RIDASCREEN® Hantavirus Puumala IgG, IgM

REF K9221
K9231

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1. Intended use

For *in vitro* diagnostic use. The RIDASCREEN® Hantavirus Puumala virus tests are enzyme immunoassays for the quantitative detection of IgG or IgM antibodies to the Hantavirus Puumala in human serum.

The tests are intended for use only in cases of a suspected hantavirus infection.

2. Summary and explanation of the test

Taxonomically, the hantavirus belongs to the bunyaviruses. It is an enclosed RNA virus. The RNA is present as an single strand in three ring-shaped segments (1,2). Hantaviruses are found throughout the world. However, there are geographic distributions for the individual hanta species given the serotype-specific host reservoirs. The species Puumala and Dobrava-Belgrade as well as Hantaan are resident in Eurasia (3). The pathogen reservoir is persistently and asymptomatically infected rodents such as mice that excrete large quantities of the pathogen through the urine, feces and saliva. The pathogens are transmitted to humans through contact infection, or by inhaling them in a feces-or urine-containing aerosol (1). In addition to harmless flu-like symptoms, an infection with hantavirus can cause hemorrhagic fever with renal syndrome (Nephropathia epidemica, HFRS) or pulmonary involvement (hantavirus pulmonary syndrome (HPS)). The lethality depends strongly on the virus strain and the severity of the infection (4.5). The humoral immune response is primarily directed against the nucleocapsid antigen of the hantavirus. In sensitive detection methods, IgM antibodies can be detected when clinical symptoms initially manifest. It is generally possible to detect IgG antibodies two weeks after infection (5).

3. Test principle

Recombinant NEV-N protein of the hantavirus species Puumala is bound to the surface of the wells in the microtiter strips. Diluted patient samples as well as the controls are pipetted into these wells and incubated at 37 °C. Existing antibodies bind to the immobilized antigens. Unbound material is removed by washing.
Then a peroxidase-conjugated anti-human antibody (anti-IgG or anti-IgM) is added. Any unbound conjugate is removed by washing. Then the substrate (H_2O_2/TMB) is added that causes a blue color to develop in positive samples from the bound enzyme. This reaction is terminated by adding stop solution. This addition causes the color to change from blue to yellow. The final measurement is taken in a photometer at 450 nm (reference wavelength 620 nm) within 20 minutes.

4. Reagents provided

Tab. 1: Reagents provided (The reagents in a kit are sufficient for 96 determinations.)

			K9221 IgG	K9231 IgM
Plate	96 assays	Microtiter plate; 12 microtiter strips (breakable) in the frame; coated with hantavirus antigen	X	X
SeroPP *Clear lid*	110 ml	Sample diluent, ready-to-use; phosphate-buffered saline solution, colored yellow	X	X
SeroWP	100 ml	Wash buffer, 10X concentration, tris-buffered saline solution	X	X
Control IgG | + *Green lid*	2.5 ml	Standard control IgG, ready-to-use; diluted human serum, colored green	X	
Control IgM | + *Red lid*	2.5 ml	Standard control IgM, ready-to-use; diluted human serum, colored red		X
Control IgG | - *Clear lid*	1.2 ml	Negative control IgG, ready-to-use; diluted human serum	X	
Control IgM | - *Clear lid*	1.2 ml	Negative control IgM, ready-to-use; diluted human serum		X
Control IgG | A *Green lid*	1.2 ml	Quality control A IgG, ready-to-use; diluted human serum, colored green	X	
Control IgG | B *Green lid*	1.2 ml	Quality control B IgG, ready-to-use; diluted human serum, colored green	X	
Control IgM | A *Red lid*	1.2 ml	Quality control A IgM, ready-to-use; diluted human serum, colored red		X
Control IgM | B *Red lid*	1.2 ml	Quality control B IgM, ready-to-use; diluted human serum, colored red		X
SeroG LD *Green lid*	12 ml	Anti-human IgG conjugate LD (goat), ready-to-use; peroxidase conjugate Anti-bodies in stabilized protein solution	X	
SeroM LD *Red lid*	12 ml	Anti-human IgM conjugate LD (goat), ready-to-use; peroxidase conjugate Anti-bodies in stabilized protein solution		X
SeroSC	12 ml	Substrate H_2O_2/tetramethylbenzidine; ready-to-use	X	X
Stop	12 ml	Stop solution 1 N sulfuric acid; ready-to-use	X	X

Information on hazardous substances complies with the labeling requirement. More details such as safety data sheets (SDS) and product information can be found at www.r-biopharm.com.

