*recom*Line HantaPlus IgG
*recom*Line HantaPlus IgM

IVD

Instructions for use (English)

1 Purpose

The *recom*Line HantaPlus IgG, IgM is an in-vitro quality test for detecting IgG and/or IgM antibodies to the Hanta virus serotypes Puumala (PuN), Sin Nombre (SinN), Hantaan (HaN), Dobrava (DobN) and Seoul (SeoN) and Sandfly Fever Virus serotypes Toscana and Sicilian in human serum or plasma.

2 Intended use

The *recom*Line HantaPlus was developed as a confirmation test for positive or questionable screening results. The *recom*Line HantaPlus can also be used as a screening test. Serotyping is possible in the *recom*Line HantaPlus IgG test. Definite allocation of the serotype *necessitates* taking into account the travel history of the patient both within and outside Europe. Serotyping must be confirmed by means of PCR (polymerase chain reaction). The analysis of the test strips can be visual or computer-assisted - using the *recom*Scan test strip analysis software.

3 Test principle

Highly purified recombinant Hanta virus antigens of the nucleocapside protein (PuN, SinN, HaN, DobN, SeoN) and sandfly fever virus antigens (Toscana and Sicily) have been fixed on nitrocellulose membrane test strips.

1. The test strips are incubated with the diluted serum or plasma samples, with specific antibodies in the samples attached to the pathogen-specific antigens on the test strips.
2. Unbound antibodies are then flushed away.
3. In a second step, the strips are incubated with anti-human immunoglobulin antibodies (IgG or IgM), which are coupled to horseradish peroxidase.
4. Unbound conjugate antibodies are then flushed away.
5. Specifically bound antibodies are detected with a staining reaction catalysed by the peroxidase. If an antigen-antibody reaction has taken place, a dark band will appear on the strip at the corresponding point.

There are three control bands at the upper end of the test strips:

a) The reaction control band under the strip number, which must show a reaction for every serum/plasma sample. This control band is only visible when the test has been carried out correctly.
b) The conjugate controls (IgG or IgM) are used for the inspection of the antibody class detected. If, for example, the test strip is used for the detection of IgG antibodies, the conjugate control band IgG shows a clearly defined band.
c) Cut-off control: The intensity of this band allows the assessment of the reactivity of each of the antigen bands (see 9.2. Evaluation).

4 Reagents

4.1 Package contents

The reagents in one package are sufficient for 20 tests.

Each test kit contains:

WASHBUF A 10 X	**100 ml** Wash Buffer A (**10 times concentration**) Contains phosphate buffer, NaCl, KCl, detergent, preservative: MIT (0.1%) and Oxypyrion (0.2%)
SUBS TMB	**40 ml** Chromogenic substrate Tetramethylbenzidin (**TMB, ready-to-use**)
MILKPOW	**5 g** skimmed milk powder
INSTRU	**1** Instructions for use
EVALFORM	**1** Evaluation form

4.1.1 *recom*Line HantaPlus IgG

In addition to the components listed in 4.1, each test kit contains:

TESTSTR	**2** tubes, each with 10 numbered test strips
CONJ IgG	**500 µl** anti-human IgG conjugate **(100-fold concentration, green cap)** From rabbit, containing NaN_3 (<0.1%), MIT (<0.1%) and chloroacetamide (<0.1%)

4.1.2 *recom*Line HantaPlus IgM

In addition to the components listed in 4.1, each test kit contains:

TESTSTR	**2** tubes, each with 10 numbered test strips
CONJ IgM	**500 µl** anti-human IgM conjugate **(100-fold concentration, purple cap)** From rabbit, contains NaN_3 (<0.1%), MIT (<0.1%) and chlorazetamide (<0.1%)

4.2 Materials required but not supplied

- Incubation trays (can be purchased as needed from MIKROGEN)
- Deionised water (high quality)
- Plastic forceps
- Horizontal shaker
- Vortex mixer or other rotators
- Vacuum pump or similar device
- Volumetric cylinders, 50 ml and 1000 ml
- Micropipettes with disposable tips, 20 µl and 1000 µl
- 10 ml pipette or dispenser
- Timer
- Absorbent paper towels
- Disposable protective gloves
- Waste container for bio-hazardous materials

