ELISA-VIDITEST anti-complement factor H

ODZ-166

Instructions for use

PRODUCER:
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1. TITLE
ELISA-VIDITEST anti-complement factor H

2. INTENDED USE
ELISA-VIDITEST anti-complement factor H is intended for the quantitative detection of IgG antibodies against human complement factor H in human serum or plasma. Factor H is a complement regulatory glycoprotein that is found in human plasma in concentrations about 500 µg/mL. Its main function is the regulation of complement activation. Inhibitory autoantibodies against complement factor H resulting from an immunopathological reaction, dysregulate complement system. Such autoimmune dysregulation of complement is associated with a specific form of atypical haemolytic uremic syndrome (AI-HUS). It is recommended testing anti-complement factor H autoantibodies in all cases of HUS at the onset of the disease. Approximately 30% of AI-HUS patients had diarrhoea as prodromal syndromes, which in turn are the typical sign in the classic form of HUS which is caused by Shigga toxin positive species of E. coli. Removal of anti-factor H antibodies from the bloodstream by plasmapheresis or the use of immune suppressive drugs to eliminate the antibody production is beneficial for the outcome of the disease.

3. TEST PRINCIPLE
ELISA-VIDITEST anti-complement factor H is an enzyme linked immunosorbent assay designed to detect IgG antibodies against complement factor H. The wells of the microtitrate plate are coated with purified human complement factor H. Antibodies against factor H present in serum sample bind to the immobilized factor H. Other antibodies, unbound to the factor H, are washed away during the next step. Then anti-human IgG antibodies labelled with horseradish peroxidase are added and those detect the antibodies from the sample that previously bound to factor H. The unbound labelled antibodies are washed away and the remaining labelled antibodies are visualized with a chromogenic substrate. The peroxidase activity leads to a change in colour of the solution. The reaction is stopped by adding an acidic solution. The colour intensity is directly proportional to the amount of anti-factor H antibodies in the sample.

4. KIT COMPONENTS
ELISA strips coated with human factor H purified from human plasma STRIPS Ag 6 strips
50 µL Anti-CFH IgG standard (10.000 AU/mL) STANDARD 1 vial
100 µL IgG-HRP conjugate 101x concentrated CONJ101x 1 vial
55 mL Wash buffer 10x concentrated WASH10x 1 vial
60 mL Dilution buffer (DB) ready to use DIL 1 vial
13 mL Chromogenic substrate (TMB-BF substrate) TMB-BF 1 vial
13 mL Stop solution r.t.u. STOP 1 vial
Instruction manual
Quality control certificate
1) AU/ml (Arteficial units/ml)
2) r.t.u. ready to use

Notice: Control sera may be colorless to yellowish or blue due to the use of different diluents.
5. MATERIAL NEEDED BUT NOT PROVIDED
Distilled/deionised water; precision micropipets 20, 200 and 1000 µL and suitable tips; graduated cylinders (1000 mL); microplate washer or other device for microplate washing; absorbent papers; ELISA reader; adhesive membrane or microplate lid to cover the wells during incubations.

Note: It is recommended to use a precise dispenser e.g. Multipette Xtrawm Eppendorf for the dispensing of the TMB-BF and STOP solution.

6. PREPARATION OF REAGENTS
Allow all the kit components to reach room temperature (~ 20 min). Mix all reagents well before use to ensure homogeneity.

WASH BUFFER
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.

DETECTION ANTIBODY anti-IgG CONJUGATED TO HRP (IgG-HRP)
For one 8-well strip prepare 1 mL of the IgG-HRP conjugate solution. Dilute the IgG-HRP concentrate 101x with the Dilution buffer.

If you intend to prepare a certain amounts of the IgG-HRP solution see the recommendations indicated in table 1. Do not store the diluted IgG-HRP.

Table 1.

<table>
<thead>
<tr>
<th>Number of 8-well strips</th>
<th>IgG-HRP conjugate concentrate 101x (µL)</th>
<th>Dilution buffer (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>20</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>30</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>40</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>50</td>
<td>5</td>
</tr>
<tr>
<td>6</td>
<td>60</td>
<td>6</td>
</tr>
</tbody>
</table>

STANDARD anti-CFH IgG
Standard is supplied as 10.000 AU/mL stock solution. Prepare the serial dilutions of the Standard as follows:
Prepare eight 1.5 mL PP microtubes and label them as 250 AU, 125 AU, 62.5 AU, 31.25 AU, 15.6 AU, 7.8 AU, 3.9 AU, 0 AU. Pipette sequentially 800 µL, 400 µL, 400 µL, 400 µL, 400 µL, 400 µL, 400 µL of the Dilution buffer to the microtubes. Mix well the Standard stock solution and pipette 20 µl of the stock solution to the first microtube (250 AU/mL). Mix the content of the tube, remove 400 µl and add it to the second tube (125 AU/mL). Then continue with dilutions as 400 + 400 µl to prepare the entire set of concentrations ranging from 250 AU/mL to 3.9 AU/mL. Do not add any anti-CFH into the tube labelled with 0, the zero standard is the Dilution buffer only. Change the pipette tip after each dilution, always mix well the content.

