Hantavirus IgG DxSelect™
(OUS)

**REF** EL1600G
Rev. P

Enzyme-linked immunosorbent assay (ELISA) for the qualitative detection of human IgG class antibodies to hantavirus

This package insert is for export only and not for distribution in the United States.

**Outside of the United States:**
For *in vitro* Diagnostic Use.

**INTENDED USE**
Focus Diagnostics’ Hantavirus IgG DxSelect™ test is intended for the qualitative detection of human IgG class antibodies to Hantavirus in human sera.

**SUMMARY AND EXPLANATION OF TEST**
Hantavirus (HTV) is a novel genus in the Bunyaviridae family, in which it stands as the only non-arthropod-borne human pathogen. Transmission to man occurs via inhalation of viral particles from aerosolized excreta from HTV-infected rodents. Phylogeny and epidemiology of HTV’s are closely linked to those of their respective rodent reservoirs. To date, up to 30 different HTV strains have been isolated or characterized, of which at least 13 have pathogenic significance for man. The clinically most important strains are murine Hantaan (HTN) and Seoul (SEO) in Asia, Puumala (PUU) and Dobrava (DOB) in Europe, and Sin Nombre (SNV) in the America’s.1 Whereas HTN, PUU, SEO, and DOB all have the kidney as the main target organ in man, SNV and SNV-like agents primarily affect the lung. The HTV strains typically have regional distribution; however, geographic overlap between strains does exist.

Over 150,000 cases of hemorrhagic fever with renal syndrome (HFRS) occur worldwide each year. 2 HFRS is characterized by fever, renal failure and, at times, hemorrhagic manifestations. The clinical manifestations of HFRS are more severe when caused by HTN and less severe when due to SEO and PUU. In central Europe HFRS due to DOB may also cause a severe form of disease with mortality of up to 20%. 3

Hantavirus pulmonary syndrome (HPS) is a disease of rapid onset characterized by fever and severe pulmonary dysfunction with mortality approaching 50%. SNV in North America and the Andes virus in South America have been the causative agents of HPS to date.

The HTV are enveloped and have a tripartite, negative-stranded RNA which encodes several proteins including an RNA-dependent RNA polymerase, 2 envelope glycoproteins and a nucleocapsid protein (NP). The small (S) segment of the genome encodes for the NP and the medium segment encodes for 2 envelope proteins, G1 and G2.4,5 The NP is the predominant target of the antibody response; however, antibodies directed to the NP are highly cross-reactive between the HTV species. The envelope proteins tend to induce a lower antibody response, but the antibody is species specific. Previously Rossi, et al. 7 found that the HTN NP was a potential candidate for the test antigen in an ELISA-based format to detect IgG.

Recently Elgh, et al.4 studied the kinetics of the antibody response to recombinant (rNP) in nephropathia epidemica patients caused by Puumala virus. They found that IgG was detected within 2 to 8 days of disease onset, remained high for 2 to 5 months and gradually declined over 2 to 3 years. Nearly all patients remained positive for IgG after 2 to 3 years. The IgM was detectable in most cases within 2 to 8 days of disease onset with nearly all patients positive at 5 to 15 days. The IgM titers declined rapidly and most patients are negative at 2 to 5 months after disease onset.

As is true with other viral diseases, it is difficult to diagnose HTV infection based on clinical grounds alone; thus, serologic methods to detect HTV have evolved as the test of choice for aiding the diagnosis of HTV infection. The Focus Diagnostics Hantavirus DxSelect™ kit uses a cocktail of baculovirus-derived recombinant NP of HTV strains. Using a rNP cocktail allows for detecting antibodies to a broad range of pathogenic species of HTV. The Focus Diagnostics Hantavirus DxSelect™ will detect antibodies to the most clinically relevant pathogenic strains of Hantaviruses, i.e., SEO, HTN, PUU, DOB, and SNV.

**TEST PRINCIPLE**
In the Focus Diagnostics Hantavirus IgG DxSelect™ assay, the polystyrene microwells are coated with Hantavirus antigens. Diluted serum samples and controls are incubated in the wells to allow specific antigen present in the samples to react with the antigen. Nonspecific reactants are removed by washing and peroxidase-conjugated anti-human IgG is added and reacts with specific IgG. Excess conjugate is removed by washing. Enzyme substrate and chromogen are added, and the color is allowed to develop. After adding the Stop Reagent, the resultant color change is quantified by a spectrophotometric reading of optical density (OD) which is directly proportional to the amount of antigen-specific IgG present in the sample. Sample OD readings are compared with reference cutoff OD readings to determine results.

