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.

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 *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 **Sample Diluent, 2 X 50 mL**

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 H_2O_2 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.

BUF | WASH | PBS **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

BUF | WASH | TWEEN **Tween 20, 2 mL**

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

Warnings

1. The Control and Calibrator sera have been screened for infectious agents by FDA required testing. Since no testing can assure the absence of infectious agents, however, these reagents, as well as all serum and equipment coming in contact with these specimens, should be handled with good laboratory practices to avoid skin contact and ingestion.

2. The coated microwells are prepared with inactivated antigens. However, they should be considered potentially infectious and handled accordingly.

Storage and Handling

Kit components should be stored at 2-8°C. Bring them to room temperature (20-25°C) before opening bottles and plate pouches.
Unused antigen strips should be returned to the package with desiccant and tightly resealed After initial opening of test strip bag, use microwells within 3 months

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, store samples at -20°C or colder. 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.

PREPARATION OF SAMPLES AND REAGENTS

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

2. **Prepare 1:100 dilutions** of all patient serum specimens using Sample Diluent.

3. **Prepare 1:10 dilutions** of the Positive Control, Negative Control and Cutoff Calibrator using Sample Diluent.

PROCEDURE

The kit supplies sufficient reagents and materials for 96 determinations.

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
5. Adhesive or plastic cover for incubations.
6. EIA reader equipped with a 450nm filter.

Precautions

1. Do not use components past expiration date.
2. TMB-substrate and Conjugate are photosensitive and are packaged in amber bottles for protection. Return to cold storage after use.
2. Liquid reagents contain thimerosal at 0.01%, which may be toxic if ingested.
3. Stop Solution contains 0.2N Sulfuric Acid. If this acid comes into contact with skin, wash thoroughly with water and seek medical attention.

ASSAY PROCEDURE

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

1. Pipette 100 µL of each diluted serum and diluted Control into appropriate microwells. Replicate microwells are recommended for the diluted Cutoff Calibrator.

2. Cover microwells to minimize evaporation, then **incubate for 60 minutes at ambient temperature** (20-25°C).

3. 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.

4. To each microwell add 100µL IgG HRP Conjugate. Cover and incubate for **30 minutes at ambient temperature** in the dark.

5. Wash microwells as in step 3 above.

6. To each microwell, in a timed sequence, **add 100 µL TMB Substrate** and allow reaction to proceed for **exactly 10 minutes** in the dark.

7. Stop reaction, in the same timed sequence as above, by adding **100 µL of Stop Solution.**

8. Read absorbance on a microplate reader equipped with a **450nm filter**.

QUALITY CONTROL

A Cutoff Calibrator is provided for discrimination between reactive and non-reactive sera. The best use of this Calibrator is in defining the intra-assay variation in the equivocal region. If two or more microwells measure the Cutoff value, the equivocal regioin of absorbance values will lie between the highest and lowest values recorded for this Calibrator. Alternatively, by dividing the OD values of test sera by the OD values of the Cutoff Calibrator, an index value can be derived where the Cutoff Calibrator is set at an index of 1.0. Indices from 0.8 to 1.2 may be considered equivocal (assumes %CV of 20%). Indices above 1.2 are then considered positive and those below 0.8 are considered negative.

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.

EXPECTED VALUES

The prevalence of specific antibodies varies depending upon the geographic region and population being tested. Endemic areas have been reported with seropositive rates of 7-26%, some of which were undoubtedly mild or subclinical cases.

REFERENCES

1. La Scola, Bernard and Didier Raoult. J. Clin. Microbiol. 1997; 35: 2715 – 2727

2. Raoult, Didier and Gregory A. Dasch. J. Clin. Microbiol. 1989; 27: 2073 – 2079.

Version B (01/19/2004)