5. Storage instructions

The test kit can be used up to the expiration date printed on the label when stored at 2 - 8 °C. The diluted wash buffer has a shelf-life of four weeks when stored at 2 - 8 °C, and one week when stored at room temperature (20 - 25 °C). After the expiration date, the quality guarantee is no longer valid.
Open the aluminum bag containing the microtiter plate without separating the clip seal. Immediately store any unneeded microtiter strips in the sealed aluminum bag at 2 - 8 °C.
Prevent contamination of the reagents, and prevent direct light from shining on the colorless substrate.

6. Reagents required but not provided

6.1. Reagents

- Distilled or deionized water
- RF-Absorbens for IgM determinations (such as RIDA® RF-Absorbens, Art. No. Z0202)

6.2. Accessories

- Specimen vials
- Incubator 37 °C
- Vortex mixer
- Micropipettes for volumes of 10-100 µl and 100-1000 µl
- Graduated cylinder (1000 ml)
- Washer for microtiter plates or multichannel pipettes
- Photometer for microtiter plates (450 nm, reference filter ≥ 620 nm)
- Filter paper (lab wipes)
- Waste container with a 0.5% hypochlorite solution

7. Warnings and precautions for the users

For *in vitro* diagnostic use only.

Only trained laboratory personnel may perform this test. Follow the guidelines for working in medical laboratories. The instructions for use for performing this test must be strictly followed. Do not pipette specimens or reagents using your mouth. Avoid contact with broken skin or mucous membranes. Wear personal protective equipment (appropriate gloves, lab coat, safety glasses) when handling reagents and

specimens, and wash hands after completing the test. Do not smoke, eat, or drink in areas where specimens are handled.

For further details, see the safety data sheets (SDS) at www.r-biopharm.com.

Users are responsible for proper disposal of all reagents and materials after use. Follow the respective national disposal regulations.

The control sera found in the kit (standard control, negative control, quality control A and B) were tested for HIV Ab, HCV Ab, and HBsAg and were negative. Still, they should be treated as potentially infectious, as should the patient specimens and all materials that you touch, and they should be handled according to the respective national safety regulations.

8. Collection and storage of specimens

This test was developed for the examination of human serum specimens. Following blood collection, the serum should be separated from coagulum as quickly as possible to prevent hemolysis. The specimens should be kept cool or frozen until testing. Repeated freezing and thawing of specimens must be avoided, as must microbial contamination. The use of heat-inactivated, lipemic, hemolytic, icteric, or cloudy specimens can lead to false results.

Tab. 2: Specimen storage

Undiluted serum		Diluted serum
2-8 °C	-20 °C	2-8 °C
1 week	> 1 week	7 hours

9. Test procedure

9.1. General information

Prior to use, bring all reagents and microtiter strips to room temperature (20 - 25 °C). Once they have reached room temperature, remove the microtiter strips from the aluminum bag. Mix the reagents well immediately before use. After use, store the kit promptly at 2 - 8 °C again.
Take only as many reagents as needed for performing the test. Do not return extra reagent to the container as this can lead to contamination.
Microtiter strips may not be used more than once. Do not use reagents or microtiter strips if the packaging is damaged or the containers are not tightly sealed.
Some of the reagents in the kit are not test-specific. The reagents identified with Sero (such as SeroPP) can also be used for other RIDASCREEN® Sero ELISA with corresponding reagents.

The control sera are lot specific. The exchange of control sera between kits with different lot numbers is not permitted.

RIDASCREEN® Sero ELISA quality controls A and B are offered as additional, specific control specimens in the corresponding RIDASCREEN® Sero ELISA test kit. These are control specimens for additional quality assurance that may be used if desired. They contain human control serum with different antibody concentrations.

9.2. Preparing the wash buffer

Mix 1 part wash buffer concentrate SeroWP with 9 parts distilled water. To do so, add 100 ml concentrate to a 1000-ml cylinder and fill with distilled water to 1000 ml. Any crystals present in the concentrate should be dissolved beforehand through heating (water bath at 37 °C). The diluted wash buffer has a shelf-life of four weeks when stored at 2 - 8 °C and five days when stored at room temperature (20 - 25 °C).

9.3. Specimen preparation

Prior to the start of the test, dilute the serum specimens with the sample diluent SeroPP . Please note that the dilutions are different for IgG and IgM.

IgG :1:200 dilution of serum specimens

For example: 10 µl serum + 1990 µl SeroPP

IgM :1:100 dilution of serum specimens

For example: 10 µl serum + 990 µl SeroPP

For IgM determinations, it is recommended to conduct a sera IgG absorption (for example with RIDA® RF-Absorbens, Art. No. Z0202) before testing.

Attention!
The negative control, standard control, and quality control A and B are ready to use and may not be diluted or absorbed.

