INSTRUCTIONS FOR USE

Rickettsia typhi IFA
IgM Antibody Kit

Catalog Number: RTM-120
Size: 120 test
Storage: 2-8°C

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

For in-vitro diagnostic use only

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INTENDED USE
The Rickettsia typhi IgM Antibody kit is intended for the detection and semi-quantitation of IgM 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 by infected fleas. 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 initially diluted at least 1:64 in IgM Sample Diluent, containing antiserum to human IgG heavy chain. This reaction (precipitin) removes competing IgG class antibody and the source of rheumatoid factor interference. The treated sera are then 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 FITC-labeled anti-human IgM (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

- 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.
- IgG Conjugate, 2.0 mL
  Yellow cap dropper bottle contains affinity-purified FITC-labeled donkey anti-human IgM (heavy chain) with bovine serum albumin and Evans' blue counterstain.
- Positive Control, 0.5 mL
  Blue cap dropper bottle contains pre-treated human serum at a 1:64 screening dilution. Endpoint titer is 1:512
- Negative Control, 0.5 mL
  Red cap dropper bottle contains pre-treated human serum at a 1:64 screening dilution
- IgM Sample Diluent, 10 mL
  Buffer contains goat anti-human IgG antibody in PBS.
- Mounting Medium, 1 mL
  White cap dropper bottle contains glycerol (50% v/v) in PBS

REAGENTS (see PREPARATION OF SAMPLES AND REAGENTS)
ASSAY PROCEDURE

1. Prepare screening dilutions (see PREPARATION OF SAMPLES AND REAGENTS) for all patient serum specimens.

2. For sera found positive on a previous assay run, prepare serial dilutions in PBS of the pretreated sera.

3. Prepare dilutions of the Positive Control to include 1 dilution above the stated endpoint and one dilution below the stated endpoint (1:256 to 1:1024). This Control is bottled at the 1:64 screening dilution.

4. 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 Positive Control prepared in step 3. Add 1 drop of the Negative Control, as bottled, to one well.

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

6. Remove humidity chamber from incubation. Rinse slide wells with gentle stream of PBS from washbottle. Then allow beads of PBS to remain in the wells for at least 5 minutes. Shake or tap excess PBS from slides and go directly to next step.

7. To each slide well add 1 drop (10 µL) conjugate, then incubate slides for 30 minutes at 37°C ± 0.5°C. Incubation should be in the dark to protect the photosensitive conjugate.

8. Wash slides as in step 6, above.

9. Add 2-3 drops of Mounting Medium to each slide and place coverslip on.

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 titre of 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 and short chains of coccobacilli) within the cytoplasm of 7-15% 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: IgM titers of 1:64 and greater reflect recent infection. Positive sera should be rerun to determine their endpoint titre for comparison with earlier or later specimens from the same patient.

Negative at 1:64: Report as negative for R. typhi IgM 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 titre between acute and convalescent serum specimens is considered strong evidence suggestive of the diagnosis of recent infection.

LIMITATIONS

A marked cross-reactivity is seen in the IFA procedure between R. typhi and R. prowazekii, members of the typhus group. Cross-reactivity with 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. Since IgM titers reflect recent or active disease, IgM class specific titers are not seen in the uninfected healthy population.

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