5 Shelf life and handling

- Store reagents at +2 to +8 °C before and after use, **do not freeze**.
- Subject all ingredients to room temperature (+18 to +25 °C) for at least 30 minutes before beginning the test. The test procedure is carried out at room temperature.
- Where different *recom*Line and *recom*Blot tests are used, the same reagents (see printed symbol) can be used across the whole range of parameters and batches. The shelf life of these components should be noted.
- Mix the concentrated reagents and patient samples thoroughly before use. Avoid a build up of foam.
- Only open the tube containing the test strip immediately before use to avoid condensation. Leave unused strips in the tube and continue to store at +2 °C to +8 °C (reseal tube tightly, test strips must not become moist before the test!).
- The test strips are marked with the serial number and the test code.
- The packages bear an expiration date. After this has been reached no guarantee of quality can be offered.
- Protect kit components from direct sunlight throughout the entire test procedure. The substrate solution (TMB) is especially sensitive to light.
- The test should only be carried out by trained and authorised personnel.
- In case of significant changes by the user to the product and/or the instructions for use, application may be beyond the purpose specified by MIKROGEN.
- Cross-contamination of patient samples or conjugates can lead to inaccurate test results. Add patient samples, test strips and conjugate solution carefully. Make sure that incubation solutions do not flow over into other wells. Carefully remove liquids.
- The strips must be completely wetted and immersed throughout the entire procedure.
- Automation is possible; you will receive further information from MIKROGEN.

6 Warnings and precautions

- For *In vitro* diagnostic use only.
- All blood products must be treated as potentially infectious.
- The test strips were prepared with inactivated bacterial or viral antigens.
- After the addition of patient or control specimens the strip material must be considered infectious and treated as such.
- Suitable disposable gloves must be worn throughout the entire test procedure.
- The reagents contain the antimicrobial agents and preservatives sodium azide, MIT (methylisothiazolone), oxypyrion and chloroacetamide and hydrogen peroxide. Avoid contact with the skin or mucous membrane. Sodium azide can form an explosive azide upon contact with heavy metals such as copper and lead azide.

- All siphoned liquids must be collected. All collecting containers must contain suitable disinfectants for the inactivation of human pathogens. All reagents and materials contaminated with potentially infectious samples must be treated with disinfectants or disposed of according to your hygiene regulations. The concentrations and incubation periods of the manufacturer must be observed.
- Use incubation trays only once.
- Handle strips carefully using plastic forceps.
- Do not substitute or mix the reagents with reagents from other manufacturers.
- Read through the entire instructions for use before carrying out the test and follow them carefully. Deviation from the test protocol provided in the instructions for use can lead to erroneous results.

7 Sampling and preparation of reagents

7.1 Samples

The sample can be serum or plasma (citrate, EDTA, heparin, CPD), which needs to be separated from the blood clot as soon as possible after sampling so as to avoid haemolysis. Avoid Microbial contamination of the samples. Insoluble substances must be removed from the sample prior to incubation.
The use of heat-inactivated, icteric, haemolytic, lipaemic or turbid samples is not recommended.
Caution!
If the tests are not conducted immediately, the sample can be stored for up to 2 weeks at +2 to +8 °C. Longer term storage of the samples is possible at -20 °C or lower. Repeated freezing and thawing of samples is not recommended due to the risk of producing inaccurate results.

7.2 Preparation of solutions

7.2.1 *Preparation of ready-to-use wash buffer A*
This buffer is required for sample and conjugate dilution as well as during the washing stages.
Prior to dilution, the volume of wash buffer A must be determined for the corresponding number of tests.
First, the skimmed milk powder is dissolved in wash buffer A concentrate, and then deionised water is added to bring the solution up to the final volume (dilution: 1 + 9). The quantities required for a defined number of test strips are to be mathematically determined according to the following formula (device-specific dead volume is not considered):

Reagent	**Formula**	Example: 5 strips
Skimmed milk powder [g]	= number of strips x 0.1	0.5 g
Wash buffer A concentrate [ml]	= number of strips x 2	10 ml
Deionised water [ml]	= number of strips x 18	90 ml
Ready-to-use wash buffer A [ml]	= number of strips x 20	100 ml

Ready-to-use wash buffer A can be stored for 4 weeks at +2 °C to +8 °C.
The ready to use wash buffer A is odourless and easily marred.

