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

CE

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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

IFA Ag x 12 **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).

CONJ FITC **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.

CONT + **Positive Control, 0.5 mL**

Blue cap dropper bottle contains human serum at a 1:64 screening dilution. Endpoint titer is 1:512

CONT – **Negative Control, 0.5 mL**

Red cap dropper bottle contains human serum at a 1:64 screening dilution

MM **Mounting Medium, 1 mL**

White cap dropper bottle contains glycerol (50% v/v) in PBS

BUF WASH PBS **PBS, 1 liter**

Add supplied powder to 1 liter purified water to produce PBS.

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 also be considered potentially infectious and handled accordingly.

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

SPECIMEN COLLECTION
Allow blood samples to clot and separate sera by centrifugation. Transfer sera aseptically to tightly closing sterile containers. Store at 2-8ºC. If testing is to be delayed longer than 5 days, freezing the sample at -20ºC or colder is recommended. Acute specimens should be drawn at the onset of illness; convalescent specimens should be obtained at two and four week intervals 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 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 530 nm) and 400X magnification
- 37ºC water bath 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.
- Conjugate contains Evans' Blue dye, which may be carcinogenic. Avoid contact with skin.
- Liquid reagents contain thimerosal at 0.01%, which may be toxic if ingested.

ASSAY PROCEDURE

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

1. Prepare 1:64 screening dilutions in PBS for patient serum specimens. For sera found positive on a previous assay run, prepare serial dilutions in PBS, starting with screening dilution (as above).

2. Prepare further dilutions of the Positive Control, which is bottled at a 1:64 screening dilution Make serial dilutions of the bottled Control through 16-fold (ie. 1:2, 1:4, 1:8. 1:16). These dilutions are considered to be 1:128, 1:256. 1:512 and 1:1024 when comparing to the specified endpoint.

3. For each serum dilution, add 10 µL to one slide well and record the location for later reference. For each assay run include the dilutions of the Positive Control prepared in step 2 and 1 drop of the Negative Control to one well.

4. Place slides into a humidity chamber and incubate for 30 minutes at 37º± 0.5ºC.

5. Remove humidity chamber from incubator or water bath.. Rinse slide wells with gentle stream of PBS from wash bottle three (3) times. Then allow beads of PBS to remain in the wells for at least 5 minutes.

6. Shake or tap excess PBS from slides held with beads of PBS and go directly to next step without allowing slide wells to dry.

7. To each slide well add 1 drop of conjugate, then return slides to humidity chamber for 30 minute incubation at 37º± 0.5ºC. Incubation should be in the dark to protect the photosensitive conjugate.

8. Wash slides as in steps 5-6, above.

9. Add 2-3 drops of Mounting Medium to each slide and place coverslip on, carefully removing air bubbles caught under the coverslip.

10. Read the stained substrate slides at 400X magnification, comparing each well to the visual intensity and appearance of the Positive and Negative Control wells. 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 Negative Control well is an example of a non-reactive serum, with either uniform red counterstain or slight, but uniform greenish staining. The Positive Control wells should give an endpoint titer from 1:256 to 1:1024. The fluorescence intensity at 1:512 may be used as the cut-off level required for a patient reaction to be called positive. 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 short pleomorphic rod 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.

PATIENT SPECIMENS

Positive at 1:64: IgG titers of 1:64 and greater reflect infection at an undetermined time (seropositive). Positive sera should be rerun to determine their endpoint titer for comparison with earlier or later specimens from the same patient.

Negative at 1:64: Report as negative for *R. conorii* antibody. Further serum specimens should be drawn, if the original was taken immediately post onset of symptoms, especially if antibiotic therapy was instituted.

Paired Sera: A four-fold increase in titer between acute and convalescent serum specimens is considered strong evidence supporting the diagnosis of recent infection.

LIMITATIONS
- In attempting to support the diagnosis of rickettsial infection in newborns the IgM class antibody should be tested for, as any IgG class antibody detected may be maternal in origin.

- A marked crossreactivity is seen in the IFA procedure between members of the spotted fever group, which includes the species *R. conorii, R. rickettsii, R. helvetica, R. slovaka, R. massiliae, R. africae* and many others. Crossreactivity with typhus fever group is much less evident, with titers 8-32-fold lower than those to the infecting species.

EXPECTED VALUES
The prevalence of specific antibodies varies depending upon the geographic region and population being tested. Endemic areas may have seropositive rates of 7-26%, some of which were undoubtedly mild 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 southern California 120 tested sera were all <1:64 (Negative), as were 4 sera with antibody to Coxiella burnetii. See also under Limitations for crossreactivity with other spotted fever group Rickettsia.

Due to a lack of clinical sera from the Mediterranean region, sensitivity was assessed with sera from North America reactive with closely related Rickettsia rickettsii. Out of 63 sera positive for R. rickettsii by IFA, all sera were within 2 dilutions against R. conorii using this IFA test. Of 16 sera submitted from southern Europe with reactivity to spotted fever group Rickettsia, all 16 were reactive with this IFA test.

REFERENCES

1. La Scola, Bernard und Didier Raoult. J. Clin. Microbiol. 1997; 35: 2715 – 2727

2. Raoult, Didier und Gregory A. Dasch. J. Clin. Microbiol. 1989; 27: 2073 – 2079.

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