INSTRUCTIONS FOR USE

Rickettsia felis EIA IgG Antibody Kit

Catalog Number: RFG-96K

Size: 96 test

Storage: 2-8˚C

An Indirect enzyme immunoassay for the detection of IgG class antibody against *Rickettsia felis* in human serum or plasma

For in-vitro diagnostic use only.

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INTENDED USE

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

SUMMARY AND EXPLANATION OF TEST

Spotted Fever Group Rickettsia are found worldwide and are mediated by ticks (most species), mites (*Rickettsia akari*) and fleas (*Rickettsia felis*)) whose bite transfers an infection. The ensuing infection induces a specific antibody response, which may be detected and used as an indirect means of identifying an infected person.

The EIA test microwells in this kit utilize the immunodominant outer membrane protein (native Omp B), which contains both species-specific and more broadly reactive determinants. Antigens used in this assay were purified from ***Rickettsia felis,*** yet may show some level of crossreactivity with antibody to ***Rickettsia akari, Rickettsia austalis*** or felis-like strains.

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 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

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

12 x 8-microwell strips coated with native OmpB protein purified from *Rickettsia felis* and packaged with desiccant, ready to use.

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

PBS containing bovine serum albumin and Tween-20.

CONJ | ENZ **Enzyme Conjugate, 12 mL**

Affinity-purified peroxidase-labeled goat anti-human IgG (gamma chain-specific), ready to use.

CONT | + **Positive Control, 120 µL**

Blue cap vial contains positive human serum diluted 1:10.

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

Green cap vial contains weakly reactive human serum diluted 1:10.

CONT | – **Negative Control, 120 µL**

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

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

A solution containing H_2O_2 and tetramethylbenzidine (TMB) supplied in an amber bottle. Ready to use.

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 Tween 20 in PBS. Add contents to 1 liter PBS to prepare Wash Buffer.

Warnings

1. Since no testing can assure the absence of infectious agents 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 packaging. If all 12 antigen strips are not used when the package is opened, the unused strips must be immediately returned to the package with desiccant pouches and tightly resealed. Storage in a desiccator is recommended.

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 (2 mL) of Tween 20 bottle to 1 liter PBS and mixing thoroughly:

2. **Prepare 1:100 dilutions** of all patient serum specimens using Sample Diluent. Note that the Controls and Cutoff Calibrator have been packaged at a 1:10 dilution.

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. 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.
3. Liquid reagents contain thimerosal at 0.05%, 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 into appropriate microwells.

2. Dilute the Positive Control, Cutoff Calibrator and Negative Control 1:10 in Sample Diluent. Replicate wells are recommended for the diluted Cutoff Calibrator.

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

4. 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.

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

6. Wash microwells as in step 5 above.

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

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

9. 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).

LIMITATIONS

This procedure may detect antibody to related members of the spotted fever group (R. akari and R. australis). 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. Endemic areas have been reported with seropositive rates of 7-26%, some of which were undoubtedly mild or subclinical cases.

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