INSTRUCTIONS FOR USE

**Rickettsia conorii IFA IgG Antibody Kit**

Catalog Number: RCG-120
Size: 120 test
Storage: 2-8°C

An Indirect fluorescence immunoassay for the detection of IgG class antibody against *Rickettsia conorii* in human serum or plasma

For in-vitro diagnostic use only

INTENDED USE

The *Rickettsia conorii* IgG Antibody kit is intended for the detection and semi-quantitation of IgG class human antibody to *Rickettsia conorii*, to be used as an aid in the diagnosis of human infection by this pathogen.

SUMMARY AND EXPLANATION OF TEST

*Rickettsia conorii* is normally found in the Mediterranean region, India and Africa. Human infection, termed Boutonneuse or Mediterranean spotted fever, is mediated by ticks, whose bite transfers an infection derived from the more natural hosts of this organism (dogs and rodents). The ensuing infection induces a specific antibody response, which may be detected and used as an indirect means of identifying an infected human.

The IFA slides in this kit utilize cell culture-propagated *Rickettsia conorii* as the substrate antigen. Patient sera are diluted at least 1:64 in PBS and incubated in the individual slide wells to allow reaction of serum antibody with the intracellular rickettsia. The slides are then washed to remove unreacted serum proteins, and an FITC-labeled anti-human IgG (conjugate) is added, to react with and tag the antigen-antibody complexes. After further incubation, the slides are washed again to remove unreacted conjugate. The resulting reactions can be visualized using standard fluorescence microscopy, where a positive reaction is seen as sharply defined apple-green fluorescent rod forms in the cytoplasm of infected cells. A negative reaction is seen as either red-counterstained cells or fluorescence unlike that seen in the positive control well. Positive reactions may then be retested at higher dilutions to determine the highest reactive or endpoint dilution.

REAGENTS

**Substrate Slides (10)**
10 X 12-well masked slides containing acetone-fixed Vero cells, some of which were infected with the Moroccan strain of *Rickettsia conorii* (chemically killed).

**Conjugate, 2.5 mL**
Yellow cap dropper bottle contains affinity-purified FITC-labeled goat anti-human IgG (heavy chain) with bovine serum albumin and Evans' blue counterstain.

**Positive Control, 0.5 mL**
Blue cap dropper bottle contains human serum at a 1:64 screening dilution. Endpoint titer is 1:512

**Negative Control, 0.5 mL**
Red cap dropper bottle contains human serum at a 1:64 screening dilution

**Mounting Medium, 1 mL**
White cap dropper bottle contains glycerol (50% v/v) in PBS

**PBS, 1 liter**
Add supplied powder to 1 liter purified water to produce PBS.
2.

**ASSAY 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 diluting
- Precision pipette (15 µL)
- 24 x 50 mm glass coverslips
- Fluorescence microscope with filter system for FITC (maximum excitation wavelength 490 nm, mean emission wavelength 520 nm) and 460X magnification
- 37ºC water bath or incubator
- Humidity chamber for slide incubation steps

**Quality Control**

- Do not use components past expiration date.
- Conjugate is photosensitive and is packaged in opaque plastic for protection. Store in the dark.
- Conjugate contains Evans’ Blue dye, which may be carcotoxic. Avoid contact with skin.
- Liquid reagents contain thimerosal at 0.01%, which may be toxic if ingested.

**Expected Values**

The prevalence of specific antibodies varies depending upon the geographic region and population being tested. Endemic areas may have seropositive rates of 72%, some of which are undoubtedly undiagnosed or subclinical cases. Specific IgG antibody titers of 1:128 and higher are unusual and suggest active or recent infection. IgM class specific titers are not seen in the uninfected healthy population.

**Specific Performance Characteristics**

Test specificity was determined by testing random sera from non-endemic regions and sera with related diseases. From the California “Health” sera, only those sera positive for R. rickettsii by IFA, all sera were within 2 dilutions against R. rickettsii.

**Interpretation of Results**

A positive reaction appears as bright staining (at least 1+) of short pleomorphic red forms in the cytoplasm of 10-20% of the cells of each field. The size, appearance, and density of the infected cells 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.

**Primary (initial) infection is characterized by a prompt rise in both IgG and IgM class antibody by IFA testing. IgM antibody levels peak approximately 3 weeks post onset of symptoms and remain detectable for 2-3 months. IgG class antibody peaks in 7-12 weeks, but declines much more slowly than IgM antibody levels and remains elevated for approximately 12 months.**

**Storage**

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

**Specifications**

Rev D (1/04)

Original Version 7/93