Version 19-12/24

User's Manual

Hantavirus (Puumala) IgG/IgM ELISA Kit

REF **DEIA596**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

Enzyme immunoassay for the qualitative determination of lgG/lgM antibodies against the serotype Puumala of Hantavirus in human serum. For research use only. Not for use in diagnostic procedures.

Principles of Testing

The microtiter plate is coated with recombinant nucleo-capsidprotein of Puumala virus. For determination of lgM antibodies, patient sera must be incubated with rheumatic-factor-lgG-absorbent before starting the test procedure in order to eliminate unspecific reactions caused by lgG 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 lgG and lgM antibodies are detected with peroxidase-conjugated anti human lgG and lgM 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 And Materials Provided

1 × 12 × 8, MTP. Mlcrotiter Plate. Ready to use. Break apart wells. Coated with recombinant Puumala antigen N120.

1 × 0.75 mL, ANTI lgG CONJ CONC. Enzyme Conjugate Concentrate (20×). Anti-lgG conjugated to peroxidase.

1 × 0.75 mL, ANTI lgM CONJ CONC. Enzyme Conjugate Concentrate (20×). Anti-lgM conjugated to peroxidase.

1 × 1.5 mL, CONTROL + lgG. Positive Control lgG. Ready to use. Contains: Human serum, stabilizers, preservatives.

1 × 1.5 mL, REFCONTROL lgG. Reference Control lgG. Ready to use. Contains: Human serum, stabilizers, preservatives.

1 × 1.5 mL, CONTROL + lgM. Positive Control lgM. Ready to use. Contains: Human serum, stabilizers, preservatives.

1 × 1.5 mL, REFCONTROL lgM. Reference Control lgM. Ready to use. Contains: Human serum, stabilizers, preservatives.

1 × 1.9 mL, CONTROL -. Negative Control lgM. Ready to use. Contains: Human serum, stabilizers, preservatives.

1 × 15 mL, DILBUF CONC. Diluent Buffer Concentrate (20×). Red colored. Contains: PBS pH 7.4, 0.01 % (w/v) Thimerosal, detergents.

1 × 100 mL, WASHBUF CONC. Wash Buffer Concentrate (10×). Contains: phosphate buffer.

1 × 1.5 mL, RF-AB. RF-Absorbent. Ready to use. Contains: anti-human lgG, stabilizers, preservatives.

1 × 15 mL, TMB SUBS. TMB Substrate Solution. Ready to use. Contains: TMB (tetramethylbenzidine).

1 × 15 mL, STOP. Stop Solution. Ready to use. Contains: 0.5 M H_2SO_4.

1 × FOIL. Adhesive Foil.

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

Storage

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.

Specimen Collection And Preparation

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 (Puumala) IgG/IgM ELISA.

Storage:

2- 8°C (5 days)

-20°C (Aliquots) (12 months)

Keep away from heat or direct sunlight.

Reagent Preparation

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.

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.

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.

Assay 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 × 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 × 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.

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.

Calculation

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

OD_{sample} / $OD_{reference\ control}$ = Q

Interpretation Of Results

Interpreted as follows:

1. For IgG antibodies

Q < 1 Negative: No IgG antibodies against Puumala virus 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 Puumala IgM antibodies and/or antibodies against the Hantaan serotype.

Q > 1.5 Positive: Specific IgG antibodies against Puumala virus detected.

2. For IgM antibodies

Q < 1 Negative: No IgM antibodies against Puumala virus detected.

1 ≤ Q ≤ 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.

INTERPRETATION OF RESULTS

1. IgG

Sensitivity and specificity were determined by testing 80 sera from healthy subjects and 52 patients' sera positive for IgG by reference method. The sensitivity for Puumala virus is 100% and specificity is 98.75%.

2. IgM

Sensitivity and specificity were determined by testing 80 sera from healthy subjects and 42 patients' sera positive for IgM by reference method. The sensitivity for Puumala virus is 100% and specificity is also 100%.

Precision

Precision		Range (Q)	Mean (%)	Range CV (%)
Intra-Assay (n = 20)	IgG	0.21 – 4.00	2.1	1.6 – 2.8
	IgM	0.22 – 7.44	3.6	1.3 – 6.7
Inter-Assay (n = 10)	IgG	0.48 – 1.84	6.8	4.5 – 9.5
	IgM	0.23 – 6.35	7.9	5.0 – 9.5
Inter-Lot (n = 3)	IgG	0.43 – 1.70	13.1	9.1 – 16.4
	IgM	0.28 – 5.99	8.2	6.3 – 9.7

Precautions

1. For research use only. Not for use in diagnostic procedures.
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 Reagents And Materials Provided and labels for details.
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 1/11, 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.

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 l/quid 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 affeds 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.

Limitations

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

Hemoglobin: 1.25 mg/mL

Bilirubin: 2.5 mg/mL

Triglyceride: 91 mg/mL