**MATERIALS SUPPLIED**
The Focus Diagnostics Hantavirus IgG DxSelect™ Test kit contains sufficient materials to perform 96 determinations. Allow the supplied reagents to warm to room temperature before use. All un-opened materials are stable at 2 to 8°C until the expiration date stated on the reagent label.

<table>
<thead>
<tr>
<th>Material Name</th>
<th>Ref. Code</th>
<th>Concentration</th>
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</thead>
<tbody>
<tr>
<td>Antigen Wells, 96 wells</td>
<td>REF EL1601</td>
<td></td>
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<tr>
<td>12 eight-well polystyrene microwell strips on a frame. Each well is coated with recombinant proteins. Each strip may be broken down into individual wells for cost effective use. To avoid condensation, allow the antigen strips to warm to room temperature before opening the sealed packets.</td>
<td></td>
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</tr>
<tr>
<td>IgG Conjugate, 16 mL</td>
<td>REF EL1604</td>
<td>CONJ IgG</td>
</tr>
<tr>
<td>1 vial of affinity-purified and peroxidase-conjugated goat anti-human IgG (Fc fragment specific). Contains protein, buffer, and preservatives.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgG Detectable Control, 0.30 mL</td>
<td>REF EL1611</td>
<td>CONTROL &gt;</td>
</tr>
<tr>
<td>1 vial of human serum. Contains 0.1% sodium azide as a preservative. Requires dilution before use (see Specimen, Controls and Calibrator Preparation, below).</td>
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</tr>
<tr>
<td>Non-Detectable Control, 0.30 mL</td>
<td>REF EL1612</td>
<td>CONTROL &lt;</td>
</tr>
<tr>
<td>1 vial of human serum. Contains 0.1% sodium azide as a preservative. Requires dilution before use (see Specimen, Controls and Calibrator Preparation, below).</td>
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<td></td>
</tr>
<tr>
<td>IgG Cut Off Calibrator, 0.30 mL</td>
<td>REF EL1606</td>
<td>CONTROL CAL</td>
</tr>
<tr>
<td>1 vial of human serum. Contains 0.1% sodium azide as a preservative. Requires dilution before use (see Specimen, Controls and Calibrator Preparation, below).</td>
<td></td>
<td></td>
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</tbody>
</table>
Sample Diluent, 100 mL
1 vial of protein, surfactant, and preservatives in PBS.

10X Wash Buffer, 100 mL
1 vial of surfactant in PBS with preservatives. Prepare a 1X wash buffer solution before use.

To prepare a 1X wash buffer solution, mix 100 mL 10X Wash Buffer with 900 mL distilled (or deionized) water and rinse out any crystals. Use only the highest grade purified water for reconstitution of the wash buffer. It has been observed that some sources of deionized water contain materials, which can interfere in the assay. Swirl until well mixed and all crystals are dissolved.

Stop Reagent, 16 mL
1 vial of 1M sulfuric acid

Substrate Reagent, 16 mL
1 vial of tetramethylbenzidine (TMB) and hydrogen peroxide in buffer. A dark blue color indicates contamination with peroxidase; and, if this occurs, use a fresh bottle.

Sealing Tape
2 sheets of sealing tape.

MATERIALS REQUIRED, BUT NOT SUPPLIED
1. Distilled water
2. 250 or 500 mL wash bottle
3. 1 L graduated cylinder
4. 12 x 75 mm borosilicate glass test tubes or equivalent
5. 10 µL pipettors with disposable tips
6. 100 µL pipettors with disposable tips (100 µL 8- or 12-channel pipettor recommended for runs over 48 wells)
7. 1 mL pipet or dispenser
8. 5 mL pipet
9. Timer
10. Paper towels or absorbent paper
11. Sink
12. Vortex mixer or equivalent
13. ELISA plate spectrophotometer, wavelength = 450 nm

SHELF LIFE AND HANDLING
1. Kits and kit reagents are stable through the end of the month indicated by the label expiration date when stored at 2 to 8°C.
2. Do not use test kit or reagents beyond their expiration dates.
3. Do not expose reagents to strong light during storage or incubation.
4. Allow reagents to warm to room temperature before use.

