INSTRUCTIONS FOR USE

Babesia bigemina IFA
IgG Antibody Kit

Catalog Number:	BIG-120
Size:	120 test
Storage:	2-8˚C

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

For in-vitro diagnostic use only

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INTENDED USE

The *Babesia bigemina* IgG Antibody kit is intended for the detection and semi-quantitation of IgG class bovine antibody to *Babesia bigemina.*

SUMMARY AND EXPLANATION OF TEST

Babesia bovis and *Babesia 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 bigemina* infected animals is specific (see Limitations). The IFA assay utilizes infected bovine erythrocytes as a source of characteristic inclusions.

Sera samples are diluted in buffered saline and incubated in the individual slide wells to allow reaction of antibody with the solid-phase *Babesia bigemina* antigens. Slides are then washed to remove unreacted serum proteins, and DyLight 488-labeled anti-bovine 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 is seen as sharply defined apple-green fluorescent inclusions within the infected erythrocytes. A negative reaction is seen as either no fluorescence or fluorescence unlike that seen in the Positive Control wells. 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 *Babesia bigemina* –infected bovine erythrocytes, packaged under vacuum.

CONJ | FITC **IgG 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 reactive bovine serum, provided at a 1:80 screening dilution. Endpoint titer is 1:640.

CONT | – **Negative Control, 0.5 mL**
Dropper bottle with a red cap contains non-reactive bovine serum at a 1:80 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
Since no testing can assure the absence of infectious agents, 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. 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.

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. Dylight 488 is comparable (493/518).
- 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

ASSAY PROCEDURE

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

1. Prepare 1:80 screening dilutions in PBS for all untested patient sera..

2. Prepare dilutions of the Positive Control to include 1 dilution above the stated endpoint and one dilution below (ie. 1:320-1:1280). Note that this Control is bottled at a dilution of 1:80.

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. 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 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 1:256 to 1:1024. The fluorescence intensity at 1:320 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 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 1:80 screening dilution: IgG titers of 1:840 and greater are considered positive (exposed).

Negative at 1:80: Report as negative for Babesia antibody.

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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