Rickettsia IFA IgM
(English)

Product Code IF0100M
Rev. H

Indirect immunofluorescent assay (IFA) for the
detection of human IgM antibodies to Rickettsia
antigens

For in vitro Diagnostic Use

INTENDED USE
The Focus Diagnostics Rickettsia Indirect Immunofluorescence Antibody (IFA) IgM test is intended for the detection and semi-quantitation of human IgM class antibodies to Spotted Fever and Typhus Fever group Rickettsia, as an in vitro aid in the diagnosis of diseases caused by these organisms.

SUMMARY AND EXPLANATION OF TEST
Rickettsiae are highly fastidious rod-shaped obligate intracellular parasites. 2 major bio-groups within the genus Rickettsia are the Spotted Fever group and the Typhus Fever group.

Human disease in the western hemisphere caused by Spotted Fever group organisms includes Rocky Mountain spotted fever (Rickettsia rickettsii) and rickettsial pox (Rickettsia akari). Rocky Mountain spotted fever is found throughout the western hemisphere, although primarily east of the Rocky Mountains, and is transmitted principally by the wood tick (Dermacentor andersoni) and the dog tick (Dermacentor variabilis). Rickettsial pox is found in the northeastern states of the United States and is transmitted by a mite, Allodermanyssus sanguineus, harbored by house mice. 2

Typhus Fever group organisms cause 3 main diseases with worldwide distribution. Marine typhus (endemic typhus) is caused by Rickettsia typhi and is transmitted by the bite of fleas commonly found on rats and domestic animals. 3 Epidemic typhus (louse-borne typhus) is caused by Rickettsia prowazekii and is transmitted by the body louse, squirrel louse, and flea. Brill-Zinsser disease (sporadic or recrudescent typhus) is caused by a reactivation of latent R. prowazekii infection occurring years after primary epidemic typhus. 4

Rocky Mountain spotted fever is classically characterized by a sudden onset of fever, chills, severe headache, and myalgia. In a recent study; however, onset was described as abrupt or very abrupt in only 42% of patients, with 35% of the patients describing a gradual onset. 5 A characteristic macular rash develops on the extremities by the third to fifth day of illness, spreading soon to the trunk. Approximately 50% of patients also show a petechial-hemorrhagic rash as well. 6

Rickettsial pox is initially noted by a papule developing at the site of the mite bite within 7 to 10 days. This develops over several days into a vesicle filled with fluid, while the patient begins to experience fever, chills, headache, sore throat, cough, photophobia, and myalgia. A characteristic maculopapular rash also occurs with the onset of constitutional symptoms, developing soon into vesicles. Mortality is negligible in the U.S. to date.

Marine typhus has an incubation period of 1 to 2 weeks, followed by the generally sudden onset of fever, chills, headache, and myalgia characteristic of most rickettsial diseases. A central macular rash soon develops, becoming maculopapular within several days. Epidemic typhus has a somewhat shorter incubation period, but also begins suddenly with severe headache, fever, and myalgia. The rash is macular, spreading from the trunk outward to the extremities. Primary epidemic typhus and Brill-Zinsser disease are almost always acquired outside the U.S.

The Focus Diagnostics Rickettsia IFA IgM assay utilizes inactivated R. rickettsii antigen and R. typhi antigen. Each slide contains 8 wells: on each well are 2 individual antigen spots.

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

MATERIALS SUPPLIED
Focus Diagnostics Rickettsia IFA IgM Test kit contains sufficient materials to perform 80 determinations.

Rickettsia Substrate Slides
10 slides of 8 wells each. Each well contains 2 individual antigen spots: 1 inactivated Rickettsia rickettsii antigen spot and 1 inactivated Rickettsia typhi antigen spot. Egg yolk sac suspension is utilized in slide preparation to increase adherence of the Rickettsial bodies and to produce a background for microscopic reading. Store sealed slide packets at 2 to 8°C. The sealed slides are stable until the date stated on the slide packet labels. To avoid condensation, allow the slides to warm to room temperature before opening the sealed packets.

Note: The majority of fluorescent microscopes invert the image of the slide. When viewed through the microscope the antigens will appear in reverse order as below.
IgM Conjugate, 2.5 mL
1 vial of affinity-purified and fluorescein-labeled goat anti-human IgM, mu-chain specific. Contains Evan’s Blue counterstain with protein stabilizer and preservatives. The conjugate is stable at 2 to 8°C until the date stated on the label. Repeated freezing and thawing is deleterious and should be avoided.

Spotted Fever group IgM Positive Control, 0.25 mL
1 vial of human serum bottled at screening dilution. Contains preservatives. The conjugate is stable until the expiration date stated on the label when stored at 2 to 8°C. 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. Repeated freezing and thawing is deleterious and should be avoided.

