Bartonella IFA IgG
(OUS)

REF IF1300G
Rev. J

Indirect immunofluorescent assay (IFA) for the detection of human serum IgG antibodies to bartonella infections

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' Bartonella Indirect Immunofluorescent Assay (IFA) is intended as an aid in the clinical diagnosis of Bartonella infections. This product uses Vero cells which have been infected with either *B. henselae* or *B. quintana* as the individual substrates permitting the qualitative detection and semi-quantitation of human serum IgG antibodies to Bartonella.

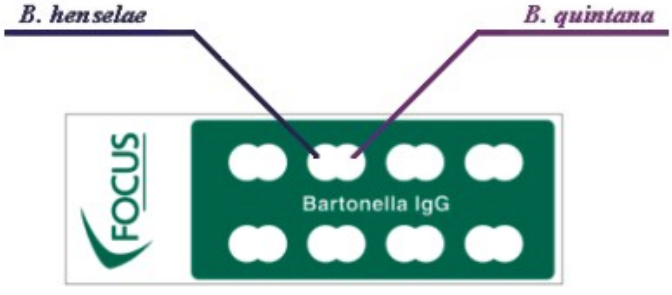
SUMMARY AND EXPLANATION OF TEST
Until recently the etiological agent of Cat Scratch Disease (CSD) has been in dispute. Several putative agents have been associated with this disease including *Chlamydia sp.* and *Afipia felis.* It is now generally recognized that the cause of CSD is actually a small gram negative rod of the genus *Bartonella* (formerly *Rochalimaea).*[1]
Cat scratch disease is a common disease (0.77 to 0.86 cases per 100,000 population) and affects 22,000 people in the United States annually.[2,3] The majority of reported cases have occurred in persons under 20 years of age, who are usually male. CSD reportings peak in the fall or winter.[4]
CSD usually follows a history of a cat scratch or bite which begins as a primary lesion on the skin surface. This is often followed by regional lymph node swelling which eventually drain to site of primary inoculation. The majority of cases are self limiting and resolve spontaneously in 2 to 4 months.[5]
Atypical forms of CSD make up approximately 11% of documented cases.[6] In these patients granulomatous conjunctivitis, oculoglandular syndrome, tonsillitis, visceral granulomatous disease, encephalitis, and cerebral arteritis are the most common.[6] In immunosuppressed persons *B. henselae* is most commonly associated with classic CSD, bacillary angiomatosis and peliosis hepatis.[7]
Traditionally, diagnosis was predicated on a history of cat bites or scratches, demonstration of the organism in the tissue by Warthin-Starry silver stain or positive skin tests. It is now possible to confirm suspected CSD cases using serological methods.[4] Studies indicate that approximately 90% of suspected cases of CSD result in serum IgG titers of > 1:64 by IFA, and/or IgM titers of ≥ 1:20 during the acute phase.[8] Background titers in healthy people are rare.[1]
Recently, there has been a resurgence of interest in another species of the genus Bartonella, *B. quintana.* This organism is the causative agent of classical Trench Fever.[9] Now *B. quintana* has been implicated in acute endocarditis and bacillary angiomatosis in HIV positive patients.[10,11] *B. quintana* serological diagnosis is similar to *B. henselae.* There is extensive IgG cross reactivity between these 2 species while the IgM response is more species specific. Therefore, the serological differentiation between *B. henselae* and *B. quintana* is typically not possible when only IgG is detected. In these cases it is necessary to test subsequent samples and rely upon clinical presentation.
The primary immune response to Bartonella is an IgM class antibody which appears early in the infection and is highly diagnostic when present. The IgG antibody response follows the initial IgM response closely. Since the IgG response is broadly cross reactive between species, these results must be interpreted with caution.
The Focus Diagnostics' *Bartonella* IFA slide contains both *B. henselae* and *B. quintana.* Looking at the slide through the microscope with the frosted end to the left, *B. quintana* appears on the left and *B. henselae* appears on the right.

