DRG® Rickettsia conorii IgM ELISA (EIA-4613)

REVISED 4 SEPT. 2012 RM (VERS. 3.1) **RUO IN THE USA**

This kit is intended for Research Use Only.

Not for use in diagnostic procedures.

Please use only the valid version of the package insert provided with the kit.

1 INTENDED USE

The Rickettsia conorii IgM ELISA is intended for measurement of IgM class human antibody to Rickettsia conorii.

2 SUMMARY AND EXPLANATION OF TEST

The ELISA test microwells in this kit utilize the immunodominant outer membrane protein (rOmpB), which contain both species-specific and more broadly reactive determinants. Antigens used in this assay were purified from ***Rickettsia conorii,*** yet react much like antigens from ***Rickettsia rickettsii, Rickettsia slovaka*** and ***Rickettsia africae***.

Specimen sample 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 450 nm on a microtiter plate reader or spectrophotometer.

3 REAGENTS AND MATERIALS SUPPLIED

1. **Microtiter wells,** 12 x 8-microwell strips
   96-microwell EIA Module, coated with rOmpB purified from *Rickettsia conorii* and packaged with desiccant, ready to use.
2. **IgM Serum Prep,** 10 mL
   Buffer containing goat anti-human IgM, ready to use.
3. **Sample Diluent,** 2 X 50 mL
   PBS buffer containing bovine serum albumin and Tween.
4. **Enzyme Conjugate,** 10.5 mL
   Affinity-purified peroxidase-labeled goat anti-human IgM (µ-chain-specific), ready to use.
   Protect from light.
5. **High Control,** 120 µL
   Blue cap vial contains reactive human serum pre-diluted 1:10.

Fax: (908) 233-0758 • **E-mail:** corp@drg-international.com •**Web:** www.drg-international.com

6. **Calibrator control,** 120 µL
   Green cap vial contains equivocally reactive human serum pre-diluted 1:10.
7. **Low Control,** 120 µL
   Red cap vial contains non-reactive human serum prediluted 1:10.
8. **TMB Substrate Solution,** 12 mL
   A solution containing H_2O_2 and tetramethylbenzidine (TMB) supplied in an amber bottle.
   Ready to use. Protect from light.
9. **Stop Solution,** 12 mL
   Diluted sulfuric acid ready to use. Avoid contact with skin.
10. **PBS,** 1 liter
    Add supplied packet to 1 liter purified water to produce PBS Buffer pH 7.2.
    Mix thoroughly.
    (See Preparation of Wash buffer in Chapter 5, step 1.)
11. **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.
    (See Preparation of Wash buffer in Chapter 5, step 1.)

3.1 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.

3.2 Storage and Handling

Kit components should be stored at 2 °C - 8 °C.

Bring them to room temperature (20 °C - 25 °C) before opening bottles and plate pouches.

Unused antigen strips should be returned to the package with desiccant and tightly resealed

4 SPECIMEN COLLECTION

Allow blood samples to clot and separate sera by centrifugation. Transfer sera aseptically to tightly closing sterile containers.

Store at 2 °C - 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.

5 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 specimen sample 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.
   Controls are prediluted at 1:10 already.
3. **Prepare further dilutions of the mixtures prepared in Step 2 (above).**
   Dilute these mixtures 1:10 in Sample Diluent to give final serum dilution of 1:100.
4. **Prepare 1:10 dilutions of Low Control, High Control and Calibrator in Sample Diluent.**
   Final dilution is now 1:100.

6 PROCEDURE

The kit supplies sufficient reagents and materials for 96 determinations.

6.1 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 450 nm filter.

6.2 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.

Fax: (908) 233-0758 • E-mail: corp@drg-international.com •Web: www.drg-international.com

6.3 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 Calibrator.
2. Cover microwells to minimize evaporation, then **incubate for 60 minutes at ambient temperature** (20 °C - 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 100 µL IgM 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 Solution** 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 **450 nm filter**.

7 QUALITY CONTROL

A Calibrator control is provided for discrimination between reactive and non-reactive sera.

The Calibrator control is set at an index of 1.0.
By dividing the Absorbance values of the test sera by the mean Absorbance value of the Calibrator control, an index value for each serum is derived.

8 LIMITATIONS

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

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 decreased.

Cross reactivity to typhus fever group or scrub typhus is, in general, not detected.

9 REFERENCES

1. La Scola, Bernard and Didier Raoult. J. Clin. Microbiol. 1997; 35: 2715 – 2727

Fax: (908) 233-0758 • E-mail: corp@drg-international.com •Web: www.drg-international.com

2. Raoult, Didier and Gregory A. Dasch. J. Clin. Microbiol. 1989; 27: 2073 – 2079

Rev. 8/21/12cc