



Hantavirus IF Test

Indirect Immunofluorescence Test for Detection of Human Antibodies against Hantavirus (Dobrava/Hantaan, Puumala)

Dobrava/Hantaan Art. No. PR77065
Puumala Art. No. PR77056

Content 6 × 10 determinations each
Store at 2 - 8°C



Slide packs available separately:

Dobrava/Hantaan Art. No. PR97065
Puumala Art. No. PR97056
Contents 6 x 10 determinations each

Instruction sheet / Gebrauchsanweisung / Carnet d'instruction / instrucciones de uso / gebruiksaanwijzing / istruzioni per l'uso / σελίδα οδηγιών / bruksanvisning / modo de emprego: www.progen.de

1. Introduction

Different serotypes of Hantavirus cause a number of infectious diseases known as Hemorrhagic Fever with Renal Syndrome (HFRS).

Hantaviruses cause an inapparent infection in rodents. Transmission from animals to humans occurs via aerosols or via direct contact. Hantaan and Dobrava serotypes (DOB/HTN) are the etiological agents of the severe form of HFRS and are predominantly found in Asia and Eastern and Mediterranean European countries. In Scandinavia, Central Europe and Russia, Puumala serotype (PUU) is responsible for a milder form of the HFRS, known as Nephropathia epidemica (NE). Another form of HFRS found predominantly in urban regions (mainly in Asia) is caused by viruses of the Seoul serotype. However, the course of the disease is milder than with DOB/HTN serotypes.

HFRS is a febrile illness and may lead to acute renal failure: the disease is characterized by fever, headache, myalgia, and, in some cases respiratory manifestations in the initial stage, followed by abdominal and back pains, vomiting and oliguria. 4-10 days after the onset of the disease acute renal failure may develop, necessitating temporary dialysis.

The initial diagnosis of a Hantavirus infection is based on clinical symptoms and should be confirmed by serology. There is a one-way cross reactivity between Dobrava/Hantaan and other serotypes: patient sera containing antibodies against Puumala serotype often react with DOB/HTN virotypes. Antibodies against the Seoul virus are often also detected with the Dobrava/Hantaan IF test.

A few days after the onset of the disease IgM antibodies are detectable in patient sera reaching peak titers after 2 to 3 weeks. IgG antibodies are generated early and may persist for years.

2. Application of the Test

PROGEN's Hantavirus IF test is an indirect immunofluorescence technique suited for the detection of specific anti-Hantavirus (DOB/HTN, PUU) antibodies in patient sera, e. g. in cases of suspected hemorrhagic fever with renal syndrome (HFRS) or nephropathia epidemica (NE).

3. Principle of the Test

Cultured cells infected with Hantavirus (HTN, PUU) mixed with non-infected cells are used as antigen.

In the first step, virus fixed onto multiwell glass slides is incubated with patient serum. If specific IgG and IgM antibodies are present they will bind to the virus and not wash off. Subsequently, when conjugated antihuman IgG and IgM is added to the reaction site, it binds to the human antibodies. After a second wash, the slide is examined under a fluorescence microscope. In case of a positive reaction, the immune complexes formed in situ are visualized in form of yellow-green fluorescing Hantavirus particles in the cytoplasm of the red-stained cells.

4. Material and Reagents Required but Not Provided

Fluorescence microscope with filter system for FITC and Evans Blue
Glass cover slips (24 × 60 mm)
Precision pipettes (10 µl, 20 µl, 50 µl, 100 µl, 200 µl)
Moist incubation chamber (e.g. Petri dish with moistened filter paper)
Distilled water
Slide baths (staining cuvettes)
Gloves
Timer

5. Materials and Reagents Provided

GS, 6 × 10 well glass slides (single sealed) with fixed virus-infected and non-infected cells.

POS G, Positive control for IgG antibodies, human sera with stabilizer and preservative (lyophilized), 1 vial. Reconstitute with 0.4 ml distilled water.

POS M, Positive control for IgM antibodies, from IgG antibodies preabsorbed human sera with stabilizer and preservative (lyophilized), 1 vial. Reconstitute with 0.2 ml distilled water.

NEG, Negative control, human sera with stabilizer and preservative (lyophilized), 1 vial. Reconstitute with 0.4 ml distilled water.

CON G/M, Alexa Fluor 488 conjugated goat anti human IgG and IgM (lyophilized), contains a preservative and Evans Blue as counterstain, 1 vial. Reconstitute with 2 ml distilled water.

M, Mounting medium, contains a preservative, 1 bottle, 2 ml. Ready to use!

PBS, Powdered phosphate buffered saline (PBS), 2 containers, 10 g each). Reconstitute the contents of each container with 1 l distilled water!

6. Stability

Store the test kit and components at 2-8°C. The unopened reagents are stable until the expiry date indicated.

Stability after reconstitution at 2-8°C:

Component	Stability
POS G, POS M NEG CON G/M	4 weeks
PBS	1 week (For prolonged storage: add 0.05% (w/v) sodium azide)

7. Preparation

7.1. Sample Material and Storage

Human serum must be used as sample material for the Hantavirus IF test. 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.

7.2. Preparation of Samples

1. Add 10 µl patient serum to 150 µl PBS solution to obtain an initial dilution of **1:16**.
2. Prepare a 1:32 dilution by adding 100 µl of the initial dilution to 100 µl PBS solution.
3. To determine the titer, use a 2-fold dilution scheme by mixing 100 µl of serum dilution with 100 µl PBS solution.

