INTENDED USE
Focus Diagnostics Q Fever IFA IgG assay is intended for use in the detection and semi-quantitation of the human IgG antibody response to phase I and phase II Coxiella burnetii antigens and as an aid in the diagnosis of Q fever.

SUMMARY AND EXPLANATION OF TEST
Coxiella burnetii, the organism which causes Q fever, is an obligate intracellular parasite (family Rickettsiaceae), with worldwide distribution. It is unique within this group of organisms in that it undergoes a phase transition, similar to the smooth-rough liposaccharide transitions seen in the enteric gram-negative bacteria. Virulent isolates are of the phase I type, while serial passage in eggs or tissue culture is required for selection of the avirulent phase II transition. These phases are serologically distinguishable and quite useful in the serodiagnosis of both acute and chronic C. burnetii infections. Important reservoirs of C. burnetii include dairy cattle, sheep, and goats. Recent evidence has also implicated rodents as reservoirs, as well as cats that feed on them. Infection in these animals is enzootic and nearly always inapparent. The rickettsia infect humans via inhalation of contaminated dust particles and aerosols, and via handling and ingestion of infected meat and milk.

Q fever has an incubation period of approximately 2 to 3 weeks. Acute symptoms are onset of fever, peaking in 2 to 4 days near 40°C and gradually declining for 1 to 2 weeks, accompanied by malaise, anorexia, myalgia, weakness, and intense headache. Liver damage with hepatomegaly occurs, often leading to hepatic granulomas when treatment is delayed or diagnosis is missed. Q fever may also manifest as pneumonitis or bronchitis. Endocarditis is an uncommon sequel, following a protracted latent phase and requiring pre-existing heart valve damage. The laboratory would perform the Q Fever IgM and IgG IFA tests concurrently to determine the presence of phase I/phase II antibody. The presence of IgG to phase II antigen is indicative of acute disease. The presence of IgG to phase I antigen is indicative of chronic disease.

During the acute disease, the IgG titers to phase II antigen are greater than IgG phase I antigen. During the chronic disease, the IgG titers to phase I antigen are greater than or equal to phase II antigen. In early acute sera, IgM phase II titers may be higher and appear sooner than IgG phase II titers.

<table>
<thead>
<tr>
<th>Titer to phase Antigen</th>
<th>Disease Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase II &gt; Phase I</td>
<td>Acute</td>
</tr>
<tr>
<td>Phase II ≤ Phase I</td>
<td>Chronic or Convalescent</td>
</tr>
</tbody>
</table>

The Focus Diagnostics Q Fever IFA IgG assay utilizes C. burnetii (Nine Mile strain). Each slide contains 8 wells; and, each well contains 2 individual spots: a C. burnetii phase I antigen spot and a C. burnetii phase II antigen spot. The C. burnetii organisms have been diluted in a yolk sac matrix to add contrast to the background.

TEST PRINCIPLE
The Indirect Immunofluorescent Antibody (IFA) assay is a 2-stage “sandwich” procedure. In the first stage, the patient sera is diluted in PBS, 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 IgG. The slide is incubated allowing antigen-antibody complexes to react with the fluorescein-labeled anti-IgG. After the slide is washed, dried, and mounted, it is examined using fluorescence microscopy. Positive reactions appear as bright apple-green fluorescent rickettsia with a background matrix of yolk sac. Semi-quantitative endpoint titers are obtained by testing serial dilutions of positive specimens.

MATERIALS SUPPLIED
Focus Diagnostics Q Fever IFA IgG Test kit contains sufficient materials to perform 80 determinations.

<table>
<thead>
<tr>
<th>Q Fever Substrate Slides</th>
<th>REF</th>
<th>IF0201</th>
<th>Ag</th>
</tr>
</thead>
<tbody>
<tr>
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</tr>
</tbody>
</table>

10 slides of 8 wells each. Each well contains 2 individual antigen spots: 1 inactivated C. burnetii phase I antigen spot and 1 inactivated C. burnetii phase II antigen spot. Egg yolk sac suspension is utilized in slide preparation to increase adherence of the C. burnetii bodies and to produce a background for microscopic reading. The sealed slides are stable until the date stated on the slide packet labels when stored at 2 to 8°C. 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.

