in order to ensure homogeneity and mix all solution well prior use.

c) **Dilute serum samples 101x in Dilution buffer** and mix (5 \( \mu L \) of serum sample + 500 \( \mu L \) of Dilution buffer). **Do not dilute** the Controls, they are ready to use.

d) Prepare Wash buffer by diluting the Wash buffer concentrate 10 x 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 week if stored at +2 to +10°C.

e) **Do not dilute** TMB-BF 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 the adequate number of strips and put unused strips into the provided pouch and seal it carefully with the desiccant kept inside.

b) Start with filling the first two wells with 100 \( \mu L \) of Calibrator \( \text{CAL} \). Fill the following well with 100 \( \mu L \) of Negative control serum \( \text{CONTROL} \). Calibrator simultaneously serves as a positive control. The remaining wells fill with 100 \( \mu L \) of diluted tested sera (S1...) according to the pipetting scheme in Figure 1 (page 3). 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 (triplets for Calibrator).

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

c) **Prepare the Antigen/ Px-conjugate mixture**: Pipette 1 mL of distilled/ deionised water into the vial with lyophilized antigen \( \text{Ag.LYOF} \) and dissolve by gently mixing. After complete solution add 1 mL of Dilution buffer and mix gently. (1 vial is sufficient for 2 strips). Dissolved antigen can be stored up to two weeks at +2 to +10°C, see Cap.13). Immediately before use dilute Px-conjugate \( \text{CONJ.101x} \) with dissolved antigen 1:101 (e.g. by adding 20 \( \mu L \) of Px-conjugate into the 1 vial (2 mL) of dissolved antigen). Mix gently to avoid foaming. Prepare only the amount necessary for the test. The Antigen/ Px-conjugate mixture cannot be stored.

d) Aspirate the liquid from the wells into a collecting bottle containing appropriate disinfectant (see Safety Precautions). **Wash and aspirate the wells 5x** with 250 \( \mu L \)/well of Wash buffer. Avoid cross-contamination between wells!

If any liquid remains in the wells, invert the plate and tap it on an adsorbent paper to remove the last remaining drops.

e) **Add 100 \( \mu L \) of the Antigen/ Px-conjugate mixture** into each well.

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

f) **Aspirate and wash 5x** with 250 \( \mu L \)/well of Wash buffer.

g) Dispense 100 \( \mu L \) of TMB substrate \( \text{TMB-BF} \) into each well. **Incubate for 20 minutes** (+/- 30 seconds) in dark at room temperature. The time measurement must be started at the beginning of TMB-BF dispensing. Keep the strips in the dark during the incubation with TMB-BF substrate.

h) **Stop the reaction by adding 100 \( \mu L \) of Stop solution \( \text{STOP} \)** Use the same pipetting rhythm as with the TMB-BF 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.

i) **Read the absorbance at 450 nm** with a microplate reader within 10 minutes. It is recommended to use reference reading at 620-690 nm.
8. PROCESSING OF RESULTS

8.1 Processing of results for Qualitative screening interpretation

1. Compute the mean absorbance of the two wells of Calibrator [CAL]. If Calibrator was applied as triplet and if any of the three parallels absorbance is different from the mean in more than 20% then exclude the deviating well from the calculation and compute a new absorbance mean with using the other two wells.

2. Compute the cut-off value of the test by multiplication the Calibrator mean by the Correction factor. The Correction factor value for the particular Lot is written in enclosed Quality control certificate.

3. Serum samples with absorbances < 80% of cut-off value are considered negative and samples with absorbances > 120% of cut-off value 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:

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

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

<table>
<thead>
<tr>
<th>Index value</th>
<th>Evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 0.80</td>
<td>Negative</td>
</tr>
<tr>
<td>0.80 - 1.20</td>
<td>+/-</td>
</tr>
<tr>
<td>1.21 - 4.10</td>
<td>+</td>
</tr>
<tr>
<td>4.11 - 7.00</td>
<td>++</td>
</tr>
<tr>
<td>7.01 - 104.00</td>
<td>+++</td>
</tr>
<tr>
<td>&gt; 10.00</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 of calculation:

Calibrator absorbances = 1.224; 1.272
Mean Calibrator absorbance = 1.248
Sample absorbance = 0.712
Correction factor = 0.18
Cut-off value = 1.248 * 0.18 = 0.225
Sample Positivity Index = 0.712/0.225 = 3.16
9. INTERPRETATION OF THE RESULTS

Interpretation of the results must be done in context of patient’s history, clinical symptoms and the results of other laboratory examinations. In immunocompromised patients CMV IgM negativity cannot exclude recent active infection. On the contrary, single CMV-IgM positivity does not provide proof of recent active infection. Samples from patients with acute EBV, HSV, VZV, toxoplasma, legionella or chlamydia infection or samples from patients with some autoimmune disorders may provide false positive results due cross-reactivity or polyclonal activation of antibody production. In pregnant women, supplementary serological tests, i.e. CMV IgG avidity or seroconversion should be taken into consideration.

10. CHARACTERISTICS OF THE TEST

10.1 Validity of the test
The test is valid if:
The absorbance of Negative control serum CONTROL is lower than 0.8x cut-off value.
The mean Calibrator CAL absorbance should be in range that is written in enclosed Quality control certificate.
The test was validated for determination of the antibodies in human serum only.

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>1.335</td>
<td>0.050</td>
<td>3.8%</td>
</tr>
<tr>
<td>16</td>
<td>0.614</td>
<td>0.023</td>
<td>3.7%</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>18</td>
<td>1.369</td>
<td>0.064</td>
<td>1.223-1.476</td>
<td>4.7%</td>
</tr>
<tr>
<td>18</td>
<td>0.463</td>
<td>0.060</td>
<td>0.337-0.569</td>
<td>12.9%</td>
</tr>
<tr>
<td>43</td>
<td>1.372</td>
<td>0.119</td>
<td>1.184-1.750</td>
<td>8.7%</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
Diagnostic sensitivity of the test was evaluated on the panel of CMV IgM-positive patients’ samples. Diagnostic specificity was evaluated on the panel of CMV-IgM negative serum samples. The results were confirmed using independent commercial diagnostic kits. Diagnostic sensitivity and specificity of the test was 96.2% and 96%, respectively.

10.4 Interference
Haemolytic and lipemic samples have no influence on the test results up to concentration 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 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. Dispose of the unused mixture of antigens like infectious waste, use an appropriate disinfectant (3% solution of chloramine, incidur etc.) Liquid wastes containing acid (Stop solution) should be neutralized in 4% sodium bicarbonate solution. The waste from strip washing disinfect in waste container using appropriate disinfection solution (e.g. Incidur, Incidin, chloramin, ...) in concentrations recommended by the producer. 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. 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. Wash buffer, Stop solution and Dilution buffer are interchangeable between ELISA-VIDITEST kits unless otherwise stated in the instruction manual. Controls, Dilution buffer and Px-conjugate contain preservative ProClin 300®. TMB-BF substrate contains preservative Kathon. Avoid microbial contamination of serum samples and kit reagents. Avoid cross-contamination of reagents. Avoid contact of the TMB-BF 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 and a diluted mixture of Antigen/ Px-conjugate. Always prepare fresh. Dissolved Antigen can be stored up to two weeks at +2 to +10°C. 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.

References:

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

Date of the last revision of this manual: 06/2014

The development of this kit was supported by grant from the Ministry of Industry and Trade of the Czech Republic