

INSTRUCTIONS FOR USE

Babesia microti IFA IgG Antibody Kit

Catalog Number: BMG-120

Size: 120 tests

Storage: 2-8°C

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

For in-vitro diagnostic use only



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INTENDED USE

The *Babesia microti* IgG Antibody kit is intended for the detection and semi-quantitation of IgG 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 is 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 a Serum Diluent and incubated in the individual slide wells to allow reaction of patient antibody with the *Babesia microti* antigens. Slides are then washed to remove unreacted 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 unreacted Conjugate. 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)
10x12-well masked slides containing fixed *Babesia microti* (GI strain)-infected human type O+ erythrocytes, packaged under vacuum.

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

CONT + Positive Control, 0.5 mL
Dropper bottle with a blue cap contains reactive human 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 human 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 into 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 date.
- Conjugate is photosensitive and is packaged 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 dilutions of the Positive Control to include 1 dilution above the stated endpoint and one dilution below (ie. 1:256-1:1024). Note that this Control is bottled at a dilution of 1:64.

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 and 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 3X 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 2-3 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. The apple-green fluorescence intensity at 1:512 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 apple-green inclusions are seen in this well, similar to those 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 peripheral clusters of distinct apple-green inclusion bodies within the infected erythrocytes. 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 of 1:64 and greater are considered to reflect infection at an undetermined time by *Babesia microti*. Sera positive at the 1:64 screening dilution should be rerun to determine their endpoint titer for comparison with earlier or later specimens from the same patient. IgM titers, when present, are also a reliable indicator of recent infection.

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

Paired Sera: A four-fold increase in IgG titer between acute and convalescent serum specimens supports the diagnosis of recent infection.

LIMITATIONS

Crossreaction with Plasmodium spp has been documented in the literature.

REFERENCES

Krause, P. J., S. R. Telford 3rd, R. Ryan, P. A. Conrad, M. Wilson, J. W. Thomford, and A. Spielman. 1994. Diagnosis of babesiosis: evaluation of a serologic test for the detection of *Babesia microti* antibody. J. Infect. Dis. 169:923-926

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