INSTRUCTIONS FOR USE

Rickettsia typhi 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

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 complex. After further incubation, the slides are washed again to remove unreacted conjugate. The resulting reactions can be evaluated using standard fluorescent microscope, where a positive reaction is seen as sharply defined apple-green fluorescent outlined 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 retested at higher dilutions to determine the highest reaction or endpoint dilution.

REAGENTS
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.
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.
Positive Control, 0.5 mL Green cap dropper bottle contains reactive serum at screening dilution. Endpoint titer is 1:512
Negative Control, 0.5 mL Red cap dropper bottle contains non-reactive serum at screening dilution
Mounting Medium, 1 mL White cap dropper bottle contains glycerol (50% v/v) in PBS
PBS, 1 liter

Warnings
The control sera have been screened for infectious agents by USDA 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. Although the endpoint dilution represents chemically inactivated antigen. 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...
Materials Required

- Purified (distilled or deionized) water
- Clean 250 or 500 mL wash bottle for PBS
- Wash both 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
- RDT Precision Filter system for FITC excitation wavelength 490 nm, mean emission wavelength 530 nm and 400X magnification
- -37ºC waterbath or incubator
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4. Wash slides as in steps 5-6, above.
5. Add 2-3 drops of Mounting Medium to each slide and apply cover glass.
6. 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 should be assayed with each daily run. The Negative Control well is an example of a non-reactive serum, with other uniform red fluorescent or slight, but uniform, greenish staining. The Positive Control wells should give an endpoint value 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 invalid, 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 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 comparable to those of the Positive Control. Patterns of reactivity different than that seen in the Positive Control wells, there must be a breakdown in technique and the assay must be repeated.

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.

Primary (initial) infection is characterized by a prompt rise in IgM class antibody by JPA 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.

LIMITATIONS

- In attempting to support the diagnosis of rickettsial infection in seronegative the IgG 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 prowazeki, members of the typhus fever group. Cross-reactivity with the spotted fever group is much less evident, but there 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.