INSTRUCTIONS FOR USE

Babesia bovis IFA
IgG Antibody Kit

Catalog Number: BVG-120

Size: 120

Storage: 2-8˚C

An Indirect fluorescence immunoassay for the detection of IgG class antibody against *Babesia bovis* in bovine serum or plasma

For in-vitro diagnostic use only

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INTENDED USE

The *Babesia (Theileria) equi* IFA IgG Antibody kit is intended for the detection and semi-quantitation of IgG class bovine antibody to *Babesia bovis.*

SUMMARY AND EXPLANATION OF TEST

B. bovis and *B. bigemina* are important causative agents of bovine babesiosis in tropical and subtropical regions of the world. They are 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. The serologic response of *Babesia bovis* infected animals is specific (see Limitations). The IFA assay utilizes infected bovine erythrocytes as a source of characteristic inclusions.

Patient sera are diluted in buffered saline and incubated in the individual slide wells to allow reaction of patient antibody with the *Babesia bovis* antigens. Slides are then washed to remove unreacted serum proteins, and DyLight 488-labeled anti-bovine IgG (Conjugate) is added to each well. This conjugate is allowed time to react with bound serum antibody. The slides are washed again to remove unreacted Conjugate. The resulting reactions can be visualized using standard fluorescence microscopy, where a positive reaction is seen as sharply defined apple-green fluorescent inclusions within the infected erythrocytes. 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 x 12-well masked slides containing fixed bovine erythrocytes infected with *Babesia bovis* and packaged under vacuum..

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

CONT | + **Positive Control, 0.5 mL**
Dropper bottle with a blue cap contains pooled reactive bovine serum and is considered to be at a screening dilution (1:40) as bottled. Endpoint titer is 1:320.

CONT | – **Negative Control, 0.5 mL**
Dropper bottle with a red cap contains pooled non-reactive bovine serum at a 1:40 dilution.

MM **Mounting Medium, 1 mL**
Dropper bottle with a white cap contains 50% glycerol in PBS, pH 7.2.

BUF | WASH | PBS **PBS, 1 liter**
Add supplied powder to 1 liter purified water to produce phosphate-buffered saline at pH 7.2.

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.

SPECIMEN COLLECTION
Allow blood sample 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, freezing the sample at -20°C or colder is recommended. Acute specimens should be drawn at the onset of illness; convalescent specimens should be obtained at two or four week 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.
- Liquid components contain 0.01% thimerosal as preservative. Do not ingest.
- Conjugate contains Evans' blue dye, which may be carcinogenic. Avoid contact with skin.

Preparation of Reagents

PBS: Add contents of packet to 1 liter purified water. Rinse out any salt crystals remaining in the packet. Mix until all salt crystals are dissolved.

ASSAY PROCEDURE

1. Prepare 1:40 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 1:40.

2. Prepare dilutions of the Positive Control to include 1 dilution above the stated endpoint and one dilution below (ie. 1:160-1:640). Note that controls have already been diluted 1:40 before bottling.

3. For each serum dilution add 10 µL to a 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. Do not aim stream of wash buffer directly at slide wells. 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 3-4 drops of Mounting Medium to each slide and apply coverglass, carefully removing air bubbles caught underneath.

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 1:160 to 1:640. The fluorescence intensity at 1:640 may be used as the cut-off level required for a test 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 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 1:40 or higher: Titers of 1:40 and greater are considered positive. Sera positive at the 1:40 screening dilution should be rerun to determine their endpoint titer for comparison with earlier or later specimens from the same animal.

Negative at 1:40: Report as negative for *Babesia bovis* antibody.

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

LIMITATIONS
Crossreaction with other *Babesia* species has been documented, although differentiation is generally not difficult by comparing titers.

REFERENCE

"Bovine Babesiosis" In Manual of Standards for Diagnostic Tests and Vaccines. Paris: World Organization for Animal Health, 2000,Chapter 2.3.8.

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