Instructions for Use

Hanta Virus IgG ELISA

IVD

CE

REF **EIA-5858**

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Please use only the valid version of the Instructions for Use provided with the kit.
Verwenden Sie nur die jeweils gültige, im Testkit enthaltene, Gebrauchsanweisung.
Si prega di usare la versione valida delle istruzioni per l'uso a disposizione con il kit.
Por favor, se usa solo la version valida de la metodico técnico incluido aqui en el kit.
Utilisez seulement la version valide des Instructions d'utilisation fournies avec le kit.

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1 INTRODUCTION

Hantaviruses are negative sense RNA viruses in the Bunyaviridae family. Humans may be infected with Hantaviruses through urine, saliva or contact with rodent waste products. Some Hantaviruses may lead to serious diseases in humans, such as hemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS).

Human infections of Hantaviruses have almost entirely been linked to human contact with rodent excrement, but recent human-to-human transmission has been reported with the Andes virus in South America.

Hantavirus has an incubation time of two to four weeks in humans before symptoms of infection occur. The symptoms of HFRS can be split into five phases:

- Febrile phase: Symptoms include fever, chills, sweaty palms, diarrhea, malaise, headaches, nausea, abdominal and back pain, respiratory problems such as the ones common in influenza virus infection, as well as gastro-intestinal problems. These symptoms normally occur for three to seven days and arise about two to three weeks after exposure.
- Hypotensive phase: This occurs when the blood platelet levels drop and symptoms can lead to tachycardia and hypoxemia. This phase can last for 2 days.
- Oliguric phase: This phase lasts for three to seven days and is characterized by the onset of renal failure and proteinuria occurs.
- Diuretic phase: This is characterized by diuresis of three to six liters per day, which can last for a couple of days up to weeks.
- Convalescent phase: This is normally when recovery occurs and symptoms begin to improve.

Regions especially affected by HFRS include China, the Korean Peninsula, Russia (Hantaan, Puumala and Seoul viruses), and northern and western Europe (Puumala and Dobrava virus).

Species	Disease	Symptoms (e.g.)	Transmission route
Puumala virus Dobrava virus Hantaan virus Seoul virus	Hemorrhagic fever with renal syndrome (HFRS)	Initial: suddenly occurring symptoms like intense headache, back and abdominal pain, fever, chills, nausea, and blurred vision. Late: low blood pressure, acute shock, vascular leakage, and acute kidney failure	After exposure to aerosolized urine, droppings, or saliva of infected rodents or their nests (airborne transmission). Also by direct contact with these materials to broken skin or onto mucous membranes. Bites by infected rodents. Human to human transmission cannot be excluded (for New World strains).
Andes virus Sin-Nombre-virus (New world strains)	Hantavirus pulmonary syndrome (HPS)	Initial: universal symptoms include fatigue, fever and muscle aches, especially in the large muscle groups - thighs, hips, back, and sometimes shoulders. There may also be headache, dizziness, chills, and abdominal problems, such as nausea, vomiting, diarrhea, and abdominal pain. Late: coughing and shortness of breath, lungs fill with fluid.	

Infection or presence of pathogen may be identified by:

- PCR
- Serology (e. g. ELISA)

2 INTENDED USE

The Hanta Virus IgG ELISA is intended for the qualitative determination of IgG antibodies against Hantavirus in human serum or plasma (citrate or heparin).

3 PRINCIPLE OF THE ASSAY

The qualitative immunoenzymatic determination of specific antibodies is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique.

Microtiterplates are coated with specific antigens to bind corresponding antibodies of the sample. After washing the wells to remove all unbound sample material a horseradish peroxidase (HRP) labelled conjugate is added. This conjugate binds to the captured antibodies. In a second washing step unbound conjugate is removed. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product.

The intensity of this product is proportional to the amount of specific antibodies in the sample. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450/620 nm is read using an ELISA microtiter plate reader.

4 MATERIALS

4.1 Reagents supplied

- **Microtiterplate:**
  12 breakapart 8-well snap-off strips coated with recombinant Hantavirus antigens in resealable aluminium foil.
- **IgG Sample Dilution Buffer**:
  1 bottle containing 100 mL of phosphate buffer (10 mM) for sample dilution; pH 7.2 ± 0.2; coloured yellow; ready to use; white cap; ≤ 0.0015% (v/v) CMIT/ MIT (3:1).
- **Stop Solution**:
  1 bottle containing 15 mL sulphuric acid, 0.2 mol/L; ready to use; red cap.
- **Washing Buffer (20x conc.):**
  1 bottle containing 50 mL of a 20-fold concentrated phosphate buffer (0.2 M); pH 7.2 ± 0.2; for washing the wells; white cap.
- **Conjugate:**
  1 bottle containing 20 mL of peroxidase labelled antibody to human IgG; in phosphate buffer (10 mM); coloured blue, ready to use; black cap.
- **TMB Substrate Solution:**
  1 bottle containing 15 mL 3,3',5,5'-tetramethylbenzidine (TMB), < 0.1%; ready to use; yellow cap .
- **Positive Control:**
  1 vial containing 2 mL control; coloured yellow; ready to use; red cap; ≤ 0.02% (v/v) MIT.
- **Cut-off Control:**
  1 vial containing 3 mL control; coloured yellow; ready to use; green cap; ≤ 0.02% (v/v) MIT.
- **Negative Control:**
  1 vial containing 2 mL control; coloured yellow; ready to use; blue cap; ≤ 0.0015% (v/v) CMIT/ MIT (3:1).