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 containing 10% normal goat serum (NGS). The NGS blocks non-specific binding thus reducing unwanted background staining. The diluted sera is 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 bacteria. Semi-quantitative endpoint titers are obtained by testing serial dilutions of positive specimens.

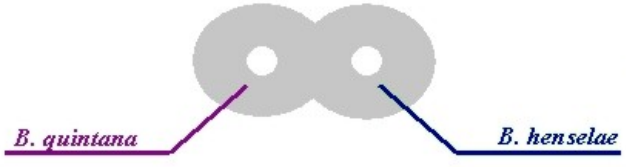
MATERIALS SUPPLIED
Focus Diagnostics' Test kit contains sufficient materials to perform 80 determinations.

Bartonella IFA IgG Substrate Slides | **REF** | **IF1301** | **Ag**

10 slides of 8 wells each. Each well contains 2 spots: 2 individual antigen spots consisting of infected Vero cells. 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.



IgG Conjugate-Dual Species, 3.5mL | REF | IF0011 | CONJ | IgG

1 vial of fluorescein-labeled goat anti-human IgG, gamma-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.

Polyvalent Detectable Control, 0.3mL | REF | IF1314 | CONTROL | >

1 vial of murine 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.

Non-Detectable Control, 0.25mL | REF | IF1313 | CONTROL | <

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. **Do not dilute.**

10X IgG Sample Diluent, 6mL | REF | IF1316 | DIL | IgG

1 vial of protein-based concentrate for IgG testing. Contains preservatives. Stable at 2 to 8°C until the expiration date stated on the label. Allow to warm to room temperature before use.

Prepare a (1X) IgG Sample Diluent Working Solution by adding 1 part Bartonella IFA IgG Sample Diluent (10X) to 9 parts PBS.

Mounting Medium, 2.5mL | REF | IF0007 | REAG | MONT

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 bottle label. Allow to warm to room temperature before use.

PBS | REF | IF0005 | BUF

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 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

WARNINGS AND PRECAUTIONS

1. This package insert is for export only and not for distribution in the United States. Outside of the United States, product regulatory status is for *in vitro* diagnostic use.
2. All blood products should be treated as potentially infectious. Source materials from which this product (including Non-Detectable 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.[12,13]
3. Evan's Blue is a carcinogen. Avoid contact with skin or eyes.
4. Do not substitute or mix reagents from different kit lots or from other manufacturers.
5. Use only protocols described in this insert. Incubation times or temperatures other than those specified may give erroneous results.
6. 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.
7. Bacterial contamination of serum specimens or reagents can produce erroneous results. Use aseptic techniques to avoid microbial contamination.
8. This kit and its components are specifically formulated for use in IgG determinations. Do not use either the kit or its components for IgM determinations.
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.

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.

Specimen Collection and Handling

Collect blood samples aseptically using approved venipuncture techniques by qualified personnel[12]. 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 Preparation

The serum screening dilution is 1:64 in (1X) IgG Sample Diluent Working Solution (see MATERIALS SUPPLIED, above).

To determine endpoint titers, use PBS to serially dilute the screening dilution.

TEST PROCEDURE (Incubation at 37°C)

1. Remove slides from cold storage. To avoid condensation, allow slides to reach room temperature before opening slide packets.
2. Apply 20 µL of Detectable Control, as bottled (undiluted), to the appropriate slide well. If a 1+ Reading Control is desired, use PBS to dilute the Detectable Control (see QUALITY CONTROL, below). Apply 20 µL of each dilution to an appropriate slide well.
3. Apply 20 µL of Non-Detectable Control, as bottled, to the appropriate slide well.
4. For each patient sample to be tested, add approximately 20 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 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 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 the slides to air dry.
8. Add approximately 20 µL **IgG Conjugate-Dual Species** 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 for up to 24 hours.

