DATA SHEET

Rickettsia conorii EIA

IgM Antibody Kit

Catalog Number: RCM-96K

Size: 96 test

Storage: 2-8°C

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

For in-vitro diagnostic use only.

INTENDED USE

The Rickettsia conorii EIA IgG Antibody Kit is intended for the detection and quantitation of IgG class human antibody to Rickettsia conorii, to be used as an aid in the diagnosis of human infection by this pathogen.

SUMMARY AND EXPLANATION OF TEST

Spotted Fever Group Rickettsia (SFG) are found worldwide and are generally mediated by ticks. 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 the immunodominant outer membrane protein (rOmpB), which contain both species-specific and more broadly reactive determinants. Antigen used in this assay was purified from Rickettsia conorii conroii, yet react to a lesser degree like antigens from Rickettsia rickettsii, Rickettsia slovaka and Rickettsia africae. Patient sera are diluted in a Sample Diluent and incubated in the coated microwells to allow binding of serum antibody to the solidphase antigens. The microwells are then washed to remove unreacted serum proteins, and an enzyme-labelled anti-human IgG (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.

REAGENTS AND MATERIALS SUPPLIED

96-microwell EIA Module
12 x 8-microwell strips coated with rOmpB purified from Rickettsia conorii and packaged with desiccant, ready to use.
IgM Serum Prep, 10 mL
Buffer containing goat anti-human IgG (Fc(□)specific), ready to use.
Sample Diluent, 2 X 50 mL

Gentaur Molecular Products
Voortstraat 49
1910 Kampenhout, Belgium

PBS buffer containing bovine serum albumin and Tween.
Enzyme Conjugate, 12 mL
Affinity-purified peroxidase-labeled goat anti-human IgM (chain-specific), ready to use. Protect from light.
Positive Control, 120 µL
Blue cap vial contains reactive human serum pre-diluted 1:10.
Cutoff Calibrator , 200 µL
Green cap vial contains equivocally reactive human serum pre-diluted 1:10.
Negative Control, 120 µL
Red cap vial contains non-reactive human serum prediluted 1:10.
TMB Substrate, 12 mL
A solution containing H2O2 and tetramethylbenzidine (TMB) supplied in an amber bottle. Ready to use. Protect from light.
Stop Solution, 12 mL
Diluted sulfuric acid ready to use. Avoid contact with skin.
PBS, 1 liter
Add supplied packet to 1 liter purified water to produce PBS Buffer pH 7.2. Mix thoroughly.
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„aC. Bring them to room temperature (20-25„aC) 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„aC. If testing is to be delayed longer than 5 days, store samples at -20„aC 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.

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 a separate dilution plate or in test tubes.

GENTAUR

Controls are prediluted at 1:10 already.

3. Prepare further dilutions of the mixtures prepared in Step 3 (above). Dilute these mixtures 1:10 in Sample Diluent to give final serum dilution of 1:100. See Step 2 (above) for dilution of Sample Diluent concentrate.

4. Prepare 1:10 dilutions of Negative Control, Positive Control and Cutoff Calibrator in Sample Diluent. Final dilution is 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.

GENTAUR

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 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 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.

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

This procedure detects antibody to closely related members of the spotted fever group (SFG). Reactivities of less related species (R. akari, R. australis, R. felis and others) is decreasedor absent. Crossreactivity to typhus 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. Positives are only to be expected in 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.

GENTAUR