Hantavirus Dobrava/Hantaan IgG/IgM ELISA Kit

Prod. No.: DEIA595
Pkg. Size: 96T

**INTENDED USE**

Enzyme Immunoassay for the Detection of Human Antibodies against Hantavirus DOB/HTN in Serum

**GENERAL DESCRIPTION**

Hantaviruses are single-stranded enveloped RNA viruses in the Bunyaviridae family, are widespread and are strictly associated with their serotype-specific reservoir hosts. They cause hemorrhagic fever with renal syndrome (HFRS) in Europe and Asia and lead to hantavirus cardiopulmonary syndrome (HCPS) in America. The severity of the disease depends on the infecting virotype with case fatality rates from 0.1% - 10% for HFRS and 25-35% for HCPS. Natural reservoir hosts are rodents, the virus is transmitted to humans via aerosol derived from rodent urine, feces or saliva. HFRS is caused by Hantaan virus and the closely related Dobrava virus as well as Seoul type and Puumala virus. Hantavirus serotypes show the same geographical distribution pattern as their reservoir hosts. Hantaan virus (host: Apodemus agrarius) is endemic in Asia, Dobrava virus (host: different Apodemus sp.) is distributed in the Balkans, Central, Eastern and Southern Europe, the form of the disease ranges from moderate to severe. Puumala serotype (host: Myodes clareolus) is predominant in Scandinavia and Western Europe and causes a mild infection, also called Nephropathia epidemica (NE). In Germany more than 90% of Hantavirus infections are caused by Puumala Virus. People at high risk are farmers, soldiers, campers.

**PRINCIPLE OF THE TEST**

The microtiter plate is coated with recombinant nucleocapsid protein of Hantavirus. For determination of IgM antibodies, patient sera must be incubated with rheumatoid-factor-IgG-absorbent before starting the test procedure in order to eliminate unspecific reactions caused by IgG antibodies or rheumatoid factor. During the incubation period specific antibodies against the recombinant Hantaan antigen are bound to the solid phase. After washing, the specific IgG and IgM antibodies are detected with peroxidase-conjugated secondary antibody. Addition of substrate solution results in a color reaction, which is proportional to the bound specific antibody content. The absorbance is then measured photometrically.

**REAGENTS PROVIDED**

MTP: 96-well microtiter plate, coated with recombinant Hantaan antigen, sealed in an aluminum bag with desiccant. If required, individual wells can be broken off from each strip. Ready-to-use!

**PG:** Positive control IgG, human sera with stabilizers and preservatives; 1 vial, 1.5 ml. Ready-to-use!

**RG:** Reference control IgG, human sera with stabilizers and preservatives; 1 vial, 1.5 ml. Ready-to-use!

**PM:** Positive control IgM, human sera with stabilizers and preservatives; 1 vial, 1.5 ml. Ready-to-use!

**PM:** Reference control IgM, human sera with stabilizers and preservatives; 1 vial, 1.5 ml. Ready-to-use!

**NEG:** Negative control, human sera with stabilizers and preservatives; 1 vial, 1.5 ml. Ready-to-use!

**SB 20x:** Sample buffer (20x), PBS pH 7.4, contains detergent and 0.01% thimerosal 1 bottle, 15 ml. Dilute before use!

**WASH 20x:** Wash buffer (20x), PBS pH 7.5, contains detergent and 0.01% thimerosal, 1 bottle, 50 ml. Dilute before use!

**CM 20x:** Anti IgG peroxidase conjugate (20x), 1 vial, 750 µl. Dilute before use!

**CM 20x:** Anti IgM peroxidase conjugate (20x), 1 vial, 750 µl. Dilute before use!

**S:** Substrate, tetramethylbenzidine (TMB) in methylpyrrolidone; 1 bottle, 12 ml. Ready-to-use!

**STOP:** Stop solution, 0.5 M sulfuric acid, 1 bottle, 12 ml. Ready-to-use!

**ABS:** Rheumatoid factor IgG absorbent; anti human IgG with stabilizers and preservatives; 1 vial, 1.5 ml. Ready-to-use! Adhesive foil for covering ELISA test strips; 2 pieces.

**MATERIALS REQUIRED BUT NOT PROVIDED**

1. Distilled Water
2. Graduated cylinder
3. Tubes for dilution of samples
4. Precision pipettes (5 µl, 20 µl, 50 µl, 200 µl, 1000 µl)
5. Multichannel- or dispensing pipettes (100 and 200 µl)
6. Pipette tips
7. ELISA reader, 450 nm filter
8. Gloves
9. Timer

**ASSAY PROCEDURE**

1. **Reagent And Sample Preparation**
   1) Sample Material and Storage
      Human serum must be used as sample material for the Hantavirus DOB/HTN IgG/IgM ELISA. Samples can be stored at 2 -8°C up to 6 weeks. Samples can be stored undiluted for several months at -20°C. Avoid repeated freezing/thawing.
   2) Preparation of Reagents:
      a. Allow kit to reach room temperature (20-26°C). Buffer
concentrates may contain salt crystals which dissolve quickly at 37°C. Let buffer cool to room temperature (20-26°C) before starting the test.

b. Dilute required volumes of reagents immediately before use!

Ready-to-use sample buffer, 1+19:
Example for 8 wells: Add 1 ml SB 20x to 19 ml distilled water.
Ready-to-use wash buffer, 1+19:
Example for 8 wells: Add 1 ml Wash 20x to 19 ml distilled water.
Ready-to-use conjugates, 1+19:
Example for 8 wells: Add 50 μl CG 20x or CM 20x to 950 μl ready-to-use wash buffer.
Dilution of patient samples, 1+200:
IgG: Add 5 μl serum to 1000 μl ready-to-use sample buffer.
IgM: Dilution as described for IgG. Add 15 μl ABS to 250 μl diluted serum, incubate 30 min at room temperature (20-26°C).

