INSTRUCTIONS FOR USE

Q-fever IFA
IgG Antibody Kit

Catalog Number: QG-120

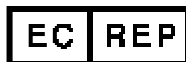
Size: 120 test

Storage: 2-8˚C

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

For in-vitro diagnostic use only

1135 E. Truslow Avenue
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 IgG Antibody kit is intended for the detection and semi-quantitation of IgG 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

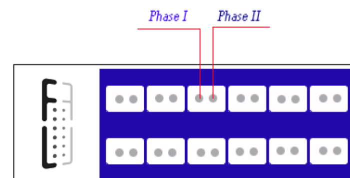
Coxiella burnetii is an obligate intracellular bacterial parasite of eucaryotic cells. Humans are most often infected via aerosol generated by infected, parturient animals or, less often, via the bite of an infected tick. Inhaled organisms spread systemically from the lung, producing Q fever, a slowly 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.

Due to the lack of characteristic symptoms in acute disease, and the laboratory hazards involved with isolation attempts, serologic procedures have been the primary supplement to clinical history in diagnosis. The IFA test utilizes the unique phase variation found in *C. burnetii* to differentiate the acute response from convalescent and chronic antibody responses. Dilutions of patient serum are 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 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 our proprietary CHS background. 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,* with phases I and II, as diagramed below. The formalin-killed substrate is acetone-fixed and packaged under vacuum.



CONJ | FITC **Conjugate, 2.5 mL**

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

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

Dropper bottle with blue cap contains reactive human serum, provided at a 1:16 screening dilution, with endpoints at 1:128 (Phase !) and 1:512 (Phase II).

CONT | – **Negative Control, 0.5 mL**

Dropper bottle with red cap contains non-reactive human serum, provided at a 1:16 screening dilution.

SAMP DIL **IgG Sample Diluent, 20 mL**

Buffer contains goat serum in PBS.

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

1. 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.

2. 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 2-8°C. Bring to room temperature (20-25°C) before opening bottles or slide envelops.

SPECIMEN COLLECTION

Allow blood samples to clot and separate sera by centrifugation. Transfer sera aseptically to tightly closing sterile containera. Store at 2-8°C. If testing is to be delayed longer than 5 days, freeze the samples at -20°C or colder. Acute specimens should be drawn at the onset of illness; convalescent specimens should be obtained within 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 coverglass
- Fluorescence microscope with filter system for FITC (maximum excitation wavelength 490 nm, mean emission wavelength 530 nm) and 400X magnification
- 37°C waterbath or incubator
- Humidity chamber for slide incubation steps

Precautions

- Do not use components past expiration date.
- 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

Allow all reagents and sera to reach ambient temperature before starting timed assay procedure.

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

3. For each diluted serum 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 37°± 0.5°C .

5. Remove humidity chamber from incubator or waterbath. Rinse slide wells quickly with a gentle stream of PBS from washbottle (the top row of wells, then the bottom row) shaking wash buffer from slides into a sink.

6. Submerge slides in a PBS wash bath for 5 minutes, then dip briefly in distilled water. Allow slide to dry before continuing.

7. To each slide well add 10 µL (1 drop) Conjugate and return to humidity chamber for 30 minutes incubation at 37°± 0.5°C. Incubation should be in the dark to protect the conjugate.

8. Rinse slide wells three times (3X) with a gentle stream of PBS from wash bottle, shaking wash buffer from slides into a sink.. Add 2-3 drops of Mounting Medium to each slide and apply coverglass.

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 2 antigen dot appears on the left and phase 1 to the right side of the field. Slides may be stored at 2-8°C 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 Negative Control well is an example of a non-reactive serum, with each field retaining a uniform red counterstain. The Positive Control wells should give an endpoint titer within a two-dilution of that listed for the Positive Control. If either of the Controls does not react as specified, 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 screening dilution: Titers against phase I organism should be accompanied by equal or higher titers against phase II organism in acute cases. In chronic disease the ratio of phase I/phase II is generally reversed (>1). Positive sera should be rerun to determine their endpoint titer for comparison with earlier or later specimens from the same patient.

IgG titers against phase II are relevant when accompanied by titers against phase I. If the screening reaction against only

phase II is less than 2+ positive (moderate staining), this is generally due to non-specific reactions with the Fc-receptors on the phase II antigen.

Negative at 1:16: Report as negative for *Coxiella burnetii* antibody. Further serum specimens should be drawn, if the original was taken immediately post onset of symptoms, especially if antibiotic therapy was instituted.

Phase II > 1:256: Report evidence of recent or active infection with C. burnetii.

LIMITATIONS

The phase II organism displays IgG Fc-receptors on its cell membrane. The IgG Sample Diluent is intended to saturate the receptors with irrelevant (goat) IgG, thereby allowing a specific IgG reaction with the human serum specimen. Elevated serum IgG levels may lead to low non-specific titers even in the presence of the special diluent, although they will be associated specifically with the phase II organism and not accompanied by IgM or IgA titers.

EXPECTED VALUES

The prevalence of specific antibodies varies depending upon the geographic region and population being tested. Phase II IgG titers are generally detectable within the first week following onset and peak around the eighth week. The vast majority of acute Q fever cases are still positive for phase II IgG after 1 year.

Phase I IgG titers, in contrast to phase II, are more often below detectable levels prior to convalescent phase or unless chronic disease develops. Chronic hepatitis is characterized by high and equivalent phase I and II titers, whereas endocarditis often has higher phase I titers. Both IgM and IgA titers to these antigens may also be helpful in differential diagnosis.

REFERENCE

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

Revised 3/2009