Arbovirus IFA IgM
(English)

Product Code IF0300M
Rev. H

Indirect immunofluorescent assay (IFA) for the detection of human serum IgM antibodies to Arbovirus infections

For in vitro Diagnostic Use

INTENDED USE
Focus Diagnostics’ Arbovirus IgM indirect immunofluorescent assay (IFA) is intended for the qualitative detection of human IgM antibodies to Eastern Equine Encephalitis (EEE), St. Louis Encephalitis (SLE), Western Equine Encephalitis (WEE), and California Encephalitis (CE) group viruses in a single sample or to identify sero-conversion in acute and convalescent specimens and as an aid in the diagnosis of current or past infection.

SUMMARY AND EXPLANATION OF TEST
St. Louis Encephalitis, Western Equine Encephalitis, and California Encephalitis group viruses are the major mosquito-borne viruses causing human disease in the United States. Eastern Equine Encephalitis is seen less frequently, but is also similar in its seasonal occurrence and overlaps in regional distribution. Human infection by these viruses induces an immune response and specific antibody production against the viral antigens. Since viral isolation attempts from these cases are seldom productive, the majority of human cases are diagnosed by serologic means.

Infections caused by arboviruses are commonly asymptomatic. Inapparent infections by SLE outnumber clinically apparent ones by 800:1 in patients under 10 years of age and 80:1 in those over 60 years. The most common clinically apparent manifestation is a mild undifferentiated febrile illness, usually with headache. Only a minority of infected individuals demonstrate central nervous system involvement.

Central nervous system involvement by these viruses is very similar, with the exception of a more abrupt onset and shorter, more severe, course found with EEE. Children are more often susceptible to severe disease, although SLE affects older adults more often and more severely. Initial symptoms include headache, fever, malaise, and vomiting. Convulsions are less common in SLE than in CE, WEE, and EEE. Supportive therapy and intensive nursing are required in all of these cases. Fatality rates are approximately 10% for WEE and SLE, somewhat lower for CE, and 70% for EEE.2,3

The California Encephalitis group includes several viruses known to cause human disease in the United States. LaCrosse virus infection occurs in north-central states, primarily in the upper Mississippi River Valley, and is transmitted by a forest dwelling mosquito Aedes triseriatus. The virus is maintained by transovarian transmission in the mosquito, overwintering of infected eggs, and a summer amplification in infected chipmunks and squirrels. Jamestown Canyon virus is seen in the same general area, in the upper midwestern states to New York, and Snowshoe Hare virus is found to the north in southern Canada. Other viruses within this group include the prototype California virus (2 human cases) and Trivittatus virus (possible human pathogen).3

Eastern equine encephalitis virus (EEE) and western equine encephalitis virus (WEE) are within the Alphavirus group. Caliseta melanura is the principle epizootic mosquito vector of EEE, which is a low prevalence cause of human disease in the eastern and Gulf Coast states. It is maintained by a cycle of mosquito/wild bird transmission, peaking in the summer and early fall, when man may become an adventitious host. WEE is generally seen in the western and midwestern states, recently (1964-1984) North Dakota and South Dakota, and is transmitted by the Culex tarsalis mosquito. Its cycle also includes wild birds, with occasional spread to horses and man.2

St. Louis encephalitis virus (SLE) is a member of the Flavivirus group of Arboviruses. The family Flavivirus is a large, closely related group containing 23 members. The family’s most notable agents include SLE, dengue fever viruses, Japanese encephalitis virus, yellow fever virus, and the Russian spring-summer encephalitis virus. Serological cross-reaction is common between SLE and other Flaviviruses, however, the extent and degree of cross-reaction varies.

Most Flavivirus infections in the United States are caused by SLE. Human infection occurs both as urban epidemics and sporadic rural cases throughout the U.S. Principle vectors are thought to be Culex nigripalpus in Florida, C. pipiens-quinquesfasciatus in the eastern U.S., and C. tarsalis in the western states.1 A 1984-1985 urban epidemic in Southern California pointed toward the possible involvement of C. p-quinquesfasciatus and C. peus as urban vectors of SLE.1 The urban SLE cycle involves not only the wild, primarily passerine birds for amplification (rural cycle), but also ducks, chickens and other domestic or urban bird species.3,4

In patients infected with these or related viruses, IgM antibody is generally detectable within 1 to 3 weeks of onset, peaking within 1 to 2 months, and declining slowly thereafter. IgM class antibody is also reliably detected within 1 to 3 weeks of onset, peaking and rapidly declining within 3 months. Both IgG and IgM antibody status should be determined at the onset of symptoms.

