Dengue Virus IgG DxSelect™
(OUS)

MATERIALS SUPPLIED

**IgG Antigen Wells, 96 wells**

12 eight-well polystyrene microwell strips on a frame. Each well is coated with equal proportions of inactivated and purified Dengue virus types 1-4. Diluted serum samples and controls are incubated in the wells to allow specific antibody 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 optical density readings are compared with reference cut-off OD readings to determine results.

**IgG Conjugate, 16 mL**

1 vial of affinity-purified and peroxidase-conjugated goat anti-human IgG (Fc fragment specific). Contains protein, buffer, and preservatives.

**IgG Detectable Control, 0.30 mL**

1 vial of human serum. Contains 0.1% sodium azide as a preservative. Requires dilution before use (see Specimen, Controls and Calibrator Preparation, below).

**Indirect Enzyme-linked immunosorbent assay (ELISA) for the qualitative detection of human IgG class antibodies to Dengue virus**

**INTENDED USE**

The Focus Diagnostics Dengue Virus IgG DxSelect™ is a qualitative assay for the detection of dengue IgG antibodies in humans. The Focus Diagnostics Dengue Virus IgG DxSelect™ can be used to establish previous exposure to dengue virus or as an epidemiological tool for dengue virus (DV) IgG sero-prevalence surveys.

**SUMMARY AND EXPLANATION OF TEST**

Dengue fever (DF) is an acute, self-limiting, viral disease that is characterized by fever, headache, body pains, rash, lymphadenopathy, and prostration. In its most severe form, dengue hemorrhagic fever (DHF), infected patients will experience severe fever and renal failure leading to the often fatal dengue shock syndrome (DSS). It is estimated that approximately 2 billion people are at risk for DF worldwide, and that over 1 million people per year are infected. This, combined with the hundreds of thousands of cases of DSS, make dengue the most important arbovirus disease in the world.

Dengue virus (DV) is a flavivirus and is closely related to the yellow fever virus, Japanese encephalitis virus and other group B Arboviruses. Members of this group possess single stranded RNA which is surrounded by an icosahedral nucleocapsid covered with a 10 nm deep lipid envelope. There are 4 strains of dengue virus, each serologically distinct. Infection with 1 strain does not protect the host from infection by the others. In fact, 1 report suggests DHF and DSS occurs most commonly in individuals that have been infected previously by another strain. The presence of circulating, non-neutralizing, cross-reactive DV antibody may act as an immune infection enhancement factor. However, non-neutralizing, cross-reactive antibodies against other non-DV flaviviruses are not associated with immune infection enhancement.

Dengue fever epidemics have been reported regularly throughout the world. The largest epidemics have occurred in the southern United States (1922 affecting over 1 million people), Australia (1925 and 1942), Greece (1927) and Japan (1942-1945). Peruvian and CDC officials have reported a major DF outbreak that occurred between March to July 1990. This epidemic centered around Lquitos, involving DV types 1 and 4, is the first laboratory confirmation of indigenous transmission of dengue in Peru.

Dengue virus can be transmitted wherever the mosquito vectors, Aedes aegypti and Aedes albopictus, are found. A. aegypti is primarily localized to tropical and subtropical Americas and is indigenous to the southern part of the United States. The primary vector for DF in Asia is A. albopictus. This mosquito has recently established itself in the United States as far north as central Illinois; however, DV transmission has not been associated with it to date.

In most patients, suspected cases of DF are most rapidly diagnosed using serological methods. Traditionally, hemagglutination inhibition and plaque reduction neutralization have been used. However, IgM capture enzyme-linked immunosorbant assays (ELISA) have become the method of choice. In most individuals the IgM response to DF is strain specific and persists as long as 90 days. IgG antibody to dengue virus has been detected in patients as long as 60 years post infection. Therefore, a single antibody determination should not be considered conclusive. However, the IgG ELISA is useful as an epidemiological tool when used to establish sero-prevalence. This information can also be valuable in studies designed to determine the role immune enhancement plays in DSS. As with most flaviviruses, IgG is highly cross-reactive among most members of the flavivirus group.

The Focus Diagnostics Dengue Virus ELISA IgG is an indirect ELISA intended for the detection of antibodies to DV types 1-4. This assay is specific for IgG and uses a horse radish peroxidase conjugate with TMB as substrate. Each antigen coated well contains equal proportions of inactivated, purified DV types 1-4. The following virus strains were used: Type 1: TH-Sman; Type 2: TH-36, Type 3:H87; and Type 4: H241.

**TEST PRINCIPLE**

In the Focus Diagnostics Dengue Virus IgG ELISA assay, the polystyrene microwells are coated with equal proportions of inactivated and purified Dengue virus types 1-4. Diluted serum samples and controls are incubated in the wells to allow specific antibody 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 optical density readings are compared with reference cut-off OD readings to determine results.

**MATERIALS SUPPLIED**

The Focus Diagnostics Dengue Virus 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</th>
<th>Code</th>
<th>Description</th>
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<tbody>
<tr>
<td>IgG Antigen Wells, 96 wells</td>
<td>EL1501</td>
<td>[table content]</td>
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<tr>
<td>IgG Conjugate, 16 mL</td>
<td>EL1504</td>
<td>[table content]</td>
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<tr>
<td>IgG Detectable Control, 0.30 mL</td>
<td>EL1511</td>
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</tbody>
</table>
9. Use proper pipetting techniques, maintaining the pipetting pattern throughout the procedure to ensure optimal and reproducible values.