For easier understanding of the standard dilution procedure look at the scheme bellow (Dilution of the anti-CFH standard).
Figure No. 1 Dilution of the anti-CFH standard

<table>
<thead>
<tr>
<th>STANDARD Anti-CFH IgG</th>
<th>10,000 AU/mL</th>
<th>20 µL</th>
</tr>
</thead>
</table>

- **800 µL**
  - 400 µL
  - 400 µL
  - 400 µL
  - 400 µL
  - 400 µL

- **250 AU**

- **125 AU**

- **62.5 AU**

- **31.25 AU**

- **15.6 AU**

- **7.8 AU**

- **3.9 AU**

- **0 AU**

**SAMPLERS**
Store serum, plasma samples frozen at -18°C or lower.
Thaw plasma samples quickly in a water bath at 37°C, the plasmatic proteins may precipitate if thawed slowly. Thaw serum samples either in a water bath at 37°C or at the laboratory temperature.
Dilute the samples 101x with the Dilution buffer (eg. 5 µL sample + 500 µL Dilution buffer).
Prepare enough volume to measure each diluted sample in replicates 100 µL/well.
If you expect anti-CFH concentrations higher than 250 AU/mL dilute the samples with the Dilution buffer to obtain the concentrations that will fall within the standard range (250-3.9 AU/mL).
Do not dilute Dilution buffer, TMB-BF solution, STOP solution! They are ready to use.

**7. ASSAY PROCEDURE**
Manufacturer will not be held responsible for results if manual is not followed exactly.

a. Allow all the kit components to reach temperature (~ 20 min).
b. Prepare the working concentrations (in the volume needed) of Wash buffer and of the IgG-HP solution.
c. Dilute the anti-CFH IgG standard STANDARD to concentrations 250, 125, 62.5, 31.25, 15.6, 7.8, 3.9 and 0 AU/mL.
d. Dilute the samples 101x with the Dilution buffer DIL.
e. Open the aluminium bag containing the strips and remove the desired number of strips. Put the unused strips together with the desiccant to the provided plastic bag and seal it. Store the unused strips at +2 to +10°C.
f. Pipette 100 µL of standards (0 – 250 AU/mL) and samples to the wells (see Pipetting scheme).
g. Cover the strips with the sealing membrane or with a lid. The cover prevents evaporation from the wells during the incubation. Incubate for 1 hour (+/-5 minutes) at laboratory temperature.
h. Aspirate and wash 5 times with 250-400 µL of the Wash buffer. Invert and tap the plate on a pile of absorbent papers (see Safety precautions).
i. Pipette 100 µL of the diluted IgG-HRP CONJ into each well. Incubate for 1 hour (+/-5 minutes) at laboratory temperature.
j. Aspirate and wash 5 times with 250-400 μL of the Wash buffer. Invert and tap the plate on a pile of absorbent papers (see Safety precautions).

k. Pipette 100 μL of TMB-BF solution [TMB-BF] to the wells. Incubate in dark place for 20 minutes (+/- 1 minute).

l. Pipette 100 μL of STOP solution [STOP] to the wells.

m. Tap the microplate side gently to ensure complete mixing of the [TMB-BF] with the [STOP] solution.

n. Read the absorbance at 450 nm, it is recommended to use a reference reading 620-690 nm.

Figure No. 2 Pipetting scheme (S = STANDARD, P = patient sample):

<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>S 0</td>
<td>S 0</td>
<td>P1</td>
<td>P1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>S 3.9</td>
<td>S 3.9</td>
<td>P2</td>
<td>P2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>S 7.8</td>
<td>S 7.8</td>
<td>P3</td>
<td>P3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>S 15.6</td>
<td>S 15.6</td>
<td>P4</td>
<td>P4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>S 31.3</td>
<td>S 31.3</td>
<td>...</td>
<td>...</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>S 62.5</td>
<td>S 62.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>S 125</td>
<td>S 125</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>S 250</td>
<td>S 250</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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</tr>
</tbody>
</table>

8. PROCESSING OF RESULTS
Subtract the absorbance at the reference wavelength from the absorbance at 450 nm (usually performed automatically by the ELISA reader).

Compute means in duplicates.

Subtract the Standard 0 mean from all of the other mean values (Blank Difference data). If the absorbance of controls or tested sera are negative after background subtraction, consider them as zero value.

Construct the standard curve by plotting the mean absorbance for each standard (Blank Difference data) on the y-axis versus the concentration in AU/mL on the x-axis. Draw the best fit curve through the standard points.