Typhus Fever group IgM Positive Control, 0.25 mL
1 vial of human serum bottled at screening dilution. Contains preservatives. The conjugate is stable until the expiration date stated on the label, when stored at 2 to 8°C. 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. Repeated freezing and thawing is deleterious and should be avoided.

Negative Control, 0.25 mL
1 vial of human serum bottled at screening dilution (representing a screening dilution of 1:64). Contains preservatives. The conjugate is stable until the expiration date stated on the label, when stored at 2 to 8°C. 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. Repeated freezing and thawing is deleterious and should be avoided.

IgM Pretreatment Diluent, 5 mL
2 vials containing chicken egg yolk sac suspension and goat anti-human IgG (heavy chain-specific) serum, with preservatives. The control is stable until the expiration date stated on the bottle label, when stored at 2 to 8°C. Allow to warm to room temperature before use.

Mounting Medium, 2.5 mL
1 dropper bottle containing PBS buffered glycerol at a pH of 7.2 ± 0.1. Contains preservatives. The control is stable until the expiration date stated on the bottle label, when stored at 2 to 8°C. Allow to reach room temperature before use.

PBS
1 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 of 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. Plastic wash bottle
7. Calibrated pipets or piston-type pipettors with disposable tips
8. Coplin jars or slide staining dish with slide holder
9. Clean volumetric flask or graduated cylinder, 1 liter
10. Humid chamber for incubation of slides
11. Distilled or purified water
12. Timer
13. Absorbent paper for blotting slides
14. 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

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.

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. The substrate slides contain inactivated R. typhi and R. rickettsii. However, the slides should be considered potentially infectious and handled accordingly.
4. Evan’s Blue is a carcinogen; however this product is below the reportable threshold (less than 0.1%).
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 on a slide can cause erroneous results. Add patient specimens and handle slides 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. 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.

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, 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\(^1\). 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. Freeze-thaw damage can result if specimens are frozen in self-defrosting freezers. 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 1:4 dilutions of patient sera by mixing 1 part patient serum with 3 parts reconstituted PBS. Further dilute the 1:4 serum dilution in IgM Pretreatment Diluent to yield a final screening dilution of 1:64, by mixing gently but thoroughly 1 part serum dilution with 15 parts IgM Pretreatment Diluent. Incubate this final screening dilution for at least 15 minutes at room temperature. After incubation, clear the serum by centrifugation.

Where it is necessary to determine endpoint titers, use IgM Pretreatment Diluent to serially dilute the pretreated final screening dilution.

TEST PROCEDURE
1. Remove slides from cold storage. To avoid condensation, allow slides to reach room temperature before opening slide packets.
2. Apply 25 µL of Spotted Fever group Positive Control, as bottled at screening dilution, to the appropriate slide well. Use PBS to serially dilute the Positive Control 32-fold beyond the bottled dilution. Apply 25 µL of each serial dilution to an appropriate slide well.
3. Apply 25 µL of Typhus Fever group Positive Control, as bottled at screening dilution, to the appropriate slide well. Use PBS to serially dilute the Positive Control to 32-fold beyond the bottled dilution. Apply 25 µL of each serial dilution to an appropriate slide well.
4. Apply 25 µL of Negative Control, as bottled at screening dilution, to the appropriate well. Do not dilute the Negative Control.
5. For each patient sample to be tested, add approximately 25 µL of the diluted/pre-treated sample (see Specimen Pretreatment, above) to an appropriate slide well. Make notations to later identify each well when reading the results.
6. Incubate slide(s) in a humid chamber for 30 ± 2 minutes at 35 to 37°C.
7. Remove slides from the humid chamber and gently rinse each slide with a stream of PBS. Do not aim the 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.
8. Dip the washed slides briefly in distilled or purified water, and allow the slides to air dry.
9. Add approximately 25 µL IgM Conjugate to each slide well.
10. Incubate slides in a humid chamber for 30 ± 2 minutes at 35 to 37°C.
11. Repeat wash steps 7 and 8.
12. 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.
13. View wells at a final magnification of 200X on a properly equipped fluorescence microscope. Higher magnifications may be used and compared with positive and negative controls for better definition. For optimum fluorescence, read slides the same day the assay is performed. If this is not possible, store 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.