WARNINGS AND PRECAUTIONS
1. This package insert is for export only and not for distribution in the United States. Outside of the United States this kit is for in vitro diagnostic use.
2. All blood products should be treated as potentially infectious. Source materials from which this product (including controls) was derived have been screened for Hepatitis B surface antigen, Hepatitis C antibody and HIV-1/2 (AIDS) antibody by FDA-approved methods and found to be negative. However, as no known test methods can offer 100% assurance that products derived from human blood will not transmit these or other infectious agents, all controls, serum specimens and equipment coming into contact with these specimens should be considered potentially infectious and decontaminated or disposed of with proper biohazard precautions. CDC and the National Institutes of Health recommend that potentially infectious agents be handled at the Biosafety Level 2.6,7
3. The Hantavirus antigen plates are produced with recombinant antigens; however, the plates should be considered potentially infectious and handled accordingly.
4. Sodium azide at a concentration of 0.1% has been added to the controls as an antibacterial agent. To prevent buildup of explosive metal azides in lead and copper plumbing, controls should be discarded into sewerage only if diluted and flushed with large volumes of water. Use copper-free and lead-free drain systems where possible. Occasionally decontaminate the drains with 10% sodium hydroxide (CAUTION: caustic), allow to stand for 10 minutes, then flush with large volumes of water.
5. The stop reagent contains sulfuric acid. Do not allow to contact skin or eyes. If exposed, flush with copious amounts of water.
6. Do not substitute or mix reagents from different kit lots or from other manufacturers.
7. Use only protocols described in this insert. Incubation times or temperatures other than those specified may give erroneous results.
8. Cross-contamination of patient specimens can cause erroneous results. Add patient specimens and handle strips carefully to avoid mixing of sera from adjoining wells. Avoid contamination of the substrate reagent with traces of the enzyme conjugate.
9. Bacterial contamination of serum specimens or reagents can produce erroneous results. Use aseptic techniques to avoid microbial contamination.
10. Perform the assay at room temperature (approximate range 20 to 25°C).
11. Use proper pipetting techniques, maintaining the pipetting pattern throughout the procedure to ensure optimal and reproducible values.

SPECIMEN COLLECTION AND PREPARATION
Serum is the preferred specimen source. No attempt has been made to assess the assay’s compatibility with other specimens. Hyperlipemic, heat-inactivated, hemolyzed, or contaminated sera may cause erroneous results; therefore, their use should be avoided.

Specimen Collection and Handling
Collect blood samples aseptically using approved venipuncture techniques by qualified personnel. Allow blood samples to clot at room temperature prior to centrifugation. Aseptically transfer serum to a tightly closing sterile container for storage at 2 to 8°C. If testing is to be delayed longer than 5 days, the sample should be frozen at –20°C or colder. Thaw and mix samples well prior to use.
Specimen, Controls and Calibrator Preparation

Dilute each specimen, control and calibrator 1:101 as follows: label tubes and dispense 1000 µL of Sample Diluent into each labeled tube. Add 10 µL of specimen, control or calibrator to each appropriate tube containing the 1000 µL IgG Sample Diluent and mix well by vortex mixing.

TEST PROCEDURE

1. Bring all reagents to room temperature before use. Remove the Antigen Well packet from cold storage. To avoid condensation, allow micro-well strips to reach room temperature before opening the foil packet. If less than a full plate is to be used, return unused strips to the foil packet with desiccant and reseal completely. Store unused antigen wells at 2 to 8°C. (Note: The end of the assay, retain the frame for use with the remaining strips.)

2. Fill wells with 1X Wash Buffer solution (see MATERIALS SUPPLIED, above) and allow to soak for 5 minutes. Decant (or aspirate) the antigen wells and tap vigorously to remove Wash Buffer. Blot the emptied Antigen Wells face down on clean paper towels or absorbent paper to remove residual Wash Buffer.

3. Dispense 100 µL of the Sample Diluent into the “blank” wells and 100 µL of each diluted specimen, control or calibrator (see Specimen, Controls, and Calibrator Preparation, above) into the appropriate wells. (Note: For runs with more than 48 wells it is recommended that 250 µL of each diluted sample first be added to a blank microtiter plate in the location corresponding to that in the ELISA wells. The samples can then be efficiently transferred into the Antigen Wells with a 100 µL 8- or 12-channel pipettor.)