<table>
<thead>
<tr>
<th>IgG Conjugate, 2.5 mL</th>
<th>REF</th>
<th>IF0001</th>
<th>CONJ</th>
<th>IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 vial of affinity-purified and fluorescein-labeled goat anti-human IgG, gamma-chain specific. Contains Evan’s Blue counterstain, protein stabilizer and preservatives. Ready for use. Stable at 2 to 8°C until the expiration date stated on the label.</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>
Q Fever IgG Positive Control, 0.25 mL
1 vial of human serum bottled at screening dilution. Contains 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. Repeated freezing and thawing is deleterious and should be avoided.

Q Fever Negative Control, 0.25 mL
1 vial of human serum bottled at screening dilution. Contains 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. Repeated freezing and thawing is deleterious and should be avoided.

IgG Sample Diluents (5X), 5 mL
1 vial containing protein-based concentrate in goat serum, with preservatives. Stable at 2 to 8°C until the expiration date stated on the label. Allow to warm to room temperature and shake before use. Prepare a 1X IgG Sample Diluent Working Solution by adding 1 part IgG Sample Diluent 5X to 4 parts reconstituted PBS. Store the 1X Working Solution at 2 to 8°C.

Mounting Medium, 2.5 mL
1 dropper bottle containing PBS-buffered glycerol at a pH of 7.2 ± 0.1. Contains preservatives. Stable at 2 to 8°C until the expiration date stated on the label. Allow to warm to 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 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 cloudy, discolored, 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 2,11,12
3. The substrate slides contain inactivated C. burnetii. 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 personnel11. 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. Acute serum should be drawn at the onset of illness. Convalescent serum should be obtained 2 to 4 weeks later.
Specimen Preparation
Prepare 1:16 screening dilutions of patient sera by mixing 1 part patient serum with 15 parts 1X IgG Sample Diluent Working Solution (see MATERIALS SUPPLIED, above).
To determine endpoint titers, use reconstituted PBS to serially dilute the 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 Positive Control 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 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 prepared sample dilutions (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 30 ± 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 the stream of PBS directly at the slide wells. Rinse 1 row at a time to avoid mixing of specimens. Wash slides by submerging 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 the slides to air dry.
8. Add approximately 25 µL IgG Conjugate to each slide 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.
1. If a 1+ Reading Control is desired, dilute the Positive Control (see TEST PROCEDURE, above) 1:8 and read versus Phase I. Due to differing laboratory conditions, including equipment, the 1+ Reading Control may vary ± 1 two-fold dilution. Upon dilution the phase I and phase II organisms may display different fluorescent intensities and different endpoint titers.
2. 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 cytoplasmic bodies on each spot, and grade the fluorescence as follows:

<table>
<thead>
<tr>
<th>Endpoint Titer</th>
<th>Interpretation</th>
</tr>
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<tbody>
<tr>
<td>≥ 1:16 to both phase I and phase II antigens</td>
<td>Positive. Strongly suggests <em>C. burnetii</em> infection. Phase I antibody titers of greater than or equal to phase II antibody titers are consistent with chronic infection or convalescent phase of Q fever.</td>
</tr>
<tr>
<td>&lt; 1:16 to both phase I and phase II antigens</td>
<td>Negative. No antibody detected. Argues against <em>C. burnetii</em> infection. This result is seen in persons with either no previous <em>C. burnetii</em> infection or with early infection. If early acute Q fever is suspected, perform IgM testing and obtain a serum second sample 2 to 3 weeks later and test in parallel with the original sample.</td>
</tr>
</tbody>
</table>

Interpreting the Patient Specimen Results
The reciprocal of the highest serum dilution that gives definite (1+) apple-green fluorescence is termed the endpoint titer. Seroconversion is defined as a change in antibody endpoint titer between acute and convalescent samples from non-reactive (no detectable antibody) to reactive (detectable antibody) when the acute sample is non-reactive.
A 4-fold IgG antibody endpoint titer increase is considered supportive evidence of current or recent acute infection. However, seroconversion alone may not be diagnostic of current or recent infection: a change in IgG endpoint titer from < 1:16 to 1:16 should not be considered a 4-fold increase and may not be significant. This is true of phase I and/or phase II.