11. Use only protocols described in this insert. Incubation times or temperatures other than those specified may give erroneous results.

13. ELISA plate spectrophotometer, wavelength = 450 nm

**MATERIALS REQUIRED, BUT NOT SUPPLIED**

1. Distilled water
2. 250 or 500 ml wash bottle
3. 1 L graduated cylinder
4. Test tubes for serum dilutions
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 handled accordingly.
3. The IgG Antigen Wells are produced with inactivated dengue virus antigens; however, the wells should be considered potentially infectious and handled accordingly. The stop reagent contains sulfuric acid. Do not allow to contact skin or eyes. If exposed, flush with copious amounts of water.
4. Sodium azide at a concentration of 0.1% has been added to some reagents 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. Do not substitute or mix reagents from different kit lots or from other manufacturers.
6. Use only protocols described in this insert. Incubation times or temperatures other than those specified may give erroneous results.
7. Cross-contamination of patient specimens can cause erroneous results. Add patient specimens and handle Antigen Well strips carefully to avoid mixing of sera from adjoining wells. Avoid contamination of the substrate reagent with traces of the enzyme conjugate.
8. Bacterial contamination of serum specimens or reagents can produce erroneous results. Use aseptic techniques to avoid microbial contamination.
9. Perform the assay at room temperature (approximate range 20 to 25°C).
10. 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 or calibrator 1:100 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 Sample Diluent and mix well by vortex mixing.

TEST PROCEDURE
1. Allow all reagents to warm to room temperature before use. Use 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: At 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, and incubate for 60 ± 1 minute at room temperature (20 to 25°C).
5. Remove sealing tape 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 then empty contents into a sink or a discard container.
7. Repeat wash (step 6) an additional 2 times, allow the last wash to soak for 5 minutes before decanting or aspirating.
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 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 two controls. If multiple plates are run, include the Cut-off Calibrator and two controls on each plate. It is recommended that until the user becomes familiar with the kit performance, all specimens, controls and the Cut-off Calibrator should be run in duplicate with the Cut-off Calibrator run twice for a total of 4 wells. If single wells are used, the Cut-off Calibrator should be run in triplicate. Include a minimum of 1 blank well (containing sample diluent only) for instrument calibration purposes.

The Cut-off Calibrator has been formulated to give the optimum differentiation between negative and positive sera. After subtracting out the blank wells, the target OD of the Cut-off Calibrator is approximately 0.250 OD units. Due to the varying laboratory conditions the absorbance values may differ significantly. A range of 0.100 OD units to 0.500 OD units is typical. All replicate Cut-off Calibrator OD should be within 0.100 absorbance units from the mean. Kit performance and result integrity are a function of the index values for the controls, not the absolute OD of the Cut-off Calibrator alone.

Report results as index values relative to the Cut-off Calibrator. To calculate index values, divide specimen optical density (OD) values (corrected for blank readings) by the mean of the corrected Cut-off Calibrator absorbance values.
1. The Detectable Control index value should be between 1.5 and 3.5.
2. The Non-Detectable Control index value should be less than 0.8.

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 (corrected for blank readings) by the mean of the corrected Cut-off Calibrator absorbance values.

<table>
<thead>
<tr>
<th>Index Value</th>
<th>Interpretation</th>
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<tbody>
<tr>
<td>&lt; 1.00</td>
<td>Negative. An index value of &lt; 1.00 indicates no IgG antibodies to dengue virus were detected.</td>
</tr>
<tr>
<td>&gt; 1.00</td>
<td>Positive. An index value of &gt; 1.00 is presumptive for the presence of IgG antibodies to dengue virus.</td>
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</tbody>
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LIMITATIONS
1. Dengue virus is a flavivirus. Other members of this group include Banzi virus, Bussaquara virus, luheus virus, Japanese Encephalitis virus, St. Louis Encephalitis virus, Murray Valley Encephalitis virus, Rocio virus, Kunjin virus, Spondweni virus, Sepik virus, West Nile virus, yellow fever virus, Zika virus, Wesselsbron virus, Rio Bravo virus, Central European Encephalitis virus, Russian spring-summer virus, Kyananur Forest Disease virus, Louping ill virus, Negishi virus, Omsk hemorrhagic fever virus and Powassan virus. While the majority of these viruses rarely cause disease in man, or are restricted to narrow geographical regions, cross reactions between members of Flaviviridae are common. Therefore, care should be taken in interpreting the results before a definitive diagnosis of dengue fever is made. These assays are not intended as a substitute for a physician’s clinical impressions.
2. 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 dengue fever disease.
3. Patients in the early stage of dengue virus infections may not have detectable IgG antibodies, as the IgG response may take several weeks to develop. Therefore, in the absence of detectable IgG, testing for IgM class antibody is strongly recommended. In an unpublished study, IgM was detectable in 93% of confirmed dengue fever cases at 10 days post disease onset.8
EXPECTED VALUES
Patients in the early stage of dengue fever may not have detectable IgG antibodies, as the IgG response may take several weeks to develop. IgG antibody to dengue virus has been detected in patients as long as 60 years post infection.3

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.

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