For hazard and precautionary statements see 12.1

For potential hazardous substances please check the safety data sheet.

4.2 Materials supplied

- 1 Cover foil
- 1 Instruction for use (IFU)
- 1 Plate layout

4.3 Materials and Equipment needed

- ELISA Microtiterplate reader, equipped for the measurement of absorbance at 450/620 nm
- Incubator 37 °C
- Manual or automatic equipment for rinsing Microtiter plates
- Pipettes to deliver volumes between 10 µL and 1000 µL
- Vortex tube mixer
- Distilled water
- Disposable tubes

5 STABILITY AND STORAGE

Store the kit at 2 °C - 8 °C.

The opened reagents are stable up to the expiry date stated on the label when stored at 2 °C - 8 °C.

6 REAGENT PREPARATION

It is very important to bring all reagents and samples to room temperature (20 °C - 25 °C) and mix them before starting the test run!

6.1 Microtiterplate

The break-apart snap-off strips are coated with recombinant Hantavirus antigens.

Immediately after removal of the strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2 °C - 8 °C.

6.2 Washing Buffer (20x conc.)

Dilute Washing Buffer 1 + 19; e. g. 10 mL Washing Buffer + 190 mL distilled water.

The diluted buffer is stable for 5 days at room temperature (20 °C - 25 °C). In case crystals appear in the concentrate, warm up the solution to 37 °C e.g. in a water bath. Mix well before dilution.

6.3 TMB Substrate Solution

The reagent is ready to use and has to be stored at 2 °C - 8 °C, away from the light.

The solution should be colourless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away.

7 SAMPLE COLLECTION AND PREPARATION

Use human serum or plasma (citrate or heparin) samples with this assay.

If the assay is performed within 5 days after sample collection, the samples should be kept at 2 °C - 8 °C; otherwise they should be aliquoted and stored deep-frozen (-70 °C to -20 °C). If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing.

Heat inactivation of samples is not recommended.

7.1 Sample Dilution

Before assaying, all samples should be diluted **1+100** with IgG Sample Dilution Buffer.

Dispense 10 µL sample and 1 mL IgG Sample Dilution Buffer into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

8 ASSAY PROCEDURE

Please read the instruction for use carefully **before** performing the assay. Result reliability depends on strict adherence to the instruction for use as described. The following test procedure is only validated for manual procedure. If performing the test on ELISA automatic systems we recommend increasing the washing steps from three up to five and the volume of Washing Buffer from 300 µL to 350 µL to avoid washing effects. Pay attention to chapter 12. Prior to commencing the assay, the distribution and identification plan for all samples and standards/controls (duplicates recommended) should be carefully established on the plate layout supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder.

Perform all assay steps in the order given and without any delays.

A clean, disposable tip should be used for dispensing each standard/control and sample.

Adjust the incubator to 37 °C ± 1 °C.

1. Dispense 100 µL standards/controls and diluted samples into their respective wells.
   Leave well A1 for the Substrate Blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 1 hour ± 5 min at 37 ± 1 °C.**
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µL of Washing Buffer. Avoid overflows from the reaction wells. The interval between washing and aspiration should be > 5 sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!
   Note: Washing is important! Insufficient washing results in poor precision and false results.
5. Dispense 100 µL Conjugate into all wells except for the Substrate Blank well A1.
6. **Incubate for 30 min at room temperature (20 °C - 25 °C).** Do not expose to direct sunlight.
7. Repeat step 4.
8. Dispense 100 µL TMB Substrate Solution into all wells.
9. **Incubate for exactly 15 min at room temperature (20 °C - 25 °C) in the dark.** A blue colour occurs due to an enzymatic reaction.
10. Dispense 100 µL Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution, thereby a colour change from blue to yellow occurs.
11. Measure the absorbance at 450/620 nm within 30 min after addition of the Stop Solution.

8.1 Measurement

Adjust the ELISA Microtiterplate plate reader **to zero** using the **Substrate Blank**.

