*Instructions for Use*

# Hantavirus Hantaan IgG/IgM ELISA

**Enzyme immunoassay for the qualitative determination of IgG/IgM antibodies against the serotype Hantaan of Hantavirus in human serum.**


**RE57451**


**96**

**2-8°C**

EU: 


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## 1. INTENDED USE

Enzyme immunoassay for the qualitative determination of IgG/IgM antibodies against the serotype Hantaan of Hantavirus in human serum.
The Hantavirus (Hantaan) IgG/IgM-ELISA is suited for the qualitative detection of specific IgG and IgM antibodies against the Hantaan virus in cases of suspected hemorrhagic fever with renal syndrome (HFRS). The detection of infections with Puumala virus or other serotypes is only limited **(see Interpretation of Results / Diagnostic Efficiency).**

## 2. SUMMARY AND EXPLANATION

The hemorrhagic fever with renale syndrome (HFRS) is caused by viruses of the Hantaan type. Compared to nephropathia epidemica (NE), caused by infection with the closely related Puumala virus, generally HFRS is a severe infection, accompanied by uremia, respiratory paralysis and hemorrhages with typical shock syndromes, often with a fatal outcome.
First signs of the disease are influenza-like symptomes. An initial thrombocytopenia and leucocytosis are also typical for HFRS.
The Hantaan virus is predominantly found in Asia and the Balkan States and is transmitted to humans through direct contact with rodents, excrements of rodents or aerosoles. Soldiers, farmers and campers are particularly at risk.

## 3. TEST PRINCIPLE

The microtiter plate is coated with recombinant nucleo-capsidprotein of Hantaan 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 Hantaan 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.

## 4. WARNINGS AND PRECAUTIONS

1. For *in-vitro diagnostic* use only. For professional use only.
2. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
3. In case of severe damage of the kit package please contact IBL or your supplier in written form, latest one week after receiving the kit. Do not use damaged components in test runs, but keep safe for complaint related issues.
4. Obey lot number and expiry date. Do not mix reagents of different lots. Do not use expired reagents.
5. Follow good laboratory practice and safety guidelines. Wear lab coats, disposable latex gloves and protective glasses where necessary.
6. Reagents of this kit containing hazardous material may cause eye and skin irritations. See MATERIALS SUPPLIED and labels for details. Material Safety Data Sheets for this product are available on the IBL-Homepage or upon request directly from IBL.
7. Chemicals and prepared or used reagents have to be treated as hazardous waste according to national biohazard and safety guidelines or regulations.
8. The cleaning staff should be guided by the professionals regarding potential hazards and handling.
9. Avoid contact with Stop solution. It may cause skin irritations and burns.
10. All reagents of this kit containing human serum or plasma have been tested and were found negative for anti-HIV I/II, HBsAg and anti-HCV. However, a presence of these or other infectious agents cannot be excluded absolutely. For this reason reagents should be treated as potential biohazards in use and for disposal.

## 5. STORAGE AND STABILITY

The kit is shipped at ambient temperature and should be stored at 2-8 °C. Keep away from heat or direct sun light. The unopened reagents are stable until the expiry date indicated. The storage and stability of specimen and prepared reagents is stated in the corresponding chapters.
The microtiter strips are stable up to the expiry date of the kit in the broken, but tightly closed bag when stored at 2-8 °C.

## 6. SPECIMEN COLLECTION AND STORAGE

**Human serum**

The usual precautions for venipuncture should be observed. It is important to preserve the chemical integrity of a blood specimen from the moment it is collected until it is assayed. Do not use grossly hemolytic, icteric or grossly lipemic specimens. Samples appearing turbid should be centrifuged before testing to remove any particulate material. Human serum must be used as sample material for the Hantavirus (Hantaan) IgG/IgM ELISA.

| | | | |
|---|---|---|---|
| Storage: | 2-8°C | ≤ -20°C (Aliquots) | Keep away from heat or direct sunlight. Avoid repeated freeze-thaw cycles. |
| Stability: | 5 days | 12 months | |