*Note: Regression is linear only if the range is narrowed to 125 AU/mL as a maximum, if you wish to use the entire set of concentration as the standard points (3.9-250 AU/mL) you will need to use a different fitting algorithm suitable for ELISA type data (i.e. cubic spline, 4PL algorithm).*

Compute the concentrations of anti-CFH IgG in AU/mL in samples according to the standard curve formula.

9. INTERPRETATION OF RESULTS
Samples with concentrations lower than 3.9 AU/mL (the lowest standard) interpret as <3.9 AU/mL of anti-CFH IgG. Samples with concentrations higher than 250 AU/mL interpret as >250 AU/mL or dilute them with Dilution buffer and repeat the test with the diluted samples, e.g. 202x a 404x (multiply the final measured concentration with the dilution factor, i.e. 2x or 4x).

To characterize the sample as anti-CFH IgG positive or negative, it is suitable to determine your own cut-off value. Cut-off value depends on the chosen population group.

*Use your own routinely used calculation or our recommended procedure:*

The cut-off value is determined from expected anti-CFH IgG negative samples (e.g. blood donors).

Calculation of the cut-off value:

a. Make histogram graph from your samples. Check if the data has normal distribution (Gaussian). If not, compute logarithm of OD (subtract blank) before the data processing. Then check again the normal distribution.

b. Compute the OD mean of all negative samples

c. Compute standard deviation from negative samples

d. Compute cut-off using formula:
OD mean + 3*standard deviation
e. Compute cut-off in AU/mL using your actual calibration curve

Example of cut-off value for the population from Czech Republic:
Cut-off for serum samples was calculated from 107 samples (healthy persons - 59 (55%) men and 49 (45%) women, average age 31 years). The OD values has non-Gaussian distribution, therefore the logarithm of OD was used.

Fig.3 Histogram of from OD negative samples

Fig.4 Histogram of logarithm of OD from negative samples

The calculated cut-off was 27 AU/mL for serum samples, 18 AU/mL for plasma samples. Do not use these values for the interpretation of your results.

10. TEST CHARACTERISTICS
Figure No. 5: Standard curve example (3.9 – 125 AU/ml). Do not use this curve for the calculation of AU/mL from your data.

10.1 Validity criteria
The mean absorbance values of Standards S 0, S 250 and the difference between the Standards S 3.9 and S 0 are in the ranges stated in the Quality control certificate for this kit lot.
10.2 Intraassay
(n = number of tests within the plate):

<table>
<thead>
<tr>
<th>n</th>
<th>sample</th>
<th>AVG AU/mL</th>
<th>±σ</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>27</td>
<td>serum B</td>
<td>5</td>
<td>0.48</td>
<td>9</td>
</tr>
<tr>
<td>24</td>
<td>serum A</td>
<td>266</td>
<td>14.3</td>
<td>5</td>
</tr>
</tbody>
</table>

10.3 Interassay
(n= number of test repetitions)

<table>
<thead>
<tr>
<th>n</th>
<th>sample dilutions</th>
<th>AVG AU/mL</th>
<th>±σ</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>serum A 404x</td>
<td>213</td>
<td>22</td>
<td>10</td>
</tr>
<tr>
<td>8</td>
<td>serum A 808x</td>
<td>122</td>
<td>17</td>
<td>14</td>
</tr>
<tr>
<td>8</td>
<td>serum A 1616x</td>
<td>66</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>8</td>
<td>serum A 3232x</td>
<td>36</td>
<td>4</td>
<td>12</td>
</tr>
</tbody>
</table>

10.4 Detection limit
Detection limit was calculated as the three standard deviations (3x ±σ) from the interassay blank.
Detection limit of the test is 0.6 AU/mL.

10.5 Linearity
Two positive samples were assayed in dilution 101x and also in serial dilutions that ranged from 202x to 6464x.

<table>
<thead>
<tr>
<th>Sample dilution</th>
<th>Observed (AU/mL)</th>
<th>Expected (AU/mL)</th>
<th>O/E (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>101x</td>
<td>&gt;MAX</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>202x</td>
<td>&gt;MAX</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>404x</td>
<td>146</td>
<td>80</td>
<td>110</td>
</tr>
<tr>
<td>808x</td>
<td>12</td>
<td>37</td>
<td>111</td>
</tr>
<tr>
<td>1616x</td>
<td>21</td>
<td>18</td>
<td>113</td>
</tr>
<tr>
<td>3232x</td>
<td>12</td>
<td>9</td>
<td>128</td>
</tr>
<tr>
<td>6464x</td>
<td>12</td>
<td>9</td>
<td>128</td>
</tr>
<tr>
<td>Sample 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>101x</td>
<td>&gt;MAX</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>202x</td>
<td>264</td>
<td>71</td>
<td>104</td>
</tr>
<tr>
<td>404x</td>
<td>137</td>
<td>66</td>
<td>108</td>
</tr>
<tr>
<td>808x</td>
<td>34</td>
<td>33</td>
<td>103</td>
</tr>
<tr>
<td>1616x</td>
<td>18</td>
<td>17</td>
<td>111</td>
</tr>
<tr>
<td>3232x</td>
<td>8</td>
<td>8</td>
<td>102</td>
</tr>
</tbody>
</table>

