INSTRUCTIONS FOR USE

Babesia microti IFA
IgM Antibody Kit

Catalog Number: BMM-120

Size: 120 test

Storage: 2-8˚C

An indirect fluorescence immunoassay for the detection of IgM class antibody against ***Babesia microti*** in human serum or plasma

For in-vitro diagnostic use only

CE

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INTENDED USE

The *Babesia microti* IgM Antibody kit is intended for the detection and semi-quantitation of IgM class human antibody to *Babesia microti*.

SUMMARY AND EXPLANATION OF TEST

One of the known causes of human babesiosis is the protozoan *Babesia microti*, which is transmitted by the bite of infected ticks. Historically diagnosis is made by the demonstration of characteristic intra-erythrocytic inclusions in thin-smear preparations of peripheral blood, although PCR methods are increasingly used to advantage. The serologic response of these patients is specific for *Babesia microti*, although titer variability in seen with other sensu stricto strains and, especially, with *Babesia microti* sensu lato strains found worldwide. The IFA assay utilizes human type "O" erythrocytes infected with GI (Gray) strain as a source of characteristic inclusions.

Patient sera are diluted in an IgM Sample Diluent containing antiserum to human IgG-class antibody. This reaction (precipitin) removes competing IgG-class antibody and the source of rheumatoid factor interference. Treated sera are incubated in the individual slide wells to allow reaction of patient IgM class antibody with the fixed antigens. Slides are then washed to remove unreacted serum proteins, and fluorescence-labeled anti-human IgM (Conjugate) is added. This conjugate is allowed time to react with antigen-antibody complexes, then washed off. The resulting reactions can be visualized using standard fluorescence microscopy, where a positive reaction appears as sharply-defined apple-green fluorescent inclusions within the infected erythrocytes. 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)**
10 x 12-well masked slides containing fixed human erythrocytes infected with *Babesia microti* (Gray strain), packaged under vacuum.

CONJ | FITC — **IgM Conjugate, 2.5 mL**
Dropper bottle with a yellow cap contains affinity-purified Alexafluor 488-labeled goat anti-human IgM (heavy chain) with bovine serum albumin and Evans' blue counterstain.

CONT | + — **Positive Control, 0.2 mL**
Microtube with a blue cap contains reactive human serum with endpoint titer of 1:128.

CONT | – — **Negative Control, 0.2 mL**
Microtube with a red cap contains non-reactive human serum with endpoint titer <1:16.

IgM | DIL — **IgM Sample Diluent, 15 mL**
Buffer contains goat anti-human IgG antibody in PBS.

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 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 them to room temperature (20°-25°C) before opening bottles or slide envelopes. For longer storage of IFA slides maintain them at or below -20°C.

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 date.**
- **Conjugate is photosensitive.**
- **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**

ASSAY PROCEDURE

1. **Prepare 1:16 screening dilutions (1 part serum with 15 parts IgM Sample Diluent) for all patient sera, Positive Control and Negative Control. Allow 10-15 minutes for precipitin aggregates to stabilize. For sera found positive on a previous assay run, prepare serial two-fold dilutions of the screening dilution in PBS.**

2. **Prepare dilutions of the Positive Control (treated in Step 1) in PBS to include 1 dilution above the stated endpoint (1:128) and one dilution below.**

3. **For each serum dilution apply 10 µL to one slide well. For each assay include the Negative Control, Positive Control and dilutions of the Positive Control prepared above.**

4. **Place slides in a humid chamber and incubate for 90 minutes at 37°± 0.5°C.**

5. **Rinse slide wells with gentle stream of PBS from wash bottle into waste container. Aim steam of buffer at the space between the rows of 6 wells to wash diluted serum off the wells without cross-contaminating other test wells. 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 a 30 minute 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 several drops of Mounting Medium to each slide and apply cover glass.**

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 1:64 to 1:256 with full antigen density, as seen in the Positive Control. The fluorescence intensity at 1:128 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.

INTERPRETATION OF RESULTS

A positive reaction appears as apple-green fluorescent intra-erythrocytic inclusions, from simple (ring-forms) to complex (tetrad) forms. The size, appearance and density of the reaction must be compared with the Positive and Negative Control reactions.

Patient Specimens

Positive at 1:16 screening dilution: IgM titers of 1:16 and greater are considered to reflect recent or active infection by *Babesia microti*. Sera positive at the 1:16 screening dilution should be rerun to determine endpoint titers for comparison with earlier or later specimens from the same patient. Elevated IgG titers, when present, are an indicator of infection at an undetermined time.

Negative at 1:16: Report as negative for *Babesia microti* IgM antibody. Further serum specimens should be drawn if the original was taken soon after onset and this etiology is still suspected.

LIMITATIONS

Cross-reactivity with Plasmodium spp has been documented.

Version B
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