## 7. MATERIALS SUPPLIED

| Quantity | Symbol | Component |
|---|---|---|
| 1 x 12 x 8 | **MTP** | **Microtiter Plate**<br>Ready to use. Break apart strips. Coated with recombinant Hantaan antigen (strain: CG 76-118). |
| 1 x 0.75 mL | **ANTI IgG CONJ** **CONC** | **Enzyme Conjugate** Concentrate (20x)<br>Anti-IgG conjugated to peroxidase. |
| 1 x 0.75 mL | **ANTI IgM CONJ** **CONC** | **Enzyme Conjugate** Concentrate (20x)<br>Anti-IgM conjugated to peroxidase. |
| 1 x 1.5 mL | **CONTROL + IgG** | **Positive Control IgG**<br>Ready to use. Contains: Human serum, stabilizers, preservatives. |
| 1 x 1.5 mL | **REFCONTROL IgG** | **Reference Control IgG**<br>Ready to use. Contains: Human serum, stabilizers, preservatives. |
| 1 x 1.5 mL | **CONTROL + IgM** | **Positive Control IgM**<br>Ready to use. Contains: Human serum, stabilizers, preservatives. |
| 1 x 15 mL | **DILBUF** **CONC** | **Diluent Buffer** Concentrate (20x)<br>Red colored. Contains: PBS pH 7.4, 0.01 % (w/v) Thimerosal, detergents. |
| 1 x 1.5 mL | **REFCONTROL IgM** | **Reference Control IgM**<br>Ready to use. Contains: Human serum, stabilizers, preservatives. |
| 1 x 1.9 mL | **CONTROL-** | **Negative Control IgM**<br>Ready to use. Contains: Human serum, stabilizers, preservatives. |
| 1 x 100 mL | **WASHBUF** **CONC** | **Wash Buffer** Concentrate (10x)<br>Contains: phosphate buffer. |
| 1 x 1.5 mL | **RF-AB** | **RF-Absorbent**<br>Ready to use. Contains: anti-human IgG, stabilizers, preservatives. |
| 1 x 15 mL | **TMB SUBS** | **TMB Substrate Solution**<br>Ready to use. Contains: TMB (Tetramethylbenzidine). |
| 1 x 15 mL | **TMB STOP** | **TMB Stop Solution**<br>Ready to use. Contains: 0.5 M $H_2SO_4$. |
| 1 x | **FOIL** | **Adhesive Foil** |

## 8. MATERIALS REQUIRED BUT NOT SUPPLIED

1. Micropipettes (Multipette Eppendorf or similar devices, < 3 % CV). Volume: 5; 20; 50; 100; 200; 1000 µL
2. Vortex mixer
3. Incubator, 37 °C
4. Tubes for sample dilution
5. 8-Channel Micropipettor with reagent reservoirs
6. Wash bottle, automated or semi-automated microtiter plate washing system
7. Microtiter plate reader capable of reading absorbance at 450 nm (reference wavelength 600-650 nm)
8. Bidistilled or deionised water
9. Paper towels, pipette tips and timer

## 9. PROCEDURE NOTES

1. Any improper handling of samples or modification of the test procedure may influence the results. The indicated pipetting volumes, incubation times, temperatures and pretreatment steps have to be performed strictly according to the instructions. Use calibrated pipettes and devices only.
2. Once the test has been started, all steps should be completed without interruption. Make sure that required reagents, materials and devices are prepared ready at the appropriate time. Allow all reagents and specimens to reach room temperature (18-25 °C) and gently swirl each vial of liquid reagent and sample before use. Mix reagents without foaming.
3. Avoid contamination of reagents, pipettes and wells/tubes. Use new disposable plastic pipette tips for each component and specimen. Do not interchange caps. Always cap not used vials. Do not reuse wells/tubes or reagents.
4. It is advised to determine samples in duplicate to be able to identify potential pipetting errors.
5. Use a pipetting scheme to verify an appropriate plate layout.
6. Incubation time affects results. All wells should be handled in the same order and time sequences. It is recommended to use an 8-channel Micropipettor for pipetting of solutions in all wells.
7. Microtiter plate washing is important. Improperly washed wells will give erroneous results. It is recommended to use a multichannel pipette or an automatic microtiter plate washing system. Do not allow the wells to dry between incubations. Do not scratch coated wells during rinsing and aspiration. Rinse and fill all reagents with care. While rinsing, check that all wells are filled precisely with Wash Buffer, and that there are no residues in the wells.
8. Humidity affects the coated wells/tubes. Do not open the pouch until it reaches room temperature. Unused wells/tubes should be returned immediately to the resealed pouch including the desiccant.

## 10. PRE-TEST SETUP INSTRUCTIONS

Allow kit to reach room temperature (18-25°C). Buffer concentrates may contain salt cristals which dissolve quickly at 37°C. Let buffer cool to room temperature (18-25°C) before starting the test.