Reactivity to Both Phase I and Phase II Antigens

<table>
<thead>
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<th>Endpoint Titer</th>
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</tr>
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<tr>
<td>&gt; 1:256</td>
<td>Evidence of recent or active <em>C. burnetii</em> infection.</td>
</tr>
<tr>
<td>&lt; 1:256</td>
<td>No specific antibody detected. Argues against <em>C. burnetii</em> infection.</td>
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Use the following matrix to determine specimen Q Fever disease state:

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LIMITATIONS
1. Neither phase of *C. burnetii* antigen has been found to cross-react with other rickettsia or bacteria sufficiently to produce false positive reactions.4
2. Low levels of phase II IgG antibody (< 1:256) may be considered non-specific.
3. The results obtained with this assay should be used in conjunction with the clinical information available to the physician.
4. Serologic responses are time dependent. Specimens obtained too early in the infection may not contain detectable antibody levels. If Q fever is suspected, obtain a second specimen 2 to 3 weeks later and test in parallel with the original sample.

EXPECTED VALUES
1. In test sera, it is common to see IgG titers of 1:128 or greater to both phase I and phase II antibody titers. However, in acute Q fever, the phase II antibody is usually higher than the phase I titer, often by 4-fold, even in early specimens. Although a rise in phase I as well as phase II titers may occur in later specimens, the phase II titer remains higher.
2. In chronic Q fever, the reverse situation is generally seen. Phase I titers rise in later specimens while phase II titers fall or remain constant. Phase I titers are significantly higher, sometimes much greater than 4-fold, in serum specimens drawn late in the illness from chronic Q fever patients. 

3. IgG class antibody titers appear very early in disease, reaching maximum phase II titers by week 8 and persisting at elevated titers for longer than a year. Phase I titers follow the same pattern, although at much lower levels, and may not be initially detected until convalescence.

4. In the case of chronic granulomatous hepatitis, IgG titers to phase I and phase II antigens are quite elevated, with phase II titers generally equal to or greater than phase I titers. Titers seen in Q fever endocarditis are similar in magnitude, although the phase I titers are quite often higher than the phase II titers.

SPECIFIC PERFORMANCE CHARACTERISTICS

Sensitivity and Specificity

The sensitivity and specificity of the Focus Diagnostics test in detecting IgG antibody to Coxiella burnetii was compared to a state health department’s in-house IFA test and to a reference laboratory’s complement fixation test.

A total of 103 sera were tested for antibody to Coxiella burnetii using the test procedures described above. The study population consisted of individuals working with or around sheep and potentially exposed to Q fever. 89 of the 103 specimens tested negative by CF and the Focus Diagnostics IFA. Of the 14 remaining sera, 13 were positive by all 3 tests. The discrepant sample was positive by the Focus Diagnostics and the state health department’s IFA test and negative by the CF procedure. Repeat testing yielded the same results.

Focus Diagnostics vs. Complement Fixation test

Relative Sensitivity: 100% (13/13)
Relative Specificity: 99% (89/90)

A total of 16 sera were tested for IgG antibody to phase I and phase II antigens on the Focus Diagnostics Q Fever IFA IgG test and on a standard reference laboratory’s IFA test. There was 100% correlation between the 2 assays. Both assays tested positive for antibody to phase I antigen in 14/16 sera and positive for antibody to phase II antigen in all 16 sera. The endpoint titers reached with both assays for phase I and phase II antigens on all 16 sera were within one 2-fold dilution.

Focus Diagnostics vs. State Health Department’s IFA test

Relative Sensitivity (Phase I): 100% (14/14)
Relative Sensitivity (Phase II): 100% (16/16)

Cross-reactivity

A total of 10 sera with IgG antibody to other rickettsial or bacterial diseases were tested on 2 different lots of the Q fever IFA IgG kit to determine if patient sera with IgG antibody to other diseases would cause false positive reactions with this assay. Neither phase of C. burnetii antigen was found to cross-react with the sera tested.

Reproducibility

To determine between run variation for the Q Fever IFA IgG assay, 2 patient sera with antibody to Q Fever IgG phase I and phase II (1 high positive and 1 low positive) and 1 patient sera negative for Q Fever IgG were run on 10 different slides. On 3 additional slides (1 for each of the sera described above), all 8 wells on a slide were brought to the same dilution to determine within run fluorescence variation. There was no between run variation in endpoint titers seen between the 10 different slides on 3 patient sera. The patient sera exhibited the same fluorescence in each well of the slide, indicating no within run variation.

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.