7.2.2 *Preparation of conjugate solutions*
The conjugate solution must be prepared just before use. It is not possible to store the ready-to-use conjugate solution.
One part of the conjugate concentrate is diluted with 100 parts of the ready to use wash buffer A (1 + 100).

The quantities required for a defined number of test strips are to be calculated according to the following formula:

Reagent	**Formula**	Example: 5 strips
Conjugate concentrate [µl]	= number of strips x 20	100 µl
Ready-to-use wash buffer A [ml]	= number of strips x 2	10 ml

The conjugate quantities are calculated without dead volume. Depending on handling (manual or on a device), please mix additional conjugate for 1 to 3 strips.

8 Test procedure

No.	Execution	Note
1	Temper all reagents for at least 30 minutes at 18°C - 25° (room temperature) before beginning the test.	The test procedure is carried out at room temperature.
2	Prepare test strips Place the strips in **2 ml** of ready-to-use **wash buffer A**. **Note:** IgG and IgM strips are not interchangeable!	Do not touch the strips with bare hands - use the forceps. The strip number points upward. A well is required in the incubation tray (see 4.2) for each strip. The strips must be completely immersed.
3 a) b)	Incubation of samples **20 µl** of undiluted sample (human serum or plasma) is pipetted on to the test strip for each incubation mixture. (Dilution 1 + 100) Incubate for **1 hour** with gentle shaking	 Pipette the sample at one end of the immersed strip in the wash buffer A and mix as quickly as possible by carefully shaking the tray. Cover the incubation tray with plastic cover and place in the shaker.
4 a) b) c)	Washing Carefully remove the plastic cover from the incubation trays. Gently siphon serum dilution from the individual wells. Pipette **2 ml** of ready to use **wash buffer A** into each well, wash for 5 minutes with gentle shaking and then siphon off the wash buffer A.	Carry out washing stages 8.4a-8.4c three times in total. Avoid cross-contamination. The manufacturer's instructions must be followed during automatic processing.
5	Incubation with conjugate Add **2 ml** of ready-to-use **conjugate solution** and incubate for **45 minutes** while shaking gently.	Cover the incubation tray with plastic cover and place in the shaker.
6	Washing see under 8.4	Carry out the washing stages three times in all (see 8.4a-8.4c)
7	Substrate reaction Add **1.5 ml** of ready-to-use **substrate solution** and incubate for **8 minutes** while shaking gently.	
8	Stopping the reaction Remove substrate solution. Wash at least three times **briefly** with **deionisied water**.	
9	Drying the strips Dry strip between 2 layers of absorbent paper for **2 hours** before analysis. **CP**	Carefully remove strips from water using plastic forceps. Store strip away from light.
Caution! Incubation solutions must not flow into other wells. Splashing must be avoided especially when opening and closing the lid.		

9 Results

Caution:
Please do not use automated interpretation without consideration of the information on interpretation given below.

9.1 Validation – Quality Control

An analysis of the test can be carried out if the following criteria have been fulfilled:
1. Reaction control band (top line) with clearly stained, dark band
2. Antibody class (second band): the IgG and / or IgM conjugate control band must show a clear staining. The respective, other conjugate band may show weak, unspecific staining.
3. Cut-off control (third band): weak, but visible staining

9.2 Evaluation

The analysis of the test strips can be visual or computer-assisted - using the test strip analysis software *recom*Scan. The *recom*Scan software is designed to support the evaluation of test strips. Further information and related instructions for the computer-assisted analysis is available on request from MIKROGEN. The following instructions relate to visual analysis.

9.2.1 *Assessment of band intensity*

1. Note the date and batch number, as well as the detected antibody class, on the attached evaluation form.
2. Enter the sample identification numbers to the evaluation form.
3. Now stick the corresponding test strip onto the appropriate fields on the evaluation form using a glue stick. Align the test strip with the reaction control bands along the marked lines. Then use a transparent adhesive tape to attach the test strip to the left of the marked lines (do not tape over the reaction control band!). Sticking the entire test strip down flat using glue or tape can lead to changes in colour.
4. Now identify the bands of the developed test strip using the control strip printed on the evaluation form and enter them in the evaluation form. For each corresponding immunoglobulin class, assess separately the intensity of the bands occurring on the basis of Table 1.

