INSTRUCTIONS FOR USE

Babesia equi (Theileria equi) IFA IgG Antibody Kit

Catalog Number: BEG-120

Size: 120

Storage: 2-8°C

An Indirect fluorescence immunoassay for the detection of IgG class antibody against *Babesia equi* in equine 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 horse antibody to *Babesia equi.*

SUMMARY AND EXPLANATION OF TEST

Equine piroplasmosis is caused by the protozoans Babesia equi and Babesia caballi. It 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. The serologic response of these horses is specific for *Babesia equi* (see Limitations). The IFA assay utilizes infected horse erythro-cytes 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 equi* antigens. Slides are then washed to remove unreacted serum proteins, and FITC-labeled anti-horse 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**

10x12-well masked slides containing fixed horse erythrocytes infected with *Babesia equi.*

CONJ | FITC **Conjugate, 2.0 mL**

Dropper bottle contains affinity-purified FITC-labeled rabbit anti-horse IgG (heavy chain) with bovine serum albumin and Evans' blue counterstain.

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

Dropper bottle contains pooled reactive horse serum and is considered to be at a screening dilution (1:80) as bottled Endpoint titer is 1:640.

CONT | – **Negative Control, 0.5 mL**

Dropper bottle contains pooled non-reactive horse serum at a 1:80 dilution.

MM **Mounting Medium, 1 mL**

Dropper bottle 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.
- 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.

Preparation of Reagents

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

ASSAY PROCEDURE

1. Prepare 1:80 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 1:80.

2. Prepare dilutions of the Positive Control to include 1 dilution above the stated endpoint and one dilution below (ie. 1:320-1:128).

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 2-3 drops of Mounting Medium to each slide and place coverslip on, carefully removing air bubbles caught under the coverslip.

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 1:320 to 1:1280. 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 1:80 or higher: Titers of 1:80 and greater are considered positive. Sera positive at the 1:80 screening dilution should be rerun to determine their endpoint titer for comparison with earlier or later specimens from the same horse.

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

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

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

REFERENCE

"Equine Piroplasmosis." In Manual of Standards for Diagnostic Tests and Vaccines. Paris: World Organization for Animal Health, 2000, pp 558-564.

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