INSTRUCTIONS FOR USE

Rickettsia Screen
IFA IgM Antibody Kit

Catalog Number: R24M-120

Size: 120 test

Storage: 2-8˚C

An Indirect fluorescence immunoassay for the detection and quantitative determination of IgM class antibody against both Rickettsia conorii and Rickettsia africae in human serum or plasma

For in-vitro diagnostic use only

CE

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INTENDED USE

The Rickettsia IFA IgM Antibody kit is intended for the simultaneous detection and semi-quantitation of IgM class human antibody to both Rickettsia conorii and Rickettsia africae, to be used as an aid in the diagnosis of human infection by these pathogens.

SUMMARY AND EXPLANATION OF TEST

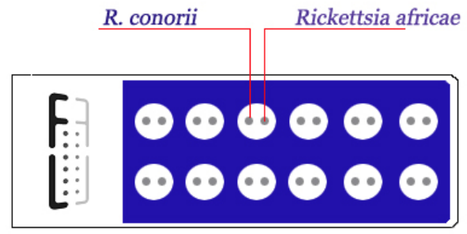
Spotted Fever Group Rickettsia are found worldwide and are mediated by ticks and mites (*Rickettsia akari*) whose bite transfers an infection ***Rickettsia*** africae (African Tick Bite fever) is found throughout most of the African continent and ***Rickettsia conorii*** (Boutonneuse fever) is found throughout the Mediterranean region, Africa, India and much of Asia. Many other species of spotted fever Rickettsia are also found over wide areas.

The IFA slides in this kit utilize purified *Rickettsia conorii* and *Rickettsia africae* as individual substrate antigens within each slide well. Patient sera are diluted at least 1:64 in buffered saline and incubated in the individual slide wells to allow reaction of serum antibody with the rickettsia. The slides are then washed to remove non-reactive serum proteins, and an FITC-labeled anti-human IgM (Conjugate) is added, to label the antigen-antibody complexes. After further incubation, the slides are washed again to remove non-reactive Conjugate. The resulting reactions can be visualized using standard fluorescence microscopy, where a positive reaction is seen as small sharply defined fluorescent rod forms within a red-counterstained background matrix. A negative reaction is seen as either counterstained (red) background or fluorescence different from that seen in the Positive Control wells. 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 x 12-well masked slides containing 2 acetone-fixed antigen spots in each well, packaged under vacuum.



CONJ | FITC **IgM Conjugate, 2.5 mL**

Yellow-cap dropper bottle contains ready to use affinity-purified and DyLight 488-labeled goat anti-human IgM (heavy chain) with anti-mouse IgG FITC conjugate, bovine serum albumin and Evans' blue counterstain.

SAMP DIL **Sample Diluent, 15 mL**

Protein-based buffer containing goat anti-human IgG, ready for use.

CONT + **Spotted Fever Group Positive Control, 0.5 mL**

Blue-cap dropper bottle contains mouse monoclonal serum reactive against spotted fever group Rickettsia and is bottled at screening screening dilution. Endpoint titer is 1:512.

CONT – **Negative Control, 0.5 mL**

Red-cap vial contains human serum non-reactive against Rickettsia and bottled at a 1:64 screening dilution.

MM **Mounting Medium, 1 mL**

White-cap dropper bottle 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

Store kit components at 2-8°C. Bring them 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 containers. Store at 2-8°C. If testing is to be delayed longer than 5 days, store sera at -20°C or colder. Acute sera should be drawn at the onset of illnes and convalescent sera at two and four week intervals to check for titer changes.

PROCEDURE

Material Supplied

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 equivalent for manual dilutions
- Precision pipette(s) for making dilutions and delivering exactly 10 µL per slide well
- 24 x 50 mm glass cover slips
- Fluorescence microscope with filter system for FITC (maximum excitation wavelength 490 nm, mean emission wavelength 530 nm) and 400X magnification
- 37°C 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 and return to storage immediately after use
- Liquid components contain 0.01% thimerosal as preservative. Do not ingest.

- Conjugate contains Evans' Blue dye, which may be carcinogenic. Avoid contact with skin.

PREPARATION OF SAMPLES AND REAGENTS

1. **Prepare Wash Buffer** by adding contents of PBS packet to 1 liter purified water and mixing thoroughly:

2. **Prepare screening dilutions** of patient sera by making an initial 1:16 dilution using Sample Diluent in micro-centrifuge tubes. Mix and allow a minimum of 5 minutes for the reaction, then centrifuge at high speed to remove the aggregated IgG. Dilute 10 µL of this supernate with 30 µL Wash Buffer, resulting in a final 1:64 screening dilution.

ASSAY PROCEDURE

1. Prepare serial 2-fold dilutions in Wash Buffer of the Positive Controls to include 1 dilution above and 1 dilution below the stated endpoint (1:512). All controls are pre-diluted and bottled at 1:64

2. Add 10 µL test serum dilutions to individual slide wells, taking care not to scratch the antigen dots with the pipette tip. For each set of slides include the Negative Control and dilutions of the Positive Controls (Step 1).

3. Place slides into a humidity chamber and incubate for 30 minutes at 37°± 0.5°C .

4. 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.

5. Shake or tap excess PBS from slides and go directly to next step without allowing slide wells to dry.

6. To each slide well add 1 drop (10 µL) IgM Conjugate, then return slides to humidity chamber for a 30 minute incubation at 37°± 0.5°C. Incubation should be in the dark to protect the photosensitive conjugate.

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

8. 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. 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 Controls must be assayed with each daily run. The Negative Control well is an example of a non-reactive serum, with no distinct and characteristic staining of the rickettsia. The Positive Control wells should give endpoint titers from 1:256 to 1:1024. The fluorescence intensity at 1:512 may be used as the cut-off level required for test reactions to be called positive. If any of the Controls do not react as specified, the assay run is considered void, reagent components and procedural steps should be rechecked, and the assay repeated from the beginning.

INTERPRETATION OF RESULTS

A positive reaction appears as bright staining (at least 1+) of short pleomorphic rod forms. The size, appearance, and density of each field must be compared with the Positive and Negative Control reactions. Patterns of reactivity different from the Positive Controls must be considered non-specific.

Primary (initial) infection is characterized by a prompt rise in both IgG and IgM class antibody by IFA. IgM 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: IgM titers of 1:64 and greater are considered positive and are supportive of recent infection. These sera should be titered to determine their endpoint for comparison with earlier or later specimens from the same patient.

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

LIMITATIONS

- Marked cross-reactivity is seen in the IFA procedure between members of the spotted fever group, which includes the species *conorii, africae, akari, massiliae, sibrica*, and others.

- IgM positive sera that are IgG negative should be viewed with suspicion, due to the potential for crossreactivity with LPS antigens of Proteus spp. and other organisms.

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