8. Test Procedure

1. Allow kit to reach room temperature (20-26°C).
2. Take Hantavirus slide and remove it carefully from its bag. Rehydrate slide for 2 min in PBS solution.
3. Transfer 20 µl of each dilution of patient serum on individual virus-bearing wells of the slide.
4. Place 20 µl of undiluted positive control G of predetermined IgG titer (3+ fluorescence) and/ or 20µl of undiluted positive control M (3+ fluorescence) onto one well.
5. Place 20 µl of negative control on another well. Include the relevant controls in each test run.
6. Place the slides in a moist chamber for 30 min at room temperature (20-26°C).
7. Aspirate serum dilutions and place the slide for 5 min in 2 consecutive PBS baths. Drain off excess liquid and air-dry.
8. Cover each well with 20 µl of reconstituted CON G/M.
9. Place the slide in a moist chamber and incubate for 30 min at room temperature (20-26°C).
10. Aspirate and wash as described in step 7.
11. Add 3 drops of mounting medium and cover the preparation with a cover slip.
12. Examine the slide with an appropriate fluorescence microscope (40× or 63× oil objective).

9. Notes for the User

For professional use.

Precision and recovery depend on the following critical factors:

Perform the incubations at room temperature (20-26°C).

Incubation periods should not be exceeded by more than ±10%. Incubation period starts **after** the last pipetting step.

Security notes:

Although fixed, slides should be handled as potentially infectious.

The sera have been tested for HIV I and II, Hepatitis B and C and found negative. However, all human blood products should be considered to be potentially infectious. Observe universal precautions concerning the handling of potentially infectious material.

Some of the reagents contain preservatives. Do not swallow! Avoid any contact with skin or mucous membranes!

The conjugate contains Evans blue (toxic, carcinogen, and teratogen). Do not swallow! Avoid any contact with skin or mucous membranes!

Safety data sheet is available on request!

Disposal considerations

Product: chemicals must be disposed of in compliance with the respective national regulations. Disposal of biological components should be in accordance with existing disposal practices employed for patient serum samples or infectious waste.

Packaging: packaging must be disposed of in compliance with the country-specific regulations. Handle contaminated packaging in the same way as the product itself. If not officially specified differently, non-contaminated packaging may be treated like household waste or recycled.

Measures after damage on transport

If a kit is considerably damaged, please contact the manufacturer or local distributor. Do not use considerably damaged components for a test procedure. Store such components or kits until the complaint is handled. After this, they should be disposed according to the official regulations.

10. Quality Control

The negative control should reveal no fluorescence.

The positive controls should show a typical bright staining pattern: yellow-green round particles in the cytoplasm of the contrasting stretched red cells; non-infected cells are only stained red (approximately 15-20% of the cells on each well are strongly Hantavirus-infected and show the typical bright staining pattern with positive sera).

11. Results and Interpretation

Using the positive control well (3+) as the reading standard, record the intensity of fluorescence as follows:

3+	brilliant yellow-green fluorescence
2+	bright yellow-green fluorescence
1+	definite but dim fluorescence
Ø	no fluorescence.

The **endpoint titer** is the reciprocal of highest serum dilution giving 1+ fluorescence.

A 4-fold rise in IgG titer is indicative for an acute Hantavirus infection.

Initial screening of sera: For the initial screening it is recommended to dilute sera 1:16 and 1:32. Sera giving positive results should be titrated to end point. An acute Hantavirus infection is indicated by consecutive sera of an individual patient. This sera should be taken on day 1 and 10 to 14, showing seroconversion (>4fold rise of titer).

Titer <16: no antibodies detectable
 Titer >16: serum should be titrated to endpoint

Subclass testing: Before testing for IgM, separation of IgG is necessary (IgG Absorb, PROGEN Art. No.: PR715).

Interpretation and Results of Hantavirus Dobrava/Hantaan IF Test

Due to the extensive cross-reactivity (4, 6, 7) between Dobrava and Hantaan Virus, the most probable serological diagnosis of the infecting hantavirus serotype can be made in connection with the patient's travel history:

If the patient has no travel history outside Europe, a Dobrava virus infection is most likely.

(Data/ Recommendations provided by Instand e.V.)

Any lab result is only one aspect of the clinical appearance of a patient and should never be the sole basis for deriving any diagnostic and/ or therapeutic consequences.

12. Limitations

Any staining pattern different from the above, e.g. nuclear staining and similar staining of 100% of cells must be considered unspecific and inconclusive for Hantavirus.

There is a one-way cross reactivity between Dobrava/Hantaan and other serotypes: patient sera containing antibodies against Puumala serotype often react with Dobrava/Hantaan virus. Antibodies against the Seoul virus are also often detected with the Dobrava/Hantaan IF test.

13. Evaluation

The Hantavirus IF Tests were used with subclass testing, and diagnostic efficiency.

a) IgG

Evaluation of the following PROGEN tests was performed in parallel: Hantavirus (Puumala) IgG ELISA, Hantavirus (Dobrava/Hantaan) IgG ELISA, Hantavirus Dobrava/Hantaan Antibody IF Test, Hantavirus Puumala Antibody IF Test. The evaluation revealed a **diagnostic efficiency^a** as follows:

PUU-IFT n = 29	HTN-IFT (DOB/HTN) n = 56	IFT ELISA
88%	99%	DOB/HTN IgG ELISA
98%	84%	Puumala IgG ELISA

^a diagnostic efficiency = specificity/2 + sensitivity/2

b) IgM

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

PUU-IFT n = 25	HTN-IFT (DOB/HTN) n = 50	IFT ELISA
74%	100%	DOB/HTN IgM ELISA
99%	62%	Puumala IgM ELISA

14. References

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