INSTRUCTIONS FOR USE

Spotted Fever Rickettsia EIA IgM Antibody Kit

Catalog Number: RRM-96K

Size: 96 test
(12 x 8-wells)

Storage: 2-8˚C

An Indirect enzyme immunoassay for the detection and quantitative determination of IgM class antibody against Spotted Fever Rickettsia in human serum or plasma

For in-vitro diagnostic use only.

CE

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INTENDED USE

The Spotted Fever Rickettsia EIA IgM Antibody Kit is intended for the detection and quantitation of IgM class human antibody to Spotted Fever Group Rickettsia, to be used as an aid in the diagnosis of human infection by these pathogens.

SUMMARY AND EXPLANATION OF TEST

Spotted Fever Group Rickettsia (SFG) are found worldwide and are generally mediated by ticks, whose bite transfers an infection derived from the more natural hosts of this organism. 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 proteins (rOmp A and rOmp B), which contain both species-specific and more broadly reactive determinants. Antigens used in this assay were purified from ***Rickettsia rickettsii,*** yet will react with antibody ***Rickettsia montanensis***, ***Rickettsia parkeri*** and other closely related species found in the Americas. Patient sera are diluted in a Sample Diluent and 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 OMP extracted from *Rickettsia rickettsii* 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.

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

White top vial contains a solution of 25% Tween 20 and 75% PBS. Add entire contents to 1 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 2-4 week intervals to check for titer changes. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine specific stability criteria for its laboratory

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 water and mixing thoroughly:

2. **Prepare 1:10 dilutions for all patient sera in IgM Serum Prep**. Mix well and allow at least 5 minutes for precipitin aggregates to develop. This step should be performed in microcentrifuge tubes or in test tubes. Separate diluted serum by centrifugation. Note: Controls are prediluted at 1:10 already.

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

4. **Prepare 1:10 dilutions of Negative Control, Positive Control and Cutoff Calibrator in Sample Diluent. Final dilutions are 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 serum and diluted Control 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 HRP 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 the dark.

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 AND ACCEPTANCE CRITERIA

RESULTS:

A Cutoff Calibrator is provided for discrimination between reactive and non-reactive sera. By dividing the Absorbance values of test sera by the average Absorbance 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 can be considered equivocal. Indices above 1.2 are considered positive and those below 0.8 are considered negative.

Alternatively, by utilizing 2-3 wells for the Cutoff Calibrator, the observed range of these values can define the Equivocal range surrounding the mean Calibrator value. The observed %CV should normally be < 10%.

RUN ACCEPTANCE CRITERIA:

The following absorbance ranges and ratios must be met prior to acceptance of test results:

Negative Control < 0.2 absorbance units
Positive Control > 0.8 absorbance units
Cutoff Calibrator 0.25-0.55 absorbance units

Cutoff Calibrator / Negative Control ratio > 1.5
Positive Control / Cutoff Calibrator ratio > 2.0

These absorbance values and ratios should be monitored over time to detect any changes or trends that may affect patient results.

EXTERNAL CONTROLS:

It is highly recommended that in-house or external controls be included in each assay run and monitored for reproducibility and trend over time. One such control should be slightly below the Equivocal absorbance range (Index < 0.8) and another slightly above the Equivocal absorbance range (Index > 1.2). It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine specific normal ranges and interpretations for its laboratory

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 testing, sera reacting only to LPS are most often false-positive[2].

This procedure detects antibody to closely related members of the spotted fever group (SFG), including R. rickettsii, R. montanensis, R. parkeri, R. conorii and R. africae. Reactivities of less related species (R. akari, R. australis, R. felis and others) is decreased. Crossreactivity to typhus fever group or scrub typhus is not detected.

EXPECTED VALUES

The prevalence of specific antibodies varies depending upon the geographic region and population being tested. Positives are only to be expected in acute cases. Sera from a blood donor panel (n=184) were found to be nagative with this test.

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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