If - due to technical reasons - the ELISA Microtiterplate reader cannot be adjusted to zero using the Substrate Blank, subtract its absorbance value from all other absorbance values measured in order to obtain reliable results!

Measure the absorbance of all wells at **450 nm** and record the absorbance values for each standard/control and sample in the plate layout.

Bichromatic measurement using a reference wavelength of 620 nm is recommended.

Where applicable calculate the **mean absorbance values** of all duplicates.

9 RESULTS

9.1 Run Validation Criteria

In order for an assay run to be considered valid, these Instructions for Use have to be strictly followed and the following criteria must be met:

- **Substrate Blank**: Absorbance value **< 0.100**
- **Negative Control**: Absorbance value **< 0.200 and < Cut-off**
- **Cut-off Control**: Absorbance value **0.150 – 1.300**
- **Positive Control**: Absorbance value **> Cut-off**

If these criteria are not met, the test is not valid and must be repeated.

9.2 Calculation of Results

The Cut-off is the mean absorbance value of the Cut-off Control determinations.

Example: Absorbance value Cut-off Control 0.44 + absorbance value Cut-off control 0.42 = 0.86. 0.86/2 = 0.43
Cut-off = 0.43

9.2.1 Results in Units [DU]

$$\frac{\text{Sample (mean) absorbance value} \times 10}{\text{Cut-off}} = \text{[DRG Units = DU]}$$

Example: $\frac{1.591 \times 10}{0.43} = 37$ DU (Units)

9.3 Interpretation of Results

Cut-off	10 DU	-
Positive	> 11 DU	Antibodies against the pathogen are present. There has been a contact with the antigen (pathogen resp. vaccine).
Equivocal	9 – 11 DU	Antibodies against the pathogen could not be detected clearly. It is recommended to repeat the test with a fresh sample in 2 to 4 weeks. If the result is equivocal again the sample is judged as **negative**.
Negative	< 9 DU	The sample contains no antibodies against the pathogen. A previous contact with the antigen (pathogen resp. vaccine) is unlikely.
Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as serological data. In immunocompromised patients and newborns serological data only have restricted value.		

9.3.1 Antibody Isotypes and State of Infection

Serology	Significance
IgM	Characteristic of the primary antibody response High IgM titer with low IgG titer: → suggests a current or very recent infection Rare: → persisting IgM
IgG	Characteristic of the secondary antibody response May persist for several years High IgG titer with low IgM titer: → may indicate a past infection

10 SPECIFIC PERFORMANCE CHARACTERISTICS

The results refer to the groups of samples investigated; these are not guaranteed specifications.
For further information about the specific performance characteristics please contact DRG.

10.1 Precision

Intra assay	n	Mean (E)	Cv (%)
#1	24	0.450	3.61
#2	24	1.333	6.41
#3	24	1.264	4.78
Inter assay	**n**	**Mean (DU)**	**Cv (%)**
#1	12	27.44	5.34
#2	12	25.44	8.15
#3	12	1.09	12.09

10.2 Diagnostic Specificity

The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte. It is 96.59% (95% confidence interval: 90.36% - 99.29%).

10.3 Diagnostic Sensitivity

The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte. It is 99.16% (95% confidence interval: 95.41% - 99.98%).

10.4 Interferences

Interferences with hemolytic, lipemic or icteric samples are not observed up to a concentration of 10 mg/mL hemoglobin, 5 mg/mL triglycerides and 0.5 mg/mL bilirubin.

10.5 Cross Reactivity

Investigation of a sample panel with antibody activities to potentially cross-reacting parameters did not reveal significant evidence of false-positive results due to cross-reactions.

11 LIMITATIONS OF THE PROCEDURE

Bacterial contamination or repeated freeze-thaw cycles of the sample may affect the absorbance values.

12 PRECAUTIONS AND WARNINGS

- The test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the test kits with analyzers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.
- Only for in-vitro diagnostic use.
- All materials of human or animal origin should be regarded and handled as potentially infectious.
- All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive.
- Do not interchange reagents or Microtiterplates of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- Use only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and standard/control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense reagents without splashing accurately into the wells.
- The ELISA is only designed for qualified personnel following the standards of good laboratory practice (GLP).
- For further internal quality control each laboratory should additionally use known samples.

12.1 Safety note for reagents containing hazardous substances

Reagents may contain CMIT/MIT (3:1) or MIT (refer to 4.1)

Therefore, the following hazard and precautionary statements apply.

Warning

H317	May cause an allergic skin reaction.
P261	Avoid breathing spray.
P280	Wear protective gloves/ protective clothing.
P302+P352	IF ON SKIN: Wash with plenty of soap and water.
P333+P313	If skin irritation or rash occurs: Get medical advice/ attention.
P362+P364	Take off contaminated and wash it before reuse.