2. Assay Steps

1) Sample incubation: Pipette 100 μl undiluted negative, positive, and reference controls as well as diluted (eventually pre-treated with ABS) patient sera per well. Cover strips with adhesive foil. Incubate at 37°C for 45 min.
2) Wash: Empty microassay strips and fill each well with 200 μl ready to use wash buffer. Empty wells again and repeat this wash step three times. Remove excess liquid by tapping the strips onto absorbent paper.
3) Conjugate incubation: Pipette 100 μl ready to use conjugate (IgG or IgM) per well. Cover strips with adhesive foil. Incubate at 37°C for 45 min.
4) Wash: Empty microassay strips and carry out wash steps as described above (4 x 200 μl per well).
5) Substrate reaction: Pipette 100 μl ready to use substrate per well. Incubate 10 min at room temperature (20-26°C).
6) Stop: Add 100 μl STOP to each well.
7) Measure color within 20 min at 450 nm (reference wavelength at 650 nm).

EVALUATION & CALCULATION

1. Calculation

For calculation of results, the ratio of the absorbance of the patient sample and the reference control is determined:

\[ \frac{\text{patient sample}}{\text{reference control}} = Q \]

and interpreted as follows:

<table>
<thead>
<tr>
<th>Q</th>
<th>Interpretation</th>
</tr>
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<tbody>
<tr>
<td>&lt; 1</td>
<td>Negative: No IgG antibodies against DOB/HTN virus detected.</td>
</tr>
<tr>
<td>1 ≤ Q ≤ 1.5</td>
<td>No clear interpretation possible. The course of the disease should be monitored after 10 days. In case of suspected Hantavirus infection, it is recommended to test the sample also for DOB/HTN IgM antibodies and/or antibodies against the Puumala serotype.</td>
</tr>
<tr>
<td>&gt; 1.5</td>
<td>Positive: Specific IgG antibodies against DOB/HTN virus detected.</td>
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b) For IgM-antibodies

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<tr>
<td>&lt; 1</td>
<td>Negative: No IgM antibodies against DOB/HTN detected.</td>
</tr>
<tr>
<td>1 &lt; Q &lt; 2</td>
<td>No clear interpretation possible. The course of the disease should be monitored after 10 days. In case of suspected Hantavirus infection, it is recommended to test the sample also for antibodies against the Puumala serotype.</td>
</tr>
<tr>
<td>&gt; 2</td>
<td>Positive: Specific IgM antibodies against DOB/HTN virus detected.</td>
</tr>
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2. Evaluation

a) IgG

Evaluation of the original Hantavirus (Hantaan) IgG ELISA in parallel with the Hantavirus (Puumala) IgG ELISA as well as Hantavirus Hantaan Antibody IF Test, Hantavirus Puumala Antibody IF Test, Hantavirus Seoul Antibody IF Test, revealed a diagnostic efficiency as follows:

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<th>HTN IFT</th>
<th>IFT ELISA</th>
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<tbody>
<tr>
<td>n = 38</td>
<td>n = 29</td>
<td>n = 56</td>
<td></td>
</tr>
<tr>
<td>95%</td>
<td>88%</td>
<td>99%</td>
<td>Hantaan IgG ELISA</td>
</tr>
<tr>
<td>75%</td>
<td>98%</td>
<td>84%</td>
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Sensitivity and Specificity was determined with 194 sera of healthy blood donors and a total of 123 IgG positive sera (56 IgG positive for Hantaan virus in indirect immunofluorescence). Sensitivity was 98%, specificity for Hantaan virus was 99%.

b) IgM

Evaluation of the original Hantavirus (Hantaan) IgM ELISA in parallel with the Hantavirus (Puumala) IgM ELISA as well as the Hantavirus Hantaan Antibody IF Test, Hantavirus Puumala Antibody IF Test, Hantavirus Seoul Antibody IF Test, revealed a diagnostic efficiency as follows:

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<td>n = 47</td>
<td>n = 25</td>
<td>n = 50</td>
<td></td>
</tr>
<tr>
<td>95%</td>
<td>74%</td>
<td>100%</td>
<td>Hantaan IgM ELISA</td>
</tr>
<tr>
<td>68%</td>
<td>99%</td>
<td>62%</td>
<td>Puumala IgM ELISA</td>
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Sensitivity and specificity was determined with 194 sera of healthy blood donors and a total of 122 IgM positive sera (50 IgM positive for Hantaan virus in indirect immunofluorescence). Sensitivity was 100% and the specificity for Hantaan virus was 100%.

QUALITY CONTROL

The following ratios and absorbent values present quality control parameters of the kit control. The ratios have to be calculated and met for a successful test run (absorbance 450 nm, A):

Q > 1.5: Positive: Specific IgG antibodies against DOB/HTN virus detected.
Q < 1: Negative: No IgM antibodies against DOB/HTN detected.
1 ≤ Q ≤ 1.5: No clear interpretation possible. The course of the disease should be monitored after 10 days. In case of suspected Hantavirus infection, it is recommended to test the sample also for antibodies against the Puumala serotype.
Q > 2: Positive: Specific IgM antibodies against DOB/HTN virus detected.

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REFERENCES


<table>
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<td>PG</td>
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<td>RM</td>
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<tr>
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<td>A negative control IgM</td>
</tr>
<tr>
<td>RM</td>
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If the above criteria are not met, the test run is invalid and has to be repeated.