INSTRUCTIONS FOR USE

Rickettsia typhi EIA IgG Antibody Kit
Catalog Number: RTG-96K
Size: 96 test
Storage: 2-8°C

An Indirect enzyme 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 *Rickettsia 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 or endemic typhus, transmitted via infected flea 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 EIA module wells in this kit utilize a species-specific protein (rOmp B) purified from *Rickettsia typhi*. Patient sera are diluted in a Sample Diluent and incubated in the coated microwells to allow binding of serum antibody to the solid-phase antigen. The microwells are then washed to remove unreacted serum proteins, and a peroxidase-labelled anti-human IgG (Enzyme Conjugate) is added to label the bound antibody. After further incubation, the microwells are washed to remove unbound HRP Conjugate. The TMB Substrate is then added to quantitate the bound peroxidase portion of the Conjugate. Development of a blue color is directly proportional to the amount of reactive serum antibody. This timed reaction is interrupted with a Stop Solution that turns the blue reactions to yellow and stabilizes the final color intensity. Color intensity (Absorbance) is measured at a wavelength of 450nm on a microtiter plate reader or spectrophotometer.

REAGENTS AND MATERIALS SUPPLIED

MW | Ag
---|---
EIA Microwells (96) | 12 x 8-well strips coated with specific membrane protein purified from *Rickettsia typhi* and packaged with desiccant, ready to use.

SAMP DIL
PBS buffer containing bovine serum albumin and Tween.

CONJ ENZ
Enzyme Conjugate, 12 mL
Affinity-purified HRP-labeled goat anti-human IgG (heavy chain) provided ready to use in an amber bottle.

CONT +
Positive Control, 120 μL
Blue cap vial contains reactive human serum, bottled at a 1:10 dilution

CAL +
Cutoff Calibrator, 200 μL
Green cap vial contains equivocally reactive human serum, bottled at a 1:10 dilution

CONT -
Positive Control, 120 μL
Red cap vial contains non-reactive human serum, bottled at a 1:10 dilution

SUBS TMB
TMB Substrate, 12 mL
A solution containing H2O2 and tetramethylbenzidine (TMB) supplied in an amber bottle. Ready to use. Protect from light.

SOLN STOP
Stop Solution, 12 mL
Diluted sulfuric acid ready to use. May be stored at room temperature.
REAGENTS
Wash Buffer see PREPARATION OF SAMPLES AND REAGENTS

PBS, 1 liter
Add supplied packet to 1 liter purified water to produce phosphate-buffered saline at pH 7.2. Mix thoroughly. For Wash Buffer see PREPARATION OF SAMPLES AND REAGENTS

Tweeen 20, 2 mL
Solution of 25% Tween 20 and 75% PBS. To make Wash Buffer, see PREPARATION OF SAMPLES AND REAGENTS

Wash Buffer
Prepare by adding contents (2mL) of Tween 20 bottle to 1 liter PBS and mixing thoroughly:

Buffer, see PREPARATION OF SAMPLES AND REAGENTS

Materials Required But Not Supplied
1. Purified (distilled or deionized) water
2. Wash bottles or automated EIA washing apparatus
3. Test tubes for manual serum dilutions or automatic dilutor for 1:100 dilutions
4. Precision pipette(s) for microliter range dilutor for 1:100 dilutions
5. Adhesive or plastic cover for incubations.
6. EIA reader equipped with a 450nm filter.

PROCEDURE
The kit supplies sufficient reagents and materials for 96 determinations.

PROCEDURE
Allow all reagents and sera to reach ambient temperature before starting timed assay procedure.

Steps:
1. Pipette 100 µL of each diluted serum and diluted Control into appropriate microwells. Replicate microwells are recommended for the diluted Cutoff Calibrator.
2. Wash plates four (4) times with a gentle stream of Wash Buffer from a wash bottle or with a multiwell EIA plate washer, removing residual wash buffer from microwells.
3. To each microwell add 100µL IgG HRP Conjugate. Cover and incubate for 30 minutes at ambient temperature in the dark.
4. Stop reaction, in the same timed sequence as above, by adding 100 µL of Stop Solution.
5. Read absorbance on a microplate reader equipped with a 450nm filter.

LIMITATIONS
This procedure detects Rickettsia typhi species-specific antibody, although cross-reactivity will be observed with high-titer Rickettsia prowazekii. Reactivity to spotted fever group or scrub typhus is, in general, not detected.

REFERENCES

Version B (01/19/2004)