Table 1: Assessment of band intensity in relation to the cut-off band

Stain intensity of the bands	**Assessment**
No reaction	-
Very low intensity (lower than the cut-off band)	+/-
Low intensity (equivalent to the cut-off band)	+
High intensity (higher than the cut-off band)	++
Very strong intensity	+++

9.3 Interpretation of test results

The results of the IgG and IgM tests, the clinical results and the travel history over the past 6 weeks should always be studied in combination when making a serological evaluation of the Hanta virus or sandfly fever virus immune status. The same interpretation criteria apply to both antibody classes IgG and IgM. The criteria for interpreting the test are to be taken from Table 2. Where the patient hasn't travelled outside Europe, a Sin Nombre reactivity with a Puumala infection should be rated as a cross-reaction. The same applies to a Hantaan or Seoul reactivity with a Dobrava infection. Where patients have returned from a trip to South America with a Sin Nombre/Puumala reactivity, infection with Andes should be considered, due to the homology between Sin Nombre, Puumala and Andes.

Table 2: Test interpretation

Hantavirus:

At least one Hanta virus-specific band (PuN, SinN, HaN, DobN, SeoN) reacts with the same or a stronger intensity (+) than the cut-off bands.	Hanta positive
No bands (-) or bands with a weaker (+/-) intensity than the cut-off band.	Hanta negative

Hantavirus serotyping:

Travel history	**IgG antigen pattern**	**Interpretation**
No travel outside Europe during the past 6 weeks.	The PuN and/or SinN bands react with the same or a stronger intensity (+) than the cut-off band and is more strongly coloured than the other hanta specific bands (HaN, DobN, SeoN)	Hanta positive suspicion of Puumala
No travel outside Europe during the past 6 weeks.	The HaN, DobN and/or SeoN react with the same or a stronger intensity (+) than the cut-off band and is more strongly coloured than the other hanta specific bands (PuN, SinN)	Hanta positive suspicion of Dobrava
Travel outside Europe (mainly North America, Canada) in the past 6 weeks	The PuN and/or SinN react with the same or a stronger intensity (+) than the cut-off band and is more strongly coloured than the other hanta specific bands (HaN, DobN, SeoN)	Hanta positive suspected Sin Nombre
Travel outside Europe (mainly Asia) in the past 6 weeks	The HaN, DobN and/or SeoN react with the same or a stronger intensity (+) than the cut-off band and is more strongly coloured than the other hanta specific bands (PuN, SinN)	Hanta positive suspected Hantaan or Seoul

Sandfly Fever Virus:

The SFFV band reacts with the same or greater intensity (+) than the cut-off band.	**positive**
No bands (-) or bands with a weaker (+/-) intensity than the cut-off band.	**negative**

10 Limitations of the method – restrictions

- Serological test results must always be seen in the context of the clinical picture of the patient. Therapeutic consequences of the serological findings must always be taken in context with the clinical data.
- If the serological results are uncertain or questionable, a new test is recommended within the following 2 weeks of the potential infection. When diagnosing a Hanta virus and/or sandfly fever virus infection and classifying the individual stages (acute and/or past infection), the clinical findings and associated travel history must be included in addition to the laboratory values.
- A positive *recom*Line HantaPlus IgG, IgM test result can serve as an indication for an acute Hanta virus or sandfly fever virus infection. A positive *recom*Line HantaPlus IgG, IgM test result does not necessarily mean that there is an active illness event. IgG antibodies against the Hanta virus or sandfly virus in particular can usually be detected for the remainder of a patient's life.
- A negative test result for *recom*line HantaPlus IgG, IgM cannot exclude an infection with the Hanta virus and/or Sandfly Fever Virus. In particular, in the early phase of the infection, antibodies may not be present or not present in a detectable quantity. When there are clinical symptoms indicating an infection and the test results are negative and/or questionable, another sample should be taken and tested after two to three weeks (a so-called diagnostic window). In rare cases, no IgM or IgG antibodies are formed (due to immunosuppression).
- In the interpretation of the serological results, it is indispensable to include the case history, clinical symptoms and additionally available laboratory data in the total diagnosis. Thus, an infection with Hanta virus or sandfly Fever virus cannot be excluded once antibodies have been detected and there are clear clinical symptoms of an infection. A second sample should be taken two weeks later to clarify the increase in antibodies (sero-conversion).
- Dark test strips: Some patient samples can produce dark, uniform or patterned staining across the entire nitrocellulose strip. Various factors in each patient serum are responsible for this. The evaluation of these strips is usually only partly feasible. Thus, "inverse" bands (white bands on dark background), for example, should be evaluated as negative. The respective serum should always be examined using other serological methods.