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

10.7 Diagnostic sensitivity and specificity of the test
Diagnostic specificity was determined using 130 serum samples (blood donors), where we did not expect anti-CFH IgG antibodies. Specificity of the test was 98.5%.
Diagnostic sensitivity was determined using 9 samples from patients with DEAP-HUS (deficiency of CFHR plasma proteins and factor H autoantibody positive HUS) confirmed by genetic tests. Diagnostic sensitivity was 100%.

11. SAFETY PRECAUTIONS
All ingredients of the kit are intended for laboratory use only.
STANDARD contains human plasma 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.
The test procedure requires qualified laboratory personnel.
Do not smoke, eat or drink during work.
Do not pipette by mouth, use suitable pipetting device.
Wear protective disposable gloves while handling reagents or samples and wash your hands thoroughly afterwards.
Avoid spilling or producing aerosol.
Autoclave all reusable materials that were in contact (spilled) with human samples, 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.
STOP solution contains 1M sulphuric acid, handle with caution. Avoid contact with skin, eyes or mucous membranes. In case of contact with eyes, skin or mucous membranes rinse immediately with plenty of water and seek medical advice

12. HANDLING PRECAUTIONS
Manufacturer guarantees performance of the entire ELISA kit.
Avoid microbial contamination and cross-contamination of samples and kit reagents.
Avoid contact of the TMB-BF substrate with oxidizing agents or metal surfaces.
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 with HRP-CONJUGATE
* Use of identical pipette tip for different solutions

13. STORAGE AND EXPIRATION
The ELISA kit should be used within three months after opening.
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 -18°C to -28°C. Avoid repeated thawing and freezing.
Do not store diluted samples in the working concentration. Always prepare fresh.
Kits are shipped in cooling bags.
Expiration date is indicated at the ELISA kit label and at all reagent labels.
If you find any damage at any part of the kit, please inform the manufacturer.

REFERENCES:
Anti-Factor H autoantibodies associated with atypical hemolytic uremic syndrome.
Successful pre-transplant management of a patient with anti-factor H autoantibodies-associated haemolytic uraemic syndrome.
Factor H autoantibodies in atypical hemolytic uremic syndrome correlate with CFHR1/CFHR3 deficiency.
Anti-factor H autoantibody-associated hemolytic uremic syndrome: review of literature of the autoimmune form of HUS.
14. USED SYMBOLS

\(
\begin{align*}
\triangle & \quad \text{number of tests} \\
\checkmark & \quad \text{Conformité Européenne – product meets the requirements of European legislation} \\
\text{ND} & \quad \text{in vitro diagnostics} \\
\pm \sigma & \quad \text{standard deviation} \\
\text{CV} & \quad \text{coefficient of variation} \\
\text{OD} & \quad \text{optical density} \\
\text{manufacturer} & \quad \text{manufacturer} \\
\text{expiration} & \quad \text{expiration} \\
\text{LOT} & \quad \text{Lot of kit} \\
\text{storage at } +2^\circ \text{C} - +10^\circ \text{C} & \\
\text{Celsius degree} & \\
\% & \quad \text{percentage} \\
n & \quad \text{number of tested samples} \\
A & \quad \text{value of tested sample} \\
\text{read usage instructions} & \\
\text{Cat. No. Catalog number} & \end{align*}
\)
**15. FLOW CHART**

<table>
<thead>
<tr>
<th>Step 1</th>
<th>Prepare the working concentrations of reagents, standards and dilute samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Step 2</td>
<td>Pipette 100 μL of standards and the diluted samples to the wells</td>
</tr>
<tr>
<td></td>
<td>Incubate 60 minutes at room temperature</td>
</tr>
<tr>
<td></td>
<td>Wash 5 times (250-400 μL/well), aspirate</td>
</tr>
<tr>
<td>Step 3</td>
<td>Pipette 100 μL of the diluted IgG-HRP</td>
</tr>
<tr>
<td></td>
<td>Incubate 60 minutes at room temperature</td>
</tr>
<tr>
<td></td>
<td>Wash 5 times (250-400 μL/well), aspirate</td>
</tr>
<tr>
<td>Step 4</td>
<td>Dispense 100 μL/well of TMB-BF substrate</td>
</tr>
<tr>
<td></td>
<td>Incubate 20 minutes in dark 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>

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

Date of the last revision of this manual: 04/2018