The Focus Diagnostics Arbovirus IgM IFA assay utilizes Vero cells virally infected with EEE, New Jersey 60 strain; WEE, Fleming strain; SLE, TBH-28 strain and CE, LaCrosse strain, group viruses, in each well. Each slide contains 8 wells; on each well are 4 individual antigen spots.

TEST PRINCIPLE
The Indirect Immunofluorescent Antibody (IFA) assay is a 2-stage “sandwich” procedure. In the first stage, patient serum is diluted in Pretreatment Diluent. The Pretreatment Diluent is a buffered isotonic solution containing anti-human IgG which removes both free and complexed IgG from the sample. The diluted (pretreated) sera are added to appropriate slide wells in contact with the substrate, and incubated. Following incubation, the slide is washed in phosphate buffered saline which removes unbound serum antibodies. In the second stage, each antigen well is overlaid with fluorescein-labeled antibody to IgM. The slide is incubated allowing antigen-antibody complexes to react with the fluorescein-labeled anti-IgM. After the slide is washed, dried, and mounted, it is examined using fluorescence microscopy. Positive reactions appear as cells exhibiting bright apple-green cytoplasmic fluorescence against a background of red negative control cells. Semi-quantitative endpoint titers are obtained by testing serial dilutions of positive specimens.
MATERIAL SUPPLIED
Focus Diagnostics’ Test kit contains sufficient materials to perform 80 determinations.

Arbovirus Substrate Slides
Ten slides of 8 wells each. Each well contains 4 spots: 4 individual antigen spots consisting of fixed Vero cells infected with an Arbovirus group virus. Store sealed slide packets at 2 to 8°C. The sealed slides are stable until date stated on the slide packet labels. To avoid condensation, allow the slides to warm to room temperature before opening the sealed packets.

IgM Conjugate-Dual Species, 3.5 mL
One vial of fluorescein-labeled goat anti-human IgM, mu-chain specific, blended with a fluorescein-labeled goat anti-mouse IgG. The anti-mouse IgG has been standardized to provide specific antigen control. Contains Evan’s Blue counterstain, protein stabilizer and preservatives. Ready for use. Stable at 2 to 8°C until the date stated on the label. Do not use if cloudiness, discoloration or other indications of bacterial contamination are present. Allow to warm to room temperature before use.

Arbovirus Polyvalent Positive Control, 0.25 mL
One vial of mouse ascites bottled at screening dilution, with non-azole preservatives. Stable at 2 to 8°C until the expiration date stated on the label. Do not use if cloudiness, discoloration or other indications of bacterial contamination are present. Allow to warm to room temperature before use. Do not pretreat or dilute.

Arbovirus Negative Control, 0.25 mL
One vial of human serum bottled at screening dilution. Contains non-azole preservatives. Stable at 2 to 8°C until the expiration date stated on the label. Do not use if cloudiness, discoloration or other indications of bacterial contamination are present. Allow to warm to room temperature before use. Do not pretreat or dilute.

Mounting Medium, 2.5 mL
One vial of PBS-Buffered glycerol at a pH of 7.2 ± 0.1. Contains non-azole preservatives. Mounting Medium is stable at 2 to 8°C until the expiration date stated on the bottle label. Allow to warm to room temperature before use.

IgM Pretreatment Diluent, 12 mL
One vial of PBS containing goat monospecific antiserum to human IgG, with non-azole preservatives. Stable at 2 to 8°C until the expiration date stated on the bottle label. Allow to warm to room temperature before using.

PBS
One vial of phosphate buffered saline (PBS) powder. Reconstitute with 1 liter distilled (or purified) water. The reconstituted solution is a 0.01 M buffer at pH 7.2 ± 0.1. Before and after reconstitution, store PBS at 2 to 8°C. Allow to warm to room temperature before use. Do not use if cloudiness, discoloration, or other indications of bacterial contamination are present.

