INSTRUCTIONS FOR USE

Equine Piroplasmosis MIF

IgG Antibody Kit

Catalog Number: EP2G-120

Size: 120

Storage: 2-8˚C

An Indirect micro-fluorescence immunoassay for the detection of IgG class antibody against ***Theileria equi*** and ***Babesia caballi*** in equine serum or plasma

For in-vitro diagnostic use only

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INTENDED USE

The Equine Piroplasmosis MIF IgG Antibody Kit is intended for the detection and semi-quantitation of IgG class equine antibody to *Theileria equi* and *Babesia caballi.*

SUMMARY AND EXPLANATION OF TEST