1. The Spotted Fever group IgM Positive Control titer should endpoint (1+ fluorescence) at 8-fold beyond the bottled dilution against the \( R. rickettsii \) antigen and should have negligible reactivity with the \( R. typhi \) antigen. However, due to differing laboratory conditions, including equipment, the endpoint against the \( R. rickettsii \) antigen may range from 4- to 16-fold beyond the bottled dilution.
2. The Typhus Fever group IgM Positive Control titer should endpoint (1+ fluorescence) at 8-fold beyond the bottled dilution against the \( R. typhi \) antigen and should have negligible reactivity with the \( R. rickettsii \) antigen. However, due to differing laboratory conditions, including equipment, the endpoint against the \( R. typhi \) antigen may range from 4- to 16-fold beyond the bottled dilution.
3. The Negative Control should exhibit negligible reactivity to all spots. Fluorescence that does not match the morphology and distribution of the positive control is considered negative.

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
Read the fluorescent intensity of the Rickettsial bodies on each spot, and grade the fluorescence as follows:

<table>
<thead>
<tr>
<th>Fluorescence Grade</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 to 4+</td>
<td>Moderate to intense apple-green fluorescence of the Rickettsial bodies.</td>
</tr>
<tr>
<td>1+</td>
<td>Definite, but dim fluorescence equivalent to that observed for the Positive Control at its reference endpoint titer.</td>
</tr>
<tr>
<td>Negative</td>
<td>No fluorescence or fluorescence equal to that observed in the Negative Control well (or less than endpoint titer).</td>
</tr>
</tbody>
</table>

Interpreting the Patient Specimen Results
The reciprocal of the highest serum dilution that exhibits definite (1+) apple-green fluorescence of the Rickettsial bodies is termed the serum endpoint titer.

<table>
<thead>
<tr>
<th>IgM Titer Range</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥1:64</td>
<td>IgM titers of 1:64 and greater are considered presumptive evidence of recent or current infection by organisms of the appropriate Rickettsial antigen group.</td>
</tr>
<tr>
<td>&lt;1:64</td>
<td>IgM titers less than 1:64 suggest that the patient does not have an acute Rickettsial infection.</td>
</tr>
</tbody>
</table>

For paired sera: A 4-fold change in IgM titer is considered diagnostic.

Group Specificity
Antibody reactivity to the \( R. rickettsii \) antigen should be considered Spotted Fever group reactive. Other organisms within the group include \( R. akari \), \( R. conorii \), \( R. australis \), and \( R. sibirica \). Infections by any of these species will induce the production of antibody reactive with \( R. rickettsii \).\(^2\)

Antibody reactivity with the \( R. typhi \) antigen should be considered Typhus Fever group reactive, as infection by \( R. prowazekii \) also induces the production of antibody reactive with \( R. typhi \).\(^2\)
LIMITATIONS
1. Cross-reactivity within the Spotted Fever group or the Typhus Fever group precludes the speciation of the infecting Rickettsia by this procedure. Sera reactive with *R. rickettsii* must be termed “Spotted Fever group positive”, while those reactive with *R. typhi* are termed “Typhus Fever group positive”.
2. Spotted Fever and Typhus Fever intra-group cross-reactivity is weak: cross-reactive titers are at least 16-fold lower than group specific titers.
3. Antibody is variably absent for 1 to 2 weeks after onset of symptoms and an initial negative titer should not be used to exclude the diagnosis of Rickettsial disease. A second serum specimen should be obtained 1 to 2 weeks later to establish the diagnosis in such patients.1
4. Some patients may maintain a long-term IgM titer, with or without IgG. It is important to check for a changing IgM titer 1 to 2 weeks following the acute specimen.
5. IgM titers must be interpreted with caution, especially in the absence of IgG. The cases should be further evaluated clinically or serologically, by testing acute and convalescent serum in parallel to demonstrate a 4-fold or greater change in IgG or IgM titer.

EXPECTED VALUES
Individuals that have not been infected with a member of either the Spotted Fever or the Typhus Fever group Rickettsia do not demonstrate detectable antibody (≥ 1:64) by IFA. IgM class antibody is transiently detected within 1 to 2 weeks of onset of symptoms, usually declining rapidly within 3 months following prompt antibiotic treatment. These levels will also be elevated for an extended period with relapse, prior immunization, or delayed antibiotic treatment.1,2 Some patients with Brill-Zinsser disease will not demonstrate specific IgM class antibodies.3

SPECIFIC PERFORMANCE CHARACTERISTICS
The Focus Diagnostics Rickettsia IFA IgM kit was compared with both IFA and CF tests from the Centers for Disease Control (CDC) using a panel of 32 coded sera. The IgM test demonstrated 100% correlation of positives, negatives, and endpoint titer (within 1 serial dilution) when the IFA procedure was compared both for Spotted Fever group and Typhus Fever group. There was also complete correlation for positives and negatives compared with the CDC CF test.

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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