### 10.1. Preparation of concentrated components (Examples for 32 wells)

**Note:** Dilute required volumes of reagents directly before use!

| Dilute / dissolve | Component | Volumes | Diluent | Relation | Remarks | Storage | Stability |
|---|---|---|---|---|---|---|---|
| e.g. 3 mL | **DILBUF CONC** | 57 mL | bidist. water | 1:20 | Mix carefully | 2-8 °C | 1 week |
| 10 mL | **WASHBUF CONC** | 90 mL | bidist. water | 1:10 | Mix carefully | 2-8 °C | 8 weeks |
| 200 µL | **ANTI IgG CONJ CONC** or **ANTI IgM CONJ CONC** | 3.8 mL | Wash Buffer **(diluted)** | 1:20 | Mix carefully | - | Discard after test run. |

### 10.2. Dilution of patient samples

| | to be diluted | with | Relation | Remarks | Storage | Stability |
|---|---|---|---|---|---|---|
| **IgG** | generally | **Diluent Buffer (diluted)** | 1:201 | e.g. 10 µL Sample + 2000 µL | 2-8 °C | 6 weeks |
| **IgM** | generally | **Diluent Buffer (diluted)** | 1:201 | e.g. 10 µL Sample + 2000 µL **Add 15 µL RF-AB to 250 µL diluted serum; incubate for 30 min. at 18-25 °C** | 2–8 °C | 6 weeks |

**Note:** Undiluted samples can be stored at -20 °C for several months.

## 11. TEST PROCEDURE

| | |
|---|---|
| **1.** | Pipette **100 µL undiluted negative, positive and reference controls** as well as **diluted** (possibly pretreated with **RF-AB**) **patient sera** into each well. |
| **2.** | Cover plate with adhesive foil. **Incubate 45 min** at **37 °C.** |
| **3.** | Remove adhesive foil. Discard incubation solution. Wash plate **4 x** with **300 µL** of diluted **Wash Buffer.** Remove excess solution by tapping the inverted plate on a paper towel. |
| **4.** | Pipette **100 µL** diluted **Enzyme Conjugate (IgG or IgM)** into each well. |
| **5.** | Cover plate with adhesive foil. **Incubate 45 min** at **37 °C.** |
| **6.** | Remove adhesive foil. Discard incubation solution. Wash plate **4 x** with **300 µL** of diluted **Wash Buffer.** Remove excess solution by tapping the inverted plate on a paper towel. |
| **7.** | For adding of Substrate and Stop Solution use, if available, an 8-channel Micropipettor. Pipetting should be carried out in the same time intervals for Substrate and Stop Solution. Use positive displacement and avoid formation of air bubbles. |
| **8.** | Pipette **100 µL** of **TMB Substrate Solution** into each well. |
| **9.** | **Incubate 10 min** at **RT (18-25 °C).** |
| **10.** | Stop the substrate reaction by adding **100 µL** of **TMB Stop Solution** into each well. |
| **11.** | **Measure** optical density with a photometer at **450 nm** (Reference-wavelength: 600 - 650 nm) within **20 min** after pipetting of the Stop Solution. |

## 12. QUALITY CONTROL

**See QC-certificate.**

Note : The test results are only valid if the test has been performed following the instructions. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or comparable standards /laws. User and/or laboratory must have a validated system to get diagnosis according to GLP. All kit controls must be found within the acceptable ranges as stated on the vial labels. If the criteria are not met, the run is not valid and should be repeated. Each laboratory should use known samples as further controls. It is recommended to participate at appropriate quality assessment trials.

In case of any deviation the following technical issues should be proven: Expiration dates of (prepared) reagents, storage conditions, pipettes, devices, incubation conditions and washing methods.

## 13. CALCULATION OF RESULTS

For calculation of results, the ratio of the optical density (OD) of the patient sample and the reference control is determined:

$$\frac{OD_{\text{patient sample}}}{OD_{\text{reference control}}} = Q$$

and interpreted as follows:

**a) For IgG antibodies**

| | |
|---|---|
| $Q < 1$ | **Negative:** No IgG antibodies against Hantaan 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 Hantaan IgM antibodies and/or antibodies against the **Puumala serotype.** |
| $Q > 1.5$ | **Positive:** Specific IgG antibodies against Hantaan virus detected. |

**b) For IgM-antibodies**

| | |
|---|---|
| $Q < 1$ | **Negative:** No IgM antibodies against Hantaan virus 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 **Puumala serotype.** |
| $Q > 2$ | **Positive:** Specific IgM antibodies against Hantaan virus detected. |