MATERIALS REQUIRED, BUT NOT SUPPLIED
1. 24 x 50 mm coverslips
2. Test tubes and rack, microcentrifuge tubes or microtiter plate for serum dilutions
3. Clinical centrifuge
4. 35 to 37°C incubator or water bath for slide incubation
5. 2 to 8°C refrigerator
6. ≤ −20°C freezer
7. Plastic wash bottle
8. Calibrated pipets or piston-type pipettors with disposable tips
9. Coplin jars or slide staining dish with slide holder
10. Clean volumetric flask or graduated cylinder, 1 liter
11. Humid chamber for incubation of slides
12. Distilled or purified water
13. Timer
14. Absorbent paper for blotting slides
15. Fluorescence microscope, recommended parameters
   - Excitation Filter 470-490 nm
   - Barrier Filter 520-560 nm
   - Light Source HBO 100W, mercury
   - Objective 20-40X, fluorescence quality, high dry

WARNINGS AND PRECAUTIONS
1. This kit is for in vitro diagnostic use only.
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 

3. Evan’s Blue is a carcinogen; however this product is below the reportable threshold (less than 0.1%) 
4. 
5. Do not substitute reagents from different kit lots or manufacturers. 
6. Use only protocols described in this insert. 
7. Cross-contamination of patient specimens on a slide can cause erroneous results. Add patient specimens and handle slide carefully to avoid mixing of sera from adjoining wells. 
8. Bacterial contamination of serum specimens or reagents can produce erroneous results. Use aseptic techniques to avoid microbial contamination. 
9. Reporting of Positives - Arboviral infection is a reportable disease in most states. Patient sera that are considered positive and suggestive of recent or current infection should be reported to state public health agencies. Information gained by public health experts from reporting laboratories not only increases our knowledge of the epidemiology of these viruses, but helps direct the vector-control efforts that can prevent large scale epidemics. For information and consultation please contact your local health department. 
10. Mounting Medium contains 30 to 60% glycerol which may cause irritation upon inhalation or skin contact. Upon inhalation or contact, first aid measures should be taken. 

**SHELF LIFE AND HANDLING**

1. Kits are stable through the end of the month indicated in the 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. 

**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, hemolyzed, heat-inactivated or contaminated sera may cause erroneous results; therefore, their use should be avoided. Acute specimens should be drawn at illness onset; convalescent specimens should be obtained 1 to 2 weeks later. 

**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 Pretreatment**

Serum IgG antibody may compete with IgM resulting in false negatives. False positives may result when rheumatoid factor (complexed IgG) is present in the specimen. Therefore, pretreatment of the serum to remove free and complexed IgG antibody is strongly recommended. 

Prepare 1:16 screening dilutions of patient sera as follows: mix 5 µL of patient serum with 75 µL IgM Pretreatment Diluent in microtiter plate; and, allow at least 5 minutes for immunoprecipitation reaction to occur. The diluted sample may be used as is, or may be centrifuged to clear precipitate from serum. The precipitate will not interfere with the assay. 

To determine endpoint titers, use PBS to serially dilute the pretreated specimens. 

**TEST PROCEDURE**

1. Remove slides from cold storage. To avoid condensation, allow slides to reach room temperature before opening packets. 
2. Apply 25 µL of Arbovirus Polyclonal Positive Control, as bottled, to the appropriate slide well. Do not dilute. 
3. Apply 25 µL of Negative Control, as bottled, to the appropriate well. Do not dilute. 
4. For each patient sample to be tested, add approximately 25 µL of the diluted sample (see Specimen Preparation, above) to an appropriate slide well. Make notations to later identify each well when reading the results. 
5. Incubate slide(s) in a humid chamber for 90 ± 2 minutes at 35 to 37°C. 
6. Remove slides from the humid chamber and gently rinse each slide with a stream of PBS. Do not aim stream of PBS directly at the slide wells. Rinse 1 row at a time to avoid mixing of specimens. Wash slides by submersing the rinsed slides into Coplin or slide staining jars containing PBS for 10 minutes. 
7. Dip the washed slides briefly in distilled or purified water and allow them to air dry. 
8. Add approximately 25 µL IgM Conjugate-Dual Species to each well. 
9. Incubate slides in a humid chamber for 30 ± 2 minutes at 35 to 37°C. 
10. Repeat wash steps 6 and 7. 
11. Place a few drops of Mounting Medium on the slide and cover with a 24 x 50 mm coverslip. Remove any air bubbles and excess Mounting Medium with absorbent paper. 
12. View wells at a final magnification of 400X on a properly equipped fluorescence microscope. For optimum fluorescence, read slides the same day the assay is performed. If this is not possible, store in the dark at 2 to 8°C up to 24 hours. 

**QUALITY CONTROL**

Each run (each time a slide, or group of slides, is processed) should include both Positive and Negative Controls. The Arbovirus Polyclonal Positive Control has been formulated to assess the antigen spot reactivity with murine serum and IgM conjugate. The laboratory should furnish its own positive human serum control(s) to assess the reactivity of anti-human IgM conjugate. 

