Hantavirus (Puumala) IgG/IgM ELISA Kit

Prod. No.: DEIA596
Pkg. Size: 96T

INTENDED USE
Enzyme Immunoassay for the Determination of IgG and IgM Antibodies against the Puumala Serotype of Hantavirus

GENERAL DESCRIPTION
Nephropathia epidemica (NE) is caused by infection with the Puumala virus (hantaviruses). The incubation time is 10-20 days and begins with symptoms similar to influenza. Other clinical manifestations are abdominal and lumbal pains and transient, limited renal function. Typical laboratory parameters are thrombocytopenia and leucocytosis. Compared to hemorrhagic fever with renal syndrome (HFRS), caused by viruses of the Hantaan type, the nephropathia epidemica is a mild disease.

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

REAGENTS PROVIDED
MTP: 96-well microtiterplate, coated with recombinant Puumala antigen (strain: CG 18-20), sealed in an aluminium bag with desiccant. If required, individual wells can be broken off from each strip. Ready to use!
P: 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!
PG: Positive control IgM, human sera with stabilizers and preservatives; 1 vial, 1.5 ml. Ready to use!
RM: 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, 2 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!
CG 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 solution, tetramethylbenzidine (TMB); 1 vial, 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 foils; for covering ELISA test strips; 1 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 (Puumala) IgG/IgM ELISA. Samples can be stored at 2-8°C up to 6 weeks. Samples can be stored undiluted for several months at a temperature of at least -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 directly 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.

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

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

and interpreted as follows:

a) For IgG antibodies

- \(Q < 1\): Negative: No IgG antibodies against Puumala virus detected.
- \(1 \leq Q \leq 1.5\): 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 Puumala IgM antibodies and/or antibodies against the Hantaan serotype.
- \(Q > 1.5\): Positive: Specific IgG antibodies against Puumala virus detected.

b) For IgM-antibodies

- \(Q < 1\): Negative: No IgM antibodies against Puumala detected.
- \(1 \leq Q \leq 2\): 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 Hantaan serotype.
- \(Q > 2\): Positive: Specific IgM antibodies against Puumala virus detected.

2. Assay Steps
1) Sample incubation: Pipette 100 \(\mu\)l undiluted negative, positive, and reference controls as well as diluted (eventually pretreated 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 \(\mu\)l 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 \(\mu\)l 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 \(\mu\)l per well).
5) Substrate reaction: Pipette 100 \(\mu\)l ready to use substrate per well. Incubate 10 min at room temperature (20-26°C).
6) Stop: Add 100 \(\mu\)l STOP to each well.
7) Measure color within 20 min at 450 nm (reference wavelength at 650 nm).

EVALUATION & CALCULATION

2. Evaluation
a) IgG
Evaluation of the Hantavirus (Puumala) IgG ELISA in parallel with the Hantavirus (Hantaan) 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:

<table>
<thead>
<tr>
<th>Seoul IFT n = 38</th>
<th>PUU IFT n = 29</th>
<th>HTN IFT n = 56</th>
<th>IFT ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td>95%</td>
<td>88%</td>
<td>99%</td>
<td>Hantaanb IgG ELISA (Cutoff 0.600)</td>
</tr>
<tr>
<td>75%</td>
<td>98%</td>
<td>84%</td>
<td>Puumala IgG ELISA (Cutoff 0.600)</td>
</tr>
</tbody>
</table>

Sensitivity and Specificity was determined with 194 sera of healthy blood donors and total of 123 IgG positive sera (29 IgG positive for Puumala virus in indirect immunofluorescence). Sensitivity was 99%, specificity for Puumala virus 97%.

b) IgM
Evaluation of the Hantavirus (Puumala) IgM ELISA in parallel with the Hantavirus (Hantaan) 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:

<table>
<thead>
<tr>
<th>Seoul IFT n = 47</th>
<th>PUU IFT n = 25</th>
<th>HTN IFT n = 50</th>
<th>IFT ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td>95%</td>
<td>74%</td>
<td>100%</td>
<td>Hantaanb IgM ELISA (Cutoff 0.32)</td>
</tr>
<tr>
<td>68%</td>
<td>99%</td>
<td>62%</td>
<td>Puumala IgM ELISA (Cutoff 0.35)</td>
</tr>
</tbody>
</table>

Sensitivity and specificity was determined with 194 sera of healthy blood donors and a total of 122 IgM positive sera (25 IgM positive for Puumala virus in indirect immunofluorescence). Sensitivity was 100%, and the specificity for Puumala virus 99%.
**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):

<table>
<thead>
<tr>
<th>Condition</th>
<th>Calculation</th>
<th>Requirement</th>
</tr>
</thead>
<tbody>
<tr>
<td>A positive control IgG</td>
<td>PG = RG</td>
<td>A positive control IgG / A reference control IgG</td>
</tr>
<tr>
<td>A positive control IgM</td>
<td>RG = RM</td>
<td>A positive control IgM / A reference control IgM</td>
</tr>
<tr>
<td>A negative control IgG</td>
<td>NEG = RG</td>
<td>A negative control IgG / A reference control IgG</td>
</tr>
<tr>
<td>A negative control IgM</td>
<td>RM = RM</td>
<td>A negative control IgM / A reference control IgM</td>
</tr>
</tbody>
</table>

If the above criteria are not met, the test run is invalid and has to be repeated.

**REFERENCES**