## 14. INTERPRETATION OF RESULTS

**a) IgG**

Evaluation of the 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**[1] as follows:

| Seoul IFT n = 38 | PUU-IFT n = 29 | HTN-IFT n = 56 | IFT / ELISA |
|---|---|---|---|
| 95 % | 88 % | 99 % | Hantaan IgG ELISA (Cutoff 0.600) |
| 75 % | 98 % | 84 % | Puumala IgG ELISA (Cutoff 0.600) |

[1] diagnostic efficiency = specificity/2 + sensitivity/2

Sensitivity and Specifity was determined with 194 sera of healthy blood donors and total of 123 IgG positiv sera (56 IgG positive for Hantaan virus in indirect immunofluorescence). Sensivity was 98 %, specificity for Hantaan virus 99 %.

**b) IgM**

Evaluation of the 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**[1] as follows:

| Seoul IFT n = 47 | PUU-IFT n = 25 | HTN-IFT n = 50 | IFT / ELISA |
|---|---|---|---|
| 95 % | 74 % | 100 % | Hantaan IgM ELISA (Cutoff 0.32) |
| 68 % | 99 % | 62 % | Puumala IgM ELISA (Cutoff 0.35) |

[1] diagnostic efficiency = specificity/2 + sensitivity/2

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 immunfluorescence). Sensitivity was 100 % and the specificity for Hantaan virus 100 %.

## 15. LIMITATIONS OF THE PROCEDURE

The following substances do not have a significant effect on the test results up to the concentration stated below:

| | |
|---|---|
| Hemoglobin | 5 mg/mL |
| Bilirubin | 0.625 mg/mL |
| Triglyceride | 91 mg/mL |

## 16. PERFORMANCE

| Precision | | Range (Q) | Mean Recovery (%) | Range CV (%) |
|---|---|---|---|---|
| Intra-Assay (n=20) | IgG | 0.7-2.4 | 3.9 | 2.7-6.3 |
| | IgM | 0.7-3.9 | 2.3 | 1.9-3.1 |

**Method Comparison**

Comparison for Hanta Hantaan IgM

Comparison for the clinical interpretation with a panel of 162 serum samples, IgM

| | | Lot IHAA 114 | | |
|---|---|---|---|---|
| | | negative | borderline | positive |
| Lot IHAA 113 | negative | 152 | 1 | 0 |
| | borderline | 5 | 2 | 0 |
| | positive | 0 | 1 | 1 |

Comparison for Hanta Hantaan IgG

Comparison for the clinical interpretation with a panel of 162 serum samples, IgG

| | | Lot IHAA 114 | | |
|---|---|---|---|---|
| | | negative | borderline | positive |
| Lot IHAA 113 | negative | 156 | 2 | 0 |
| | borderline | 0 | 1 | 2 |
| | positive | 0 | 0 | 1 |

## 17. PRODUCT LITERATURE REFERENCES


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11. Zöller L, Yang S, Gött P, Bautz EKF, Darai G, A novel μ-capture ELISA based on recombinant proteins allows sensitive and specific diagnosis of hemorrhagic fever with renal syndrome, J. Clin. Microbiol. 31: 1194-1199 (1993)

## 18. SHORT PROTOCOL

| PRE-TEST SETUP INSTRUCTIONS | | | | | |
|---|---|---|---|---|---|
| **Dilution** | **Volumes** | **bidist. water** | **Wash Buffer (diluted)** | **Relation** | **Remarks** |
| **WASHBUF CONC** | 10 mL | 90 mL | | 1:10 | Example for 32 wells |
| **DILBUF CONC** | 3 mL | 57 mL | | 1:20 | |
| **ANTI IgG CONJ CONC or ANTI IgM CONJ CONC** | 200 µL | | 3.8 mL | 1:20 | |

| SAMPLE DILUTION | Volumes | Diluent Buffer (diluted) | Relation | Remarks |
|---|---|---|---|---|
| **Serum (IgG)** | 10 µL | 2000 µL | 1:201 | |
| **Serum (IgM)** | 10 µL | 2000 µL | 1:201 | **Add 15 µL RF-AB to 250 µL diluted serum; incubate for 30 min. at 18-25 °C** |

| TEST PROCEDURE | |
|---|---|
| **CONTROL+ IgG or CONTROL+ IgM, REF CONTROL IgG or REF CONTROL IgM, CONTROL- / Samples (diluted)** | 100 µL |
| Incubate 45 min. at 37°C.<br>Aspirate the contents of each well.<br>Wash 4 times with 300 µL of WASHBUF (diluted) and aspirate. | |
| **Enzyme Conjugate IgG or IgM (diluted)** | 100 µL |
| Incubate 45 min. at 37°C.<br>Aspirate the contents of each well.<br>Wash 4 times with 300 µL of WASHBUF (diluted) and aspirate. | |
| **TMB SUBS** | 100 µL |
| Incubate 10 min. at 18 - 25°C. | |
| **TMB STOP** | 100 µL |
| Measure optical density with a photometer at 450 nm (reference wavelength: 600-650 nm). | |