9.4. First incubation

After a sufficient number of wells have been placed in the frame, pipette 100 µl each of the diluted sera and ready-to-use controls into the respective wells; position A1 (substrate blank value) remains empty. Add the negative control Control IgG | - or Control IgM | - once, and
the positive control Control IgG | + or Control IgM | + in duplicate.
Add the quality controls Control IgG | A and Control IgG | B or the quality controls Control IgM | A and Control IgM | B once. The plate will be incubated in an incubator for 30 minutes at 37 °C. The bottom of the wells should not be in contact with materials that easily conduct heat. Cover the microtiter plate during incubation.

Use the appropriate controls (IgG or IgM).

A1	Reagent blank value
B1	Negative control
C1	Standard control
D1	Standard control
E1	Quality control A
F1	Quality control B
G1	Diluted patient serum

Attention!
Do not place the microtiter plate in a cold incubation container that heats up to 37 ° during the incubation. The container must be adapted to 37 °C beforehand.

9.5. Washing

Empty the wells into a waste container that has hypochlorite solution for disinfection. Next tap the plate over absorbable paper to remove the remaining moisture. Then wash the plate 4 times using 300 µl wash buffer each time. After every wash, tap the plate over an unused area of paper to ensure complete emptying.

When a microplate washer is used, make sure that the machine is correctly set to the plate type. After washing, tap the plate over absorbable, clean paper to remove residual moisture.

9.6. Second incubation

Add 100 µl of the anti-human IgG conjugate LD SeroG LD or the anti-human IgM conjugate LD SeroM LD to the corresponding wells (including A1). Next incubate the plate in an incubator for 30 minutes at 37 °C (see item 9.4).

9.7. Washing

Wash four times as described in item 9.5.

9.8. Third incubation

Add 100 µl substrate SeroSC to each well. Then incubate the plate in an incubator for 30 minutes at 37°C. Subsequently add 100 µl Stop solution to each well to stop the reaction. After carefully mixing the plate (by gently tapping on edge of plate), the extinction will be measured in a plate photometer at 450 nm (reference wave length ≥ 620 nm). Perform the measurement within 20 minutes after stopping. The blank value adjustment is made against the reagent blank value (position A1).

10. Quality control - indication of instability or expiration of reagents

For quality control, the standard control (in duplicate) and negative control must be run every time the test is being performed. The test has been performed correctly when the extinction average of the standard control at 450/620 nm lies within the range indicated on the attached quality certificate for the specific lot. If the two individual measurements deviate by more than 20 % from the average, the test must be repeated. The negative control must have an extinction value of < 0.3 at 450/620 nm.
RIDASCREEN® Sero ELISA quality controls A and B are additional control specimens for quality assurance that may be used optional. The target range can be found in the attached lot specific quality certificate. The individual values (U/ml, IU/ml or mIU/ml) are reference values for the user for laboratory-internal quality assurance.
A deviation from the expected values as well as cloudiness or blue coloring of the substrate prior to addition to the wells can be an indication of an expired reagent.
If the specified values are not met, check the following before repeating the test:

- Expiration date of the reagents used
- Functional performance of the equipment used (e.g., calibration)
- Correct test procedure
- Visual inspection of the kit components for contamination or leaks; a blue-colored substrate solution should no longer be used.

If the conditions are still not fulfilled after the test is repeated, please contact the manufacturer.

11. Evaluation and interpretation

The tests can be evaluated in three different ways:

1. Using the accompanying standard curve
2. Using the value table (see the accompanying datasheet)
3. Mathematically by means of the 4-parameter method or the α method

The reagent blank value must be subtracted from all extinction values before evaluating.

11.1. Evaluation using the standard curve

For an evaluation with the standard curve, the daily fluctuation must be corrected by taking the average of the standard control. The correction factor F is calculated using the target value for the standard control and the currently measured value of the control. Note the lot dependent target value on the accompanying quality certificate.

$$F = \frac{\text{Target value for the standard control}}{\text{Extinction mean for the standard control}}$$

Multiply all specimen OD values by the factor F. The corresponding U/ml value is read from the standard curve using these corrected values.

11.2. Evaluation using the value table

In the value table, the extinction mean for the standard control determines the column with the range of values applicable to the current measurement. Within this column, the measured sample extinction value is associated with the appropriate extinction range, and the corresponding titer in U/ml is read in the second column from the left.
For example, the extinction average for the standard control be 0.91 in a measurement. In this case, the column with the range of 0.89 to 0.94 in the table is used to determine the result. A patient specimen with an extinction value of 0.61 then lies within a titer range of 2.01 to 35.0 Units/ml. (The above values are examples and may deviate from the current values in the data sheet.)

The assessment of the determined result – positive (+), negative (-) or borderline (?) - can be found in the first column of the table of values.