TEST PROCEDURE (Incubation at Room Temperature)

1. Remove slides from cold storage. To avoid condensation, allow slides to reach room temperature before opening slide packets.
2. Apply 20 µL of Detectable Control, as bottled (undiluted), to the appropriate slide well. If a 1+ Reading Control is desired, use PBS to dilute the Detectable Control (see QUALITY CONTROL, below). Apply 20 µL of each dilution to an appropriate slide well.
3. Apply 20 µL of Non-Detectable Control, as bottled, to the appropriate slide well.
4. For each patient sample to be tested, add approximately 20µL 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) for 60 ± 2 minutes at Room Temperature, covered.
6. 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 the slides to air dry. **Note: If using an instrument to automate the wash process, it may not be possible to allow the slides to air dry prior to addition of conjugate.**
8. Add approximately 20 µL of **IgG Conjugate-Dual Species** to each slide well.
9. Incubate slide(s) for 30 ± 2 minutes at Room Temperature, covered.
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 for up to 24 hours.

QUALITY CONTROL

Each run (each time a slide, or group of slides, is processed) should include both Detectable and Non-Detectable Controls.

1. When used undiluted (as bottled) the Detectable Control should exhibit 3 to 4+ fluorescence on both the *B. quintana* and the *B. henselae* antigen spots.
2. If a 1+ Reading Control is desired, dilute the Detectable Control (see TEST PROCEDURE, above) 1:8 and read versus *B. quintana.* Due to differing laboratory conditions, including equipment, the 1+ Reading Control may vary ± 1 two-fold dilution.
3. The Non-Detectable Control should exhibit negligible reactivity with all spots.

If controls do not exhibit these results, sample 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 fluorescent intensity of the bacteria, and grade the fluorescence as follows:

2 to 4+	Moderate to intense apple-green fluorescence.
1+	Definite, but dim fluorescence equivalent to that observed for the Detectable Control at the reference end point titer.
Negative	No fluorescence or fluorescence equal to that observed in the Non-Detectable Control well.

Interpreting the Patient Specimens Results

The reciprocal of the highest serum dilution that gives definite (1+) apple-green fluorescence is termed the serum endpoint titer.

≥1:256	IgG endpoint titers of 1:256 and greater are considered presumptive evidence of recent infection.
<1:256 and ≥1:64	A single specimen endpoint titer ≥ 1:64 and < 1:256 should be considered evidence of infection at an undetermined time. A second specimen drawn 10 to 21 days after the original draw should be tested in parallel with the first. If the second specimen exhibits a titer ≥ 1:256 or a 4-fold increase over that of the initial specimen, current (acute) infection is indicated. Unchanging titers ≥ 1:64 and < 1:256 suggest past infection.
<1:64	IgG endpoint titers less than 1:64 suggest that the patient does not have a current infection. This may be found in patients with either no history of Bartonella infection or those with past infection whose antibody titers have dropped below detectable levels.

Non-specific Fluorescence

Occasionally a specimen may react with the Vero cells. When this occurs the assay will not be interpretable.

LIMITATIONS

1. It is essential that all results from Bartonella serologies correlate with clinical history and other data available to the physician.
2. Microscope optics, and light source condition and type will determine overall fluorescent intensity and endpoint titers. Read control wells first during every run to ensure correct interpretation.
3. Samples obtained too early during primary infection may not contain detectable antibodies. If Bartonella infection is suspected, a second sample should be obtained 10 to 21 days later and tested in parallel with the original sample.

EXPECTED VALUES

In one study, 95% of patients with typical clinical, skin test and histopathologic evidence of CSD have IgG antibodies to *B. henselae*.[3] In another study, 88% of patients with clinically suspected CSD had serum titers to *B. henselae* of ≥ 64 while 3% of healthy controls exhibited positive titers.[1]

PERFORMANCE CHARACTERISTICS

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

REFERENCES

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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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