11 Test performance

11.1 Sensitivity and specificity for Hantavirus infections

Table 3: The sensitivity and specificity were determined as a diagnostic concurrence, using two commercially available test systems for the specific detection of anti-Hanta virus IgG and IgM antibodies.

***recom*Line HantaPlus IgG**	**Positive**	**Negative**
Positive	74	0
Negative	3	18
Total	77	18
Sensitivity	96.1%	
Specificity	100%	

***recom*Line HantaPlus IgM**	**Positive**	**Negative**
Positive	59	2
Negative	1	32
Total	60	34
Sensitivity	98.3%	
Specificity	94.1%	

11.2 Hantavirus serotyping

Table 4: The sensitivity and specificity of serotyping was determined in the recomLine HantaPlus IgG test, on the basis of clinically defined ring trail samples with a known Hanta virus infection. There was no history of travel outside Europe during the past 6 weeks.

***recom*Line HantaPlus IgG**	**Serotype Puumala**	**Serotype Dobrava**
Puumala/SinNombre	10	0
Hantaan/Dobrava/Seoul	0	12
Total	10	12
Serotyping	100%	100%

11.3 Prevalence

Table 5: The seroprevalence was determined by testing for the specific anti-Hanta virus and anti-sandfly fever virus (SFFV) IgG and IgM antibodies in asymptomatic healthy blood donors in Southern Germany. The seroprevalence of a population may vary, depending on the age and place of residence of the test subjects.

Parameter	*recom*Line HantaPlus IgG		*recom*Line HantaPlus IgM	
	Anti-Hantavirus IgG	Anti-SFFV IgG	Anti-Hantavirus IgM	Anti-SFFV IgM
Number of samples	193	193	191	191
Positive	2	5	3	0
Negative	191	188	188	191
Seroprevalence	1.0%	2.6%	1.6%	0%

11.4 Analytical specificity

The analytical specificity is defined as the capacity of the test to precisely determine the analytes in the presence of potential interference factors in the sample matrix or cross-reactions with potentially interfering antibodies.

a) Interferences: Control studies on potentially interfering factors have shown that anticoagulants (sodium citrate, EDTA, heparin, CPD), haemolytical, icteric or lipaemic samples or cycles of freezing and thawing do not affect the performance of the test.

b) Cross-reactions: The potential interference of antibodies with other organisms has been investigated in control studies. In addition, conditions attributable to an atypical activity of the immune system (antinuclear autoimmune antibody, pregnancy, fresh Herpes virus infection, CMV infection) are tested. There was no evidence of cross-reactions. A cross-reactivity caused by rheumatoid arthritis factors in the anti-SFFV IgG test, by an acute EBV infection in the anti-Hanta IgM test or by samples with anti-malaria antibodies in the anti-Hanta virus IgM test and the anti-SFFV IgM test cannot be excluded. The seroprevalence of the anti-Hanta virus IgM antibodies in anti-malaria IgG-positive samples was 30%, while for samples from patients with an acute EBV infection it was 9.8%. Anti-SFFV IgM antibodies could be detected in 20% of the anti-malaria IgG-positive samples. The seroprevalence of the anti-SFFV IgG antibodies in samples that tested positive for rheumatoid arthritis factor amounted to 14.3%. A cross-reactivity caused by an acute EBV infection or anti-malaria IgG antibody in a sample must be excluded by differential diagnosis.

12 Literatur

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On request more literature about Bunyavirus diagnostic (Hantavirus, Sandfly Fever Virus) is available.

13 Explanation of symbols

Σ	Content is sufficient for <n> applications Number of applications
EVALFORM	Evaluation form
INSTRU	Instructions for use
	See instructions for use
CONT	Contents, includes
IVD	In vitro test
LOT	Batch/version number
	Do not freeze
REF	Order number
	Use by Expiry date
y°C x°C	Store at x°C to y°C

14 Manufacturer and version information

*recom*Line HantaPlus IgG *recom*Line HantaPlus IgM	Item no. **7672** Item no. **7673**
Instructions for use valid from	GARLHA002EN 2023-03

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