# Symbols / Symbole / Symbôles / Símbolos / Símbolos / Σύμβολα

| | |
|---|---|
| REF | Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.º Cat.: / N.–Cat.: / Αριθμός-Κατ.: |
| LOT | Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lote N.º: / Lotto n.: / Αριθμός -Παραγωγή: |
|  | Use by: / Verwendbar bis: / Utiliser à: / Usado por: / Usar até: / Da utilizzare entro: / Χρησιμοποιείται από: |
|  | No. of Tests: / Kitgröße: / Nb. de Tests: / No. de Determ.: / N.º de Testes: / Quantità dei tests: / Αριθμός εξετάσεων: |
| CONC | Concentrate / Konzentrat / Concentré / Concentrar / Concentrado / Concentrato / Συμπύκνωμα |
| LYO | Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizado / Liofilizzato / Λυοφιλιασμένο |
| IVD | In Vitro Diagnostic Medical Device. / In-vitro-Diagnostikum. / Appareil Médical pour Diagnostics In Vitro. / Dispositivo Médico para Diagnóstico In Vitro. / Equipamento Médico de Diagnóstico In Vitro. / Dispositivo Medico Diagnostico In vitro. / Ιατρική συσκευή για In-Vitro Διάγνωση. |
|  | Evaluation kit. / Nur für Leistungsbewertungszwecke. / Kit pour évaluation. / Juego de Reactivos para Evaluació. / Kit de avaliação. / Kit di evaluazione. / Κιτ Αξιολόγησης. |
|  | Read instructions before use. / Arbeitsanleitung lesen. / Lire la fiche technique avant emploi. / Lea las instrucciones antes de usar. / Ler as instruções antes de usar. / Leggere le istruzioni prima dell'uso. / Διαβάστε τις οδηγίες πριν την χρήση. |
|  | Keep away from heat or direct sun light. / Vor Hitze und direkter Sonneneinstrahlung schützen. / Garder à l'abri de la chaleur et de toute exposition lumineuse. / Manténgase alejado del calor o la luz solar directa. / Manter longe do calor ou luz solar directa. / Non esporre ai raggi solari. / Να φυλάσσεται μακριά από θερμότητα και άμεση επαφή με το φως του ηλίου. |
|  | Store at: / Lagern bei: / Stocker à: / Almacene a: / Armazenar a: / Conservare a: / Αποθήκευση στους: |
|  | Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabbricante: / Παραγωγός: |
|  | Caution! / Vorsicht! / Attention! / ¡Precaución! / Cuidado! / Attenzione! / Προσοχή! |

Symbols of the kit components see MATERIALS SUPPLIED.
Die Symbole der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben.
Voir MATERIEL FOURNI pour les symbôles des composants du kit.
Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS.
Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS.
Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT.
Για τα σύμβολα των συστατικών του κιτ συμβουλευτείτε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.

COMPLAINTS: Complaints may be submitted initially written or vocal. Subsequently they need to be filed including the test performance and results in writing in case of analytical reasons.

WARRANTY: The product is warranted to be free from material defects within the specific shelf life and to comply with product specifications delivered with the product. The product must be used according to the Intended use, all instructions given in the instructions for use and within the product specific shelf life. Any modification of the test procedure or exchange or mixing of components of different lots could negatively affect the results. These cases invalidate any claim for replacement.

LIMITATION OF LIABILITY: IN ALL CIRCUMSTANCES THE EXTENT OF MANUFACTURER'S LIABILITY IS LIMITED TO THE PURCHASE PRICE OF THE KIT(S) IN QUESTION. IN NO EVENT SHALL MANUFACTURER BE LIABLE FOR ANY INCIDENTAL OR CONSEQUENTIAL DAMAGES, INCLUDING DAMAGES FOR LOST PROFITS, LOST SALES, INJURY TO PERSON OR PROPERTY OR ANY OTHER INCIDENTAL OR CONSEQUENTIAL LOSS.

The labelling of hazardous substances is according to European directive.
For further country-specific classifications, please refer to the corresponding safety data sheet.


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