INSTRUCTIONS FOR USE

Rickettsia Screen
IFA IgG Antibody Kit

Catalog Number: R24G-120
Size: 120 test
Storage: 2-8˚C

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

For in-vitro diagnostic use only

INTENDED USE
The Rickettsia IFA IgG Antibody kit is intended for the simultaneous detection and semi-quantitation of IgG 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 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 an adsorbent suspension 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 a fluorescence 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 dispersed 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

Substrate Slides
10 x 12-well masked slides containing 2 acetone-fixed antigen spots in each well.

IgG Conjugate, 2.5 mL
Yellow-cap dropper bottle contains ready to use affinity-purified and DyLight 488-labeled goat anti-human IgG (heavy chain) with bovine serum albumin and Evans’ blue counterstain.

IgG Sample Diluent, 10 mL
Buffer contains 5% sonicated background matrix as adsorbent. Mix by inversion before use.
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 0.001% 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 1:64 dilutions of patient sera by first diluting the sera 1:4 in Wash Buffer, then diluting 1:16 further using the IgG Sample Diluent. Mix well and allow this mixture to incubate at least 15 minutes at ambient temperature. This 1:64 dilution is referred to as the screening dilution. For further dilutions of positive sera dilute the screening dilution with Wash Buffer (PBS).

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. For each serum dilution add 10 μL to one slide well and record the location for later reference. For each assay repeat the Positive Control and the serial dilutions of the Positive Control prepared in step 1. Also add 1 drop of Negative Control to one well. Samples should be applied to the top or bottom edge of the well, avoiding the mid-section containing the antigen microdots.
3. Place slides into a humidity chamber and incubate for 30 minutes at 37º± 0.5ºC.
4. Remove humidity chamber from incubator or water bath. Also remove conjugate from storage. Rinse slide wells with gentle stream of PBS from wash bottle, washing 1 row of wells at a time to avoid mixing of specimens. Then immerse slides in a container filled with fresh PBS for at least 5 minutes.
5. Dip slides briefly in distilled water and allow them to air dry.
6. To each slide well add 1 drop (10 μL) IgG Conjugate, then return slides to humidity chamber for a 30 minute incubation at 37º± 0.5ºC. 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 2-8ºC 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 rickettsiae. The Positive Control wells should give endpoint titers from 1:256 to 1:1024 (4-16-fold beyond the bottled screening dilution). 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.

Patient Specimens

Positive at 1:64: IgG titers of 1:64 and greater reflect primary (initial) infection at an undetermined time (seropositive). Positive 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.

PATIENT SPECIMENS

Positive at 1:128 and greater: Sera giving such elevated endpoint titers suggest recent or active infection. IgM titers, when present, are also a reliable indicator of recent infection.

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 cross-reactivity is seen in the IFA procedure between members of the spotted fever group, which includes the species conorii, qfrius, ahsort, massiliae, sibiri, and others.

Original Version 2/2010