1. The Arbovirus Polyclonal Positive Control should exhibit 3 to 4+ cytoplasmic fluorescence in 5% to 25% of the cells in all 4 antigen spots. As this control is NOT human serum, its use is intended to give assurance of antigen quality for each of the 4 antigens. 
2. The Negative Control should exhibit negligible reactivity with all spots. 

If controls do not exhibit these results, patient test results should be considered invalid, and the assay repeated.
**INTERPRETATION OF TEST RESULTS**

Microscope optics, light source condition and type will determine overall fluorescent intensity and endpoint titers. Read control wells first during every run to ensure correct interpretation.

**Reading the Slides**

Positive cells will exhibit a distinct cytoplasmic fluorescence in the 5% to 25% reactive population and are easily differentiated from the control (uninfected) cell population included in each antigen spot.

Read fluorescent intensity of the cells, and grade the fluorescence as follows:

<table>
<thead>
<tr>
<th>Fluorescence</th>
<th>Description</th>
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<tbody>
<tr>
<td>2 to 4+</td>
<td>Moderate to intense apple-green cytoplasmic fluorescence.</td>
</tr>
<tr>
<td>1+</td>
<td>Definite, but dim cytoplasmic fluorescence.</td>
</tr>
<tr>
<td>Negative</td>
<td>No fluorescence or fluorescence equal to that observed in the Negative Control well.</td>
</tr>
</tbody>
</table>

Notes: Cytoplasmic fluorescence in LaCrosse-infected cells (CE spot) may have a distinct speckled “starry night” appearance, in contrast to the more homogenous cytoplasm of SLE, EEE, or WEE-infected cells.

If specific IgM is present, fluorescence is often associated with membrane staining. 13

**Interpretation of Patient Specimens**

The reciprocal of the highest serum dilution that gives definite (1+) apple-green cytoplasmic fluorescence in 5% to 25% of the cells is termed the serum endpoint titer.

<table>
<thead>
<tr>
<th>Endpoint titer</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥ 1:16</td>
<td>Positive for IgM Arbovirus antibody. Indicative of recent or active infection by the appropriate group virus.</td>
</tr>
<tr>
<td>&lt; 1:16</td>
<td>Negative for Arbovirus IgM antibody.</td>
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</table>

The IFA procedure using infected cell preparations is a very sensitive test, partially due to the multiplicity of antigens present. The trait, however, increases the degree of cross-reactivity of related arboviruses and must be considered in the interpretation of results.

**Non-specific Fluorescence**

Some sera will give weak cytoplasmic staining in all or a percentage of cells in all 4 antigen spots. If this staining is too intense to permit a confident reading for arboviral infection (i.e., 1+ or more), the serum must be adsorbed with packed Vero cells (or other related cell) prior to retesting. If this treatment is not available, the test must be run by an alternate method or referred to a reference center for testing.

Multiple positive spots are an indication of a non-specific serum reaction, since all 4 antigens were derived from the same cell line (Vero). Usually the non-specific reactivity will be low titered and can easily be diluted out.

**LIMITATIONS**

1. All results from this and other serologies must be correlated with clinical history and other data available to the attending physician.
2. Samples obtained too early during primary infection within 2 weeks after onset may not contain detectable antibodies. If Arboviral infection is suspected, a second sample should be obtained 10 to 21 days later and tested in parallel with the original sample.
3. In the absence of overwhelming epidemiological data, a specific diagnosis depends upon the culture, isolation and identification of the disease causing virus. These procedures are difficult and may require use of either a BSL II or III containment facility.
4. Since cross-reactions with dengue fever virus do occur with SLE antigens, this kit cannot be relied upon for the definitive diagnosis or to categorically rule out infection by dengue fever virus.
5. Due to serologic cross-reactivity among flaviviruses, a history of previous exposure to or infection with dengue or yellow fever vaccination must be considered in interpreting serologic results.
6. In many cases, antibody to other Flaviviruses will cross-react with the SLE viral antigen and, therefore, cannot be differentiated further. The specific virus responsible for such a titer must be deduced by the travel history of the patient, along with available medical and epidemiological data, unless the virus can be isolated.
7. EEE and WEE viruses, both related within the alphavirus group (Group A), will show cross-reactivity, although much less completely than the Flaviviruses. Initial infection by 1 of these Group A viruses will show a specific reaction at least 8-fold higher than titers against any other Group A viruses. A subsequent infection by another virus within this group, however, will boost the titer against the initial infecting virus (anamnestic response) and make a specific diagnosis difficult.
8. LaCrosse virus is related to viruses within the CE Group and, generally, is reactive with antibody to other viruses within this group.
9. WEE and EEE viruses cross-react with high titered or anamnestic responses to Group A or Alphavirus antigens.
10. Rheumatoid factor (RF) may result in false-positive IgM responses in sera that contain specific IgG. Patient sera should be treated with an IgG immunoprecipitating agent to remove RF and then be re-tested to eliminate false positives.
11. Infection with certain arboviruses, especially the flaviviruses, can induce persistent IgM production, detection of IgM is not always evidence of etiology. 13

**EXPECTED VALUES**

Individuals not infected with a virus in this panel or an immunologically related virus do not demonstrate detectable antibody by IFA.