4. Cover plates with sealing tape (or place in a humid chamber), and incubate for 60 ± 1 minutes at room temperature (20 to 25°C).

5. Remove sealing tape (or place in a humid chamber), and empty the contents of the wells into a sink or a discard basin.

6. Fill each well with a gentle stream of 1X Wash Buffer solution from a wash bottle, then empty contents into a sink or a discard basin.

7. Repeat wash (step 6) an additional 2 times.

8. Tap the antigen wells vigorously to remove 1X Wash Buffer. Blot the emptied Antigen Wells face down on clean paper towels or absorbent paper to remove residual 1X Wash Buffer.

9. Dispense 100 µL Conjugate to all wells, using a 100 µL 8- or 12-channel pipettor.

10. Cover plates with sealing tape (or place in a humid chamber) and incubate for 30 ± 1 minutes at room temperature (20 to 25°C).

11. Repeat wash steps 5 through 8.

12. Pipet 100 µL of Substrate Reagent to all wells, using a 100 µL 8- or 12-channel pipettor. Begin incubation timing with the addition of Substrate Reagent to the first well. (Note: Never pour the substrate reagent into the same trough as was used for the conjugate.)

13. Incubate for 10 ± 1 minutes at room temperature (20 to 25°C).

14. Stop the reaction by adding 100 µL of Stop Reagent to all wells using a 100 µL 8- or 12-channel pipettor. Add the Stop Reagent in the same sequence and at the same pace as the Substrate was added. In antibody-positive wells, color should change from blue to yellow.

15. Gently blot the outside bottom of wells with a paper towel to remove droplets that may interfere with reading by the spectrophotometer. Do not rub with the paper towel as it may scratch the optical surface of the well. (Note: Large bubbles on the surface of the liquid may affect the OD readings.)

16. Measure the absorbance of each well within 1 hour of stopping the assay. Set the microwell spectrophotometer at a wavelength of 450 nm. Zero the instrument on the blank wells.

QUALITY CONTROL

Each plate run (or strips or wells from a single plate) must include the Cut-off Calibrator and 2 controls. If multiple plates are run, include the Cut-off Calibrator and both controls on each plate. It is recommended that until the user becomes familiar with the kit performance, all specimens, controls and the Cutoff Calibrator absorbance values.

If the Calibrator or controls are not within these parameters, patient test results should be considered invalid and the assay repeated.

INTERPRETATION OF TEST RESULTS

Report all patient results as index values relative to the Cut-off Calibrator: to calculate index values, divide specimen optical density (OD) values by the mean of the Cutoff Calibrator absorbance values.

<table>
<thead>
<tr>
<th>Index Value</th>
<th>Interpretation</th>
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<tbody>
<tr>
<td>&gt;1.10</td>
<td>Positive. An index value of &gt; 1.10 is presumptive for the presence of IgG antibodies to Hantavirus.</td>
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<tr>
<td>≥0.90 and ≤1.10</td>
<td>Equivocal. An index value of ≥ 0.90 but ≤ 1.10 is considered an equivocal result. These samples should be retested. If, on retesting, the result remains equivocal, a second sample should be drawn several weeks later and tested to identify a rise in IgG antibody titer. If the second sample is either negative or equivocal, report results as negative. Alternatively, the specimen may be tested using a different methodology such as IFA or Western Blot.</td>
</tr>
<tr>
<td>&lt;0.90</td>
<td>Negative. An index value of &lt; 0.90 indicates no IgG antibodies to Hantavirus were detected.</td>
</tr>
</tbody>
</table>

LIMITATIONS

1. All results from this and other serologies must be correlated with clinical history, epidemiological data, and other data available to the attending physician in making the diagnosis of Hantavirus infection.

2. Patients with early Hantavirus infection may test negative for IgG antibodies, since the IgG response may be undetectable until 5 weeks post-onset. Therefore, testing for IgM class antibody is recommended. If a negative test result is reported on a patient with signs and symptoms of Hantavirus infection, repeat testing for IgG and IgM antibodies on a second sample obtained 2 to 4 weeks later is recommended.

PERFORMANCE CHARACTERISTICS

For customers outside of the United States, the product performance characteristics are supplied as a separate sheet.
REFERENCES


This package insert is available in French, German, Italian, and Spanish at www.focusdx.com, and may be available in other languages from your local distributor.