INSTRUCTIONS FOR USE

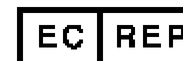
Q FEVER IFA
IgA ANTIBODY KIT

Catalog Number:	QA-120
Size:	120 test
Storage:	2-8˚C

An Indirect fluorescence immunoassay for the detection of IgA class antibody against *Coxiella burnetii*, phase I and phase II, in human serum or plasma

For in-vitro diagnostic use only

1312 E. Valencia Dr. Fullerton, California 92831 USA
Phone: +1-714-525-7660 Fax +1-714-525-7614
Email:info@fullerlabs.com
Internet: www.fullerlabs.com



MediMark Europe Sarl
11, rue Émile Zola – BP 2332
F-38033 Grenoble Cedex 2 – France

INTENDED USE

The Q Fever IgA Antibody kit is intended for the detection and semi-quantitation of IgA class human antibody to *Coxiella burnetii*, phase I and phase II, to be used as an aid in the diagnosis of human infection by this pathogen.

SUMMARY AND EXPLANATION OF TEST

Coxiella burnetii is an obligate intracellular bacterial parasite of eucaryotic cells. Humans are most often infected via aerosol generated by infected, parturient animals or, less often, via the bite of an infected tick. Inhaled organisms spread systemically from the lung, producing Q fever, a slowly resolving disease that closely mimics influenza. Chronic disease occurs in approximately 5% of these cases, primarily in the form of chronic granulomatous hepatitis or, less often, as endocarditis.

In the case of Q fever endocarditis, elevated IgA titers may be of value in supporting the diagnosis, especially with IgG titers of 512 or greater and a phase I/phase II titer ratio >1. Published results may not directly relate when serum pretreatment is not routinely employed, as it is in this assay.

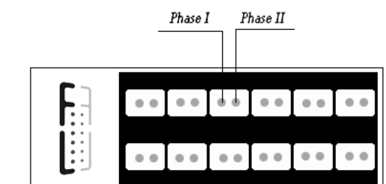
The IFA test utilizes the unique phase variation found in *C. burnetii* to differentiate the acute response from convalescent and chronic antibody responses. Patient sera are diluted with IgM Sample Diluent in order to remove the majority of competing IgG class antibody. Although the treated serum still contains both IgA and IgM class antibody, removal of the higher avidity IgG class makes both IgA and IgM assays more accurate.

Treated serum dilutions are then simultaneously reacted with both phase I and phase II organism in each slide well. After washing to remove unreacted serum proteins, the specific reaction is labeled with an immunoglobulin class-specific fluorescent conjugate. The resulting reactions can be visualized using standard fluorescence microscopy, where a positive reaction is seen as sharply defined apple-green fluorescent elementary bodies within a red counterstained field of background cell sonicate. Such positive reactions may then be retested at higher dilutions to determine the highest reactive or endpoint dilution.

REAGENTS

IFA | Ag x 12 — **Substrate Slides (10 each)**

10 x 12-well masked slides containing *Coxiella burnetii* phase I on the left side of each well and phase II on the right, when the slide is viewed with the frosted end to the left. The formalin-killed substrate is acetone-fixed and packaged under vacuum.



CONJ | FITC — **IgA Conjugate, 2.5 mL**

Dropper bottle with yellow cap contains affinity-purified Alexafluor 488-labeled goat anti-human IgA (heavy chain) with bovine serum albumin and Evans' blue counterstain.

CONT | + — **Positive Control, 0.5 mL**

Dropper bottle with blue cap contains purified IgA from a convalescent phase primary infection (human serum). This material is considered to be at 1:16 dilution, with a phase II titer of 1:128 and phase I <1:16 (negative).

CONT –

Negative Control, 0.5 mL
Dropper bottle with red cap contains non-reactive human serum at 1:16 dilution.

SAMP DIL

IgM Sample Diluent, 10 mL
Buffer contains goat antihuman IgG serum in PBS and a suspension of background matrix as adsorbent.

MM

Mounting Medium, 1 mL
Dropper bottle with white cap contains 50% glycerol in PBS.

BUF WASH PBS

PBS, 1 liter
Add supplied powder to 1 liter purified water to produce phosphate-buffered saline at pH 7.2.

Warnings

- The control sera have been screened for infectious agents by USFDA required testing. Since no testing can assure the absence of infectious agents, however, these reagents, as well as all serum specimens and equipment coming in contact with these specimens, should be handled with good laboratory practices to avoid skin contact and ingestion.
- The substrate slides are prepared with chemically inactivated antigens. However, the slides should be considered potentially infectious and handled accordingly.

Storage and Handling

Kit components should be stored at 2-8ºC. Bring them to room temperature (20-25ºC) before opening bottles or slide envelopes.

SPECIMEN COLLECTION

Allow blood sample to clot and separate serum by centrifugation. Transfer serum aseptically to a tightly closing sterile container. Store at 2-8ºC. If a delay in testing is expected to be longer than 5 days, store samples at -20ºC or colder. Acute specimens should be drawn at the onset of illness; and convalescent specimens at 2-4 weeks to check for titer changes.

PROCEDURE

The kit supplies sufficient reagents and materials for 120 determinations.

