INSTRUCTIONS FOR USE

Typhus Group Rickettsia EIA IgM Antibody Kit

Catalog Number: RTM-96K

Size: 96 test

Storage: 2-8˚C

An indirect enzyme immunoassay for the detection and quantitative determination of IgM class antibody against *Rickettsia typhi* in human serum or plasma

For in-vitro diagnostic use only.

CE

GENTAUR

EC REP

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INTENDED USE

The Rickettsia typhi EIA IgM Antibody Kit is intended for the detection and quantitation of IgM class human antibody to *Rickettsia typhi and Rickettsia prowazekii*, to be used as an aid in the diagnosis of human infection by these typhus group pathogens.

SUMMARY AND EXPLANATION OF TEST

Rickettsia typhi is found worldwide and is spread 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 EIA test microwells in this kit utilize outer membrane protein (rOmp B) purified from ***Rickettsia typhi.***. Patient sera are first diluted 1:10 in an IgM Serum Prep, which agggregates IgG and rheumatoid factor complexes, then 10-fold further in a Sample Diluent. The final 1:100 serum dilution is then incubated in the coated microwells to allow binding of serum antibody to the solid-phase antigens. The microwells are then washed to remove unreacted serum proteins, and an enzyme-labelled anti-human IgM (Enzyme Conjugate) is added to label the bound antibody. After an incubation period, the microwells are washed to remove unbound Enzyme Conjugate. An enzyme substrate (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 **96-microwell EIA Module**

12 x 8-microwell strips coated with outer membrane protein (rOMP B) extracted from *Rickettsia typhi* and packaged with desiccant, ready to use.

IgM DIL **IgM Serum Prep, 10 mL**

Buffer containing goat anti-human IgG (Fc(γ)-specific), ready to use.

SAMP DIL **Sample Diluent, 2 x 50 mL**

PBS buffer containing bovine serum albumin and Tween 20, ready to use.

CONJ ENZ **Enzyme Conjugate, 12 mL**

Affinity-purified peroxidase-labeled goat anti-human IgM (μ chain-specific), ready to use. **Protect from light**.

CONT + **Positive Control, 120 μL**

Blue cap vial contains reactive human serum pre-diluted 1:10.

CAL ± **Cutoff Calibrator, 200 μL**

Green cap vial contains equivocally reactive human serum pre-diluted 1:10.

CONT – **Negative Control, 120 μL**

Red cap vial contains non-reactive human serum pre-diluted 1:10.

SOLN 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. Avoid contact with skin.

BUF WASH PBS **PBS, 1 liter**

Add supplied packet to 1 liter purified water to produce PBS Buffer pH 7.2. Mix thoroughly.

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

Solution of 25% Tween 20 and 75% PBS. Add contents to 1.0 liter PBS to prepare Wash Buffer.

Warnings

1. The control 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. Although assay microwells are prepared with inactivated antigens, 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

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 intervals to check for titer changes.

PREPARATION OF REAGENTS AND SPECIMENS

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

2. **Prepare initial 1:10 dilutions for all patient sera in IgM Serum Prep** (**Do not treat Controls or Cutoff Calibrator in this step**). Mix well and allow at least 5 minutes for precipitin aggregates to develop. This step should be performed in a separate dilution plate or in test tubes.

3. **Prepare further dilutions of the mixtures prepared in Step 2. Dilute these mixtures 1:10 in Sample Diluent to give final serum dilution of 1:100**.

4. **Prepare 1:10 dilutions of bottled Controls and Cutoff Calibrator in Sample Diluent (final dilution now 1:100).**

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 microwell 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. Store in the dark and return to storage after use.
3. Liquid reagents contain thimerosal at 0.01%, which may be toxic if ingested.
4. 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 patient serum, diluted Controls and diluted Cutoff Calibrator into appropriate microwells**. **Replicate wells 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 Wash Buffer from a wash bottle or with an EIA plate washer, removing residual Wash Buffer from wells.

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

5. **Wash microwells as in step 6 above**.

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

7. Stop reaction, in the same timed sequence as above, by adding **100 µL 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 Cutoff Calibrator is set at an index of 1.0. By dividing the Absorbance values of the test sera by the mean Absorbance value of the Cutoff Calibrator, an index value for each serum is derived. Indices from 0.9 to 1.1 may be considered equivocal. Indices above 1.1 are considered positive and those below 0.9 are considered negative.

In assessing the kit controls, please note the expected absorbance ranges and the minimum ratios between Cutoff vs. Negative Control (E/N) and Positive Control vs. Cutoff Calibrator (P/E) absorbance values. These values are listed, along with our in-house test values, in the Certificate of Analysis.

It is also incumbent upon the testing facility to maintain and utilize internal controls for Quality Assurance purposes. Optimally, one internal control should be from a healthy population (true negative), but with an EIA value slightly below the Cutoff Calibrator. Another internal control should be a weakly positive serum from a confirmed diagnosis (true positive). By following the values of these internal controls relative to the Cutoff Calibrator, variations and trends related to test accuracy and reproducibility can be documented.

LIMITATIONS

This procedure detects antibody to protein antigens and will give negative results if the patient response is only to the lipopolyscaccharide (LPS) antigen. Based upon data from western immunoblot IgM testing, sera reacting only to LPS are most often false-positive[2].

It is recommended that IgG negative sera that test IgM positive be reported as IgM negative, based on published studies[2]. The basis of such reactivity is under investigation.

Note that Rickettsia typhi reactivity cannot be differentiated from Rickettsia prowazekii reactivity using this assay.

EXPECTED VALUES

The prevalence of specific antibodies varies depending upon the geographic region and population being tested. Positives are only to be expected in IgG-positive acute 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.

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