In the United States it is unusual for any patient serum to show positive reactions to more than one of the arboviral antigens. High titered or anamnestic responses to Group A or Alphavirus antigens are the exception, since WEE and EEE antibodies will show a noticeable cross-reactivity.

Infections with arboviruses can occur at any age. The age distribution depends on the degree of exposure to the particular transmitting arthropod relating to age, sex, and occupational, vocational, and recreational habits of the individuals. Once humans have been infected, the severity of the host response may be influenced by age: WEE tends to produce the most severe clinical infections in young persons and SLE in older persons, serious LaCrosse infections primarily involve children, especially boys. Adult males exposed to LaCrosse have high prevalence rates of antibody but usually show no serious illness. Infection among males is primarily due to working conditions and sports activity taking place where the vector is present. 14

**SPECIFIC PERFORMANCE CHARACTERISTICS**

**Sensitivity**

California Encephalitis (CE) Group: The sensitivity of the Focus Diagnostics test in detecting California group infection was compared with a state department of health’s in-house IFA IgM test. Of 24 sera compared, 19 were found to be positive on both assay systems. 4 of the remaining sera were found negative by both assays and the remaining discrepant was found positive by the health department’s assay and negative by Focus Diagnostics.
St. Louis Encephalitis Group (SLE): The sensitivity and specificity of the Focus Diagnostics test in detecting SLE viral infection was compared with another state department of health’s in-house IFA IgM test. Between 1984 and 1986, 24 specimens from 18 patients were tested by both methods in confirming SLE cases in California. Of the 24 sera compared, 20 were positive and 1 was negative by both assays. 2 of the remaining 3 were found negative by the health department’s IFA test and positive by Focus Diagnostics and the remaining discrepant was negative by Focus Diagnostics IFA and positive by the health department’s IFA test.

Eastern Equine Encephalitis (EEE) and Western Equine Encephalitis (WEE): Comparisons with EEE and WEE are hampered by the scarcity of reported cases in the United States. 9 sera containing IgM antibody to EEE and 2 sera containing IgM antibody to WEE were obtained from the Centers for Disease Control (CDC). The CDC utilized an in-house IgM ELISA test in determining the antibody status of the sera. 8 out of 9 EEE sera and both of the WEE sera were found positive on the Focus Diagnostics Arbovirus IgM IFA kit.

Specificity
CE, SLE, EEE, and WEE: Of 714 sera submitted for various testing to a reference laboratory in California (non-endemic), only 5 patients were found with IgG antibody (no IgM) to LaCrosse virus. All but 1 of these patients were from or had traveled in endemic regions, with the exception being a CDC confirmed yellow fever infected individual. None of the 714 sera had IgG or IgM antibody to SLE, WEE or EEE.
Sera from a group of normal laboratory workers in Southern California were assayed as another index of specificity. A total of 48 sera were tested and found to be negative (< 1:16) on all 4 antigens.

Cross-reactivity Studies
Dengue Fever Sera: Sera from 17 individuals exhibiting IgG and IgM antibody titer to Dengue Fever were tested on the Focus Diagnostics Arbovirus IgM IFA assay. 5 of the 17 sera were positive on the SLE antigen spot. Since there is cross-reactivity among the flavivirus family, this assay may not be relied upon for the definitive diagnosis or to categorically rule out infection by dengue fever virus.

Yellow Fever Vaccinated Individuals: Sera from 14 Yellow Fever vaccinated individuals were tested on the Focus Diagnostics Arbovirus IgM IFA assay. 1 of 14 exhibited low levels of antibody to SLE. Although the reactions are infrequent and of low intensity, this factor should be considered in assessing the immune status of the individual.

REFERENCES

ORDERING INFORMATION
Telephone: (562) 240-6500 (International)
Fax: (562) 240-6510

TECHNICAL ASSISTANCE
Telephone: (800) 838-4548 (U.S.A. only)
Fax: (562) 240-6526

Visit our web site at: www.focusdx.com