INSTRUCTIONS FOR USE

Spotted Fever Rickettsia IgG
EIA Antibody Kit

Catalog Number: SFG-96K

Size: 96 test
(12 x 8-microwells)

Storage: 2-8˚C

An Indirect enzyme immunoassay for the detection of IgG 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 IgG Antibody Kit is intended for the detection of human IgG class 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 are found worldwide and are mediated by ticks and mites (*Rickettsia akari*) whose bite transfers an infection. This 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 a group-specific lipopolysaccharide (rLPS) antigen extracted from ***Rickettsia rickettsii,*** a member of the Spotted Fever Group. Other species sharing this serologic crossreactivity include ***Rickettsia conorii*** (Boutonneuse fever), ***Rickettsia siberica*** (Siberian tick typhus), ***Rickettsia australis*** (Queensland tick typhus), ***Rickettsia akari*** (Rickettsialpox) and many others. 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 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 or spectrophotometer.

REAGENTS AND MATERIALS SUPPLIED

MW | Ag **EIA-microwells (96)**

12 x 8-microwell strips coated with rLPS extracted from *Rickettsia conorii* (or R. rickettsii) 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 peroxidase-labeled goat anti-human IgG (gamma chain-specific), ready to use. **Protect from light**.

CONT | + **Positive Control, 120 µL**

Blue cap vial contains human serum reactive with Spotted Fever Rickettsia, bottled at a 1:10 dilution

CAL | ± **Cutoff Calibrator , 200 µL**

Green cap vial contains human serum equivocally reactive with Spotted Fever Rickettsia, bottled at a 1:10 dilution.

CONT | – **Positive Control, 120 µL**

Red cap vial contains human serum non-reactive with Spotted Fever Rickettsia, bottled at a 1:10 dilution.

SUBS | TMB **TMB Substrate, 12 mL**

A solution containing H_2O_2 and tetramethylbenzidine (TMB). 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. To make 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

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. Although assay microwells are prepared with inactivated antigens, they should also 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; while convalescent specimens should be obtained at 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. Precision pipette(s) for microliter range
4. Humid chamber for microwell incubations.
5. EIA reader equipped with a 450nm filter.

Precautions

1. Do not use components past expiration date.
2. TMB-substrate and Conjugate are photosensitive. Store in the dark and return to storage after use.
3. Liquid reagents contain thimerosal at 0.001%, 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 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. Terminate 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, while those above 1.1 are considered positive and those below 0.9 are considered negative.

LIMITATIONS

This procedure detects group-specific antibody and is, thus, unable to differentiate between members of the spotted fever group.

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.

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