	U/ml	Range of values for the standard control					
				0.89 - 0.94			
-	< 16.0			< 0.49			
?	16.0 - 20.0			0.49 - 0.56			
+	20.1 - 35.0			0.57 - 0.85			
	35.1 - 60.0			0.86 - 1.20			
	60.1 - 100.0			1.21 - 1.55			
	100.1 - 150.0			1.56 - 1.81			
	150.1 - 400.0			1.82 - 2.39			
	> 400.0			> 2.39			

Fig. 1: Example of an IgG determination (excerpt from a lot specific datasheet)

11.3. Mathematical evaluation

The required values for a mathematical evaluation following the 4-parameter evaluation or the α method are noted on the accompanying datasheet.

11.4. Evaluation and interpretation

Tab. 3: Evaluation of the determined units

	Negative	Borderline	Positive
IgG	< 16	16 - 20	> 20
IgM	< 16	16 - 20	> 20

12. Limitations of the method

RIDASCREEN® Hantavirus Puumala detects IgG or IgM antibodies against Hantavirus Puumala. The test is intended for use only in cases of a suspected hantavirus infection. Test results should always be interpreted in context of the clinical picture and other diagnostic findings. The development of anti-bodies may vary from patient to patient in terms of time and concentration. A negative result does not rule out a hantavirus infection since the stage of the infection may be very early, or the subspecies may be different. If a clinical suspicion remains, the test should be repeated after a few days. It is recommended depending on the patient's geographical origin to simultaneously test for an infection with a different hantavirus strain, e.g. Dobrava/Hantaan. A positive test result does not rule out the presence of other infectious pathogens.

13. Performance characteristics

Tab. 5: Inter-assay variance (n = 30)

Inter-assay variance	IgG		IgM	
	U/ml	CV	U/ml	CV
Serum 1	81.71	13.6 %	75.84	13.1 %
Serum 2	43.03	25.9 %	49.04	12.8 %
Serum 3	64.43	17.7 %	101.67	19.5 %
Serum 4	5.87	n/a	1.52	n/a

Tab. 6: Intra-assay variance (n = 23)

Intra-assay variance	IgG		IgM	
	U/ml	CV	U/ml	CV
Serum 1	98.57	11.6 %	74.64	5.2 %
Serum 2	25.30	6.8 %	42.62	10.3 %
Serum 3	34.68	6.5 %	75.87	13.6 %
Serum 4	4.48	n/a	1.35	n/a

Tab. 7, 8: Clinical performance in comparison with other commercial ELISA

IgG		Competitor			
		Positive	Borderline	Negative	Total
R-Biopharm	Positive	10	3	0	13
	Borderline	2	1	0	3
	Negative	8*	15	43	66
	Total	20	19	43	82

Positive agreement: 55.6* %
Negative agreement: 100 %

*The discrepancy between the results (competitor positive and R-Biopharm negative) was checked using a reference test (LineBlot) which resulted in negative findings.

IgM		Competitor			
		Positive	Borderline	Negative	Total
R-Biopharm	Positive	10	2	6	18
	Borderline	0	1	6	7
	Negative	0	3	54	57
	Total	10	6	66	82

Positive agreement: 100 %
Negative agreement: 90 %

Tab. 9: Results from 200 examined blood donors from a blood donation center in Germany

200 blood donor sera	IgG	IgM
Negative	99.0 %	96.5 %
Borderline	0.5 %	1.0 %
Positive	0.5 %	2.5 %

14. Version history

Version number	Section and name
2018-02-01	Release version

15. Explanation of symbols

General symbols

IVD	In-vitro diagnostics
	Observe the instructions for use
LOT	Lot number
	Use before
	Storage temperature
REF	Article number
	Number of tests
	Date of manufacture
	Manufacturer

Test-specific symbols

Plate	Microtiter plate
SeroPP	Sample dilution buffer
SeroWP	Wash buffer 10x
Control IgG | +	Standard control IgG
Control IgM | +	Standard control IgM
Control IgG | -	Negative control IgG
Control IgM | -	Negative control IgM
Control IgG |A	Quality control A IgG
Control IgM |A	Quality control A IgM
Control IgG |B	Quality control B IgG
Control IgM |B	Quality control B IgM
SeroG LD	Anti-human IgG conjugate
SeroM LD	Anti-human IgM conjugate
SeroSC	TMB substrate
Stop	Stop solution

16. References

1. *Ulrich R, Meisel H, Schütt M, Schmidt J, Kunz A, Klempa B*, u. a. Verbreitung von Hantavirusinfektionen in Deutschland. Bundesgesundheitsblatt - Gesundheitsforschung - Gesundheitsschutz [Internet]. Juli 2004 [zitiert 23. Mai 2017];47(7). Verfügbar unter: http://link.springer.com/10.1007/s00103-004-0858-8
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