Materials Required But Not Supplied
- Purified (distilled or deionized) water
- Clean 250 or 500 mL wash bottle for PBS
- Wash bath with slide rack
- Test tubes or microtiter plate for preparing dilutions
- Precision pipette(s) for making/delivering dilutions
- 24 x 50 mm glass cover glass
- Fluorescence microscope with filter system for FITC (maximum excitation wavelength 490 nm, mean emission wavelength 530 nm) and 400X magnification
- 37ºC water bath or incubator
- Humidity chamber for slide incubation steps

Precautions
- Do not use components past expiration dates on labels.
- Conjugate is photosensitive and is packaged in opaque plastic for protection. Store in the dark and return to storage immediately after use.
- Conjugate contains Evans' blue dye, which may be carcinogenic. Avoid contact with skin.

ASSAY PROCEDURE

1. Prepare 1:16 screening dilutions in IgM Sample Diluent for all untested patient serum specimens. For sera found positive on a previous assay run, prepare serial two-fold further dilutions in PBS.

2. Prepare dilutions of the Positive Control in PBS to include 1 dilution above the stated endpoint and one dilution below the stated endpoint. Note: The Positive Control may not have the same titer for phase 1 and phase 2 antigens. Recommended titration series includes dilutions from 1:2 through 1:32 from the bottled Positive Control.

3. For each serum dilution to be tested, add 10 µL to one slide well and record the location for later reference. For each assay run, include the Negative Control and dilutions of the Positive Control prepared in step 2.

4. Place slides into a humidity chamber and incubate for 30 minutes at 37º± 0.5ºC. At this time also remove conjugate from storage to ambient temperature

5. Remove humidity chamber from incubator or water bath. Rinse slide wells quickly with a gentle stream of PBS from wash bottle (the top row of wells, then the bottom row) shaking wash buffer from slides into a sink. (Note: Direct stream of liquid at teflon mask) Repeat this action 2-3 times.

6. To each slide well add 1 drop (10 µL) Conjugate and return to humidity chamber for a 30 minute incubation at 37º± 0.5ºC. Incubation should be in the dark to protect the conjugate.

7. Rinse slide wells three times (3X) with a gentle stream of PBS from wash bottle, shaking wash buffer from slides into a sink.

8. Add 3-4 drops of Mounting Medium to each slide and apply cover glass.

9. Read the stained substrate slides at 400X magnification, comparing each well to the visual intensity and appearance of the Positive and Negative Control wells. Note that as viewed through the microscope optics, the phase II antigen dot appears on the left and phase I to the right side of the field. Slides may be stored at 2-8ºC in the dark for up to 24 hours.

QUALITY CONTROL
The Negative Control serum and dilutions of the Positive Control serum should be assayed with each daily run. The Positive Control wells should give an endpoint titer within a two-fold dilution of the stated values. For the phase II antigen this stated value is 1:64-1:256, assuming that the bottled Control starts at a 1:16 screening dilution. For the phase I antigen the Positive Control is non-reactive (negative), as the source serum was from a primary infection with a phase II titer 8-fold higher than the phase I titer. If this Control is not within range, the assay run should be considered void, reagent components and procedural steps should be rechecked, and the assay repeated from the beginning.

The Negative Control well is an example of fluorescence patterns that are to be considered negative. If bright staining is seen in this well, similar to that seen in the Positive Control wells, there has been a breakdown in technique and the assay must be repeated.

INTERPRETATION OF RESULTS

A positive reaction appears as bright staining (at least 1+) of the elementary bodies against a background of red counterstained background material. The particle size, appearance, and density of the fields must be compared with the Positive and Negative Control reactions. Patterns of reactivity different than that seen in the Positive Control must be considered non-specific.

PATIENT SPECIMENS

Positive at 1:16: IgA titers of 1:16 or higher against either phase I or phase II are supportive of recent or active disease. Positive sera should be rerun to determine their endpoint titer(s) for comparison with earlier or later specimens from the same patient**.**

Negative at 1:16: Report as negative for *Coxiella burnetii* IgA antibody.

LIMITATIONS

IgA titers may persist for over a year following chronic disease, most specifically Q fever endocarditis.

EXPECTED VALUES

The prevalence of specific IgA antibodies is limited to patients in convalescent phase of primary disease, with higher titers in chronic granulomatous hepatitis and highest titers in Q fever endocarditis. In the latter disease the phase I titers are routinely 2-8 times higher than titers toward phase II antigens, accompanied by significantly elevated IgG and IgM levels with the same relationship of phase I to phase II. As an example of normal convalescent titers in primary disease, the serum used to prepare the Positive Control had phase I/II IgM titers 1024/2048 and IgA 32/512. In chronic disease these ratios would be reversed.

REFERENCE

Peacock MG, Philip RN, Williams JC, Faulkner RS. Serological Evaluation of Q Fever in Humans. ***Infect. Immun***. 1983;41: 1089-1098.

DuPont HT, Thirion X, Raoult D. Q Fever Serology: Cutoff Determination for Immunofluorescence. ***Clin. Diagn. Lab. Immunol.*** 1994.1(2):198.

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