Further information can be found in the safety data sheet.

12.2 Disposal Considerations

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

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Abbreviations

CMIT	5-chloro-2-methyl-4-isothiazolin-3-one
MIT	2-methyl-2H-isothiazol-3-one

14 SCHEME OF THE ASSAY

Test Preparation

Prepare reagents and samples as described. Establish the distribution and identification plan for all samples and standards/controls on the plate layout supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder.

Assay Procedure

	Substrate Blank (A1)	Negative Control	Cut-off Control	Positive Control	Sample (diluted 1+100)
Negative Control	-	100 µL	-	-	-
Cut-off Control	-	-	100 µL	-	-
Positive Control	-	-	-	100 µL	-
Sample (diluted 1+100)	-	-	-	-	100 µL
Cover wells with foil supplied in the kit **Incubate for 1 h at 37 °C ± 1 °C** Wash each well three times with 300 µL of Washing Buffer					
Conjugate	-	100 µL	100 µL	100 µL	100 µL
Incubate for 30 min at room temperature (20 °C - 25 °C) Do not expose to direct sunlight Wash each well three times with 300 µL of Washing Buffer					
TMB Substrate Solution	100 µL	100 µL	100 µL	100 µL	100 µL
Incubate for exactly 15 min at room temperature (20 °C - 25 °C) in the dark					
Stop Solution	100 µL	100 µL	100 µL	100 µL	100 µL
Photometric measurement at 450 nm (reference wavelength: 620 nm)					

SYMBOLS USED

Symbol	English	Deutsch	Italiano	Español	Français
CE	European Conformity	CE-Konformitäts-kennzeichnung	Conformità europea	Conformidad europea	Conformité normes européennes
	Consult instructions for use *	Gebrauchsanweisung beachten *	Consultare le istruzioni per l'uso	Consulte las instrucciones de uso	Consulter les instructions d'utilisation
IVD	*In vitro* diagnostic medical device *	*In-vitro*-Diagnostikum *	Dispositivo medico-diagnostico in vitro	Producto sanitario para diagnóstico In vitro	Dispositif médical de diagnostic in vitro
REF	Catalogue number *	Artikelnummer *	Numero di Catalogo	Nûmero de catálogo	Référence de catalogue
LOT	Batch code *	Chargencode *	Codice del lotto	Codigo de lote	Numéro de lot
	Contains sufficient for <n> tests *	Ausreichend für <n> Prüfungen *	Contenuto sufficiente per "n" saggi	Contenido suficiente para <n> ensayos	Contenu suffisant pour "n" tests
	Temperature limit *	Temperaturbegrenzung *	Temperatura di conservazione	Temperatura de conservacion	Température de conservation
	Use-by date *	Verwendbar bis *	Utilizzare prima del	Establa hasta	Utiliser jusque
	Manufacturer *	Hersteller *	Fabbricante	Fabricante	Fabricant
	Caution *	Achtung *			
RUO	For research use only	Nur für Forschungszwecke	Solo a scopo di ricerca	Sólo para uso en investigación	Seulement dans le cadre de recherches
Distributed by	Distributed by	Vertreiber	Distributore	Distribuidor	Distributeur
Content	Content	Inhalt	Contenuto	Contenido	Contenu
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantità	Volumen/Número	Volume/Quantité
MTP	Microplate	Mikrotiterplatte	Micropiastra	Microplaca	Microplaque
CONJ	Conjugate	Konjugat	Coniugato	Conjugado	Conjugué
CONTROL -	Negative Control	Negativkontrolle	Controllo, negativo	Control negativo	contrôle négatif
CONTROL +	Positive control,	Positivkontrolle,	Controllo, positivo	Control positivo	contrôle positif
CUT OFF	Cut off control	Cut-off-Kontrolle	Controllo cut-off	Control cut-off	Contrôle Cut-off
DIL G	IgG Sample Diluent	IgG-Proben-verdünnungspuffer	Tampone diluente IgG	Diluyente para IgG de la muestra	Diluant pour échantillon IgG
DIL M	IgM Sample Diluent	IgM-Proben-verdünnungspuffer	Tampone diluente IgM	Diluyente para IgM de la muestra	Diluant pour échantillon IgM
SOLN STOP	Stop Solution	Stopplösung	Soluzione bloccante	Solución de parada	Solution d'arrêt
SUB TMB	TMB Substrate solution	TMB-Substratlösung	Soluzione substrato TMB	Solución substrato TMB	Solution de substrat TMB
WASH BUF 20x	Washing Buffer 20x concentrated	Waschpuffer 20x konzentriert	Tampone di lavaggio concentrazione x20	Tampón de lavado concentrado x20	Tampon de lavage concentré 20 x