INSTRUCTIONS FOR USE

Rickettsia typhi IFA
IgG Antibody Kit

Catalog Number: RTG-120

Size: 120 test

Storage: 2-8˚C

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

For in-vitro diagnostic use only

CE

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

SUMMARY AND EXPLANATION OF TEST
Rickettsia typhi is found throughout the world. Human infection by this agent takes the form of murine typhus, transmitted via infected louse feces. 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 typhi* as the substrate antigen. Patient sera are diluted at least 1:64 and incubated in the individual slide wells to allow reaction of patient antibody with the intracellular rickettsia. The slides are then washed to remove unreacted serum proteins, and an DyLight 488-labeled anti-human IgG (Conjugate) is added, to detect 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 infected with the Wilmington strain of *Rickettsia typhi* (chemically killed) and packaged under vacuum.

CONJ FITC **Conjugate, 2.5 mL**
Yellow cap dropper bottle 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**
Green cap dropper bottle contains reactive serum at screening screening dilution. Endpoint titer is 1:512

CONT – **Negative Control, 0.5 mL**
Red cap dropper bottle contains non-reactive serum at 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 must be considered potentially infectious and handled accordingly.

Storage and Handling
Kit components should be stored at 2-8°C or colder. Bring

them to room temperature (20-25°C) 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 2-8°C. If testing is to be delayed longer than 5 days, freezing the sample at -20°C 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

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 plate for manual dilutions
- Precision pipette(s) for making dilutions and delivering exactly 10 µL per slide well
- 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
- 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 after use.
- Conjugate contains Evans' Blue dye, which may be carcinogenic. Avoid contact with skin.

ASSAY PROCEDURE

1. Prepare 1:64 screening dilutions (1 part patient serum with 63 parts PBS) for patient serum specimens.

2. Prepare dilutions of the Positive Control to include 1 dilution above the stated endpoint and one dilution below the stated endpoint (1:128).

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 dilutions of the Positive Control prepared in step 2. Also add 1 drop (10 µL) of the Negative Control to one well.

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 with gentle stream of PBS from washbottle 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 (10 µL) conjugate, then return slides to humidity chamber for another 30 minutes incubation at 37°± 0.5°C. 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 apply cover glass.

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 2-8°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 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 do 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 and chains of small coccobacilli within the cytoplasm of 10-20% of the cells in 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. typhi* antibody. Further serum specimens should be drawn if the original was taken immediately post onset of symptoms, especially if antibiotic therapy was instituted.

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 *Rickettsia typhi* and *Rickettsia prowazekii*, members of the typhus fever group, Cross-reactivity with the spotted fever group is much less evident, but titers 8-32-fold lower than those to the infecting species are observed.

EXPECTED VALUES

The prevalence of specific antibodies varies depending upon the geographic region and population being tested. 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.

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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