INSTRUCTIONS FOR USE

Ehrlichia muris IFA
IgG Antibody Kit

Catalog Number: EMG-120

Size: 120 test

Storage: 2-8˚C

An indirect fluorescence immunoassay for the detection of IgG class antibody against *Ehrlichia muris* in human serum or plasma

For in-vitro diagnostic use only

CE

FULLER LABORATORIES

1135 E. Truslow Avenue
Fullerton, California 92831 USA
Phone: +1-714-525-7660
Fax +1-714-525-7614
Email:info@fullerlabs.com
www.fullerlaboratories.com

EC REP

MediMark Europe Sarl
11, rue Émile Zola – BP 2332
F-38033 Grenoble Cedex 2 – France

INTENDED USE

The *Behrlichia muris* IgG Antibody kit is intended for the detection and semi-quantitation of IgG class human antibody to *Ehrlichia muris.*

SUMMARY AND EXPLANATION OF TEST

Human monocytic ehrlichiosis was first recognized in 1987 by means of the characteristic cytoplasmic inclusions found in peripheral blood monocytes. The serologic response of these patients was initially found to be reactive with *Ehrlichia canis*, a previously recognized veterinary pathogen. Since then the genus Ehrlichia has added E. ewingii, E. muris and the Panola Mountain strain (PME), with all three having the ability to cause disease in humans. This IFA assay utilizes a macrophage cell line infected with Ehrlichia muris to detect antibodies induced by this agent.

Patient sera are diluted in buffered saline and incubated in the individual slide wells to allow reaction of patient antibody with the antigens. Slides are then washed to remove non-reacting serum proteins, and fluorescence-labeled anti-human IgG (conjugate) is added. This conjugate is allowed time to react with antigen-antibody complexes. The slides are washed again to remove non-reacting conjugate. The resulting reactions can be visualized using standard fluorescence microscopy, where a positive reaction is seen as sharply defined apple-green fluorescent inclusions (morulae) in the cytoplasm of infected cells. A negative reaction is seen as either red-counterstained cells or fluorescence unlike that seen in the positive control well. Positive reactions may then be retested at higher dilutions to determine the highest reactive or endpoint dilution.

REAGENTS

IFA | Ag x 12 **Substrate Slides (10)**

10x12-well masked slides containing fixed *Ehrlichia muris*-infected DH82 cells, packaged under vacuum and ready to use.

CONJ | FITC **IgG Conjugate, 2.5 mL**

Dropper bottle with a yellow cap contains affinity-purified DyLight 488-labeled goat anti-human IgG (heavy chain) with bovine serum albumin and Evans' blue counterstain.

CONT | + **Positive Control, 0.5 mL**

Dropper bottle with a blue cap contains reactive serum, provided at a 1:64 screening dilution. Endpoint titer is 1:512.

CONT | – **Negative Control, 0.5 mL**

Dropper bottle with a red cap contains non-reactive serum at a 1:50 dilution.

MM **Mounting Medium, 1 mL**

Dropper bottle with white cap contains 50% glycerol in PBS.

BUF | WASH | PBS **PBS, 1 liter**

Add supplied powder to 1 liter purified water to produce phosphate-buffered saline at pH 7.2.

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 specimens and equipment coming in contact with these specimens, should be handled with good laboratory practices to avoid skin contact and ingestion.

2. The substrate slides are prepared with chemically inactivated antigens. However, the slides should be considered potentially infectious and handled accordingly.

Storage and Handling

Kit components should be stored at 2-8°C. Bring to room temperature (20°-25°C) before opening bottles or slide envelopes.

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, freeze samples at or below -20°C. Acute specimens should be drawn at the onset of illness; convalescent specimens should be obtained at intervals to check for titer changes**.**

PROCEDURE

The kit supplies sufficient materials for 120 determinations.

Materials Required But Not Supplied

- Distilled or deionized water
- Clean 250 or 500 mL wash bottle for PBS
- Test tubes or microtiter plate for serum dilutions
- Precision pipette(s)
- 24 x 50 mm glass coverslips
- Fluorescence microscope with filter system for FITC (maximum excitation wavelength 490 nm, mean emission wavelength 530 nm) and 400X magnification.
- 37° water bath or incubator
- Humid chamber for slide incubation steps.

Precautions

- Do not use components past expiration dates.
- Conjugate is photosensitive and is bottled in opaque plastic for protection. Store in the dark and return to storage after use.
- Conjugate contains Evans' blue dye, which may be carcinogenic. Avoid contact with skin.
- Liquid reagents contain thimerosal at 0.001%, which may be toxic if ingested

Preparation of Reagents

PBS: Add contents of packet to 1 liter purified water. Mix until all salt crystals are dissolved.

ASSAY PROCEDURE

Allow all reagents and sera to reach ambient temperature before starting timed assay procedure.

1. Prepare 1:64 screening dilutions in PBS for all untested patient sera. For sera found positive on a previous assay run, prepare serial dilutions in PBS, starting with 1:64.

2. Prepare further dilutions of the Positive Control to include 1:8 (endpoint) and 1:16 (negative).

3. For each serum dilution, add 10 µL to one slide well and record the location for later reference. For each assay run include the Negative Control and dilutions of the Positive Control prepared above.

4. Place slides in a humid chamber and incubate for 30 minutes at 37°± 0.5°C.

5. Remove humid chamber from incubator. Also remove conjugate from storage. Rinse slide wells with gentle stream of PBS from wash bottle. Shake or tap beaded PBS from slides into a sink, then repeat this wash step 2X without allowing the wells to dry.

6. To each slide well add 1 drop (10 µL) Conjugate, then return slides to the humid chamber for another 30 minutes incubation at 37°± 0.5°C. Incubation should be in the dark to protect the photosensitive conjugate.

7. Wash slides as in step 5, above.

8. Add 3-4 drops of Mounting Medium to each slide and apply coverglass.

9. Read the stained substrate slides at 400X magnification, comparing each well to the visual intensity and appearance of Positive and Negative Control wells. Slides may be stored at 2-8°C in the dark for up to 24 hours.

QUALITY CONTROL

The Negative Control serum and dilutions of the Positive Control serum should be assayed with each daily run. The Negative Control well is an example of a non-reactive serum, with either uniform red counterstain or slight, but uniform greenish staining. The Positive Control wells should give an endpoint titer from 1:256 to 1:1024 (1:4-1:16 of bottled Control). The fluorescence intensity at 1:512 (1:8 of bottled Control may be used as the cut-off level required for a patient reaction to be called positive. If either of the Controls does not react as specified, the assay run should be considered void, reagent components and procedural steps should be rechecked, and the assay repeated from step #1.

The Negative Control well is an example of fluorescence patterns that are to be considered negative. If characteristic inclusions are seen in this well, similar to that seen in the Positive Control wells, there has been a breakdown in technique and the assay must be repeated.

INTERPRETATION OF RESULTS

A positive reaction appears as cytoplasmic clusters of distinct apple-green inclusion bodies within the infected cells. The size, appearance and density of the reaction must be compared with the Positive and Negative Control reactions.

Patient Specimens

Positive at 1:64 screening dilution: IgG titers ≥1:64 are considered to reflect infection at an undetermined time by *Ehrlichia muris* or closely related organism *(E. ewingii, E.chaffeensis* or PME*).* Sera positive at screening dilution should be titered to endpoint for comparison with earlier or later specimens from the same patient. IgM titers or 4-fold increase in titer are also a reliable indicator of recent infection**.**

Negative at 1:64: Report as negative for *Ehrlichia muris* antibody. Further serum specimens should be drawn if the original was taken soon after onset and this etiology is still suspected**.**

LIMITATIONS

Crossreaction with Ehrlichia chaffeensis and Ehrlichia ewingii is strong and cannot be differentiated by IFA from infection with Ehrlichia muris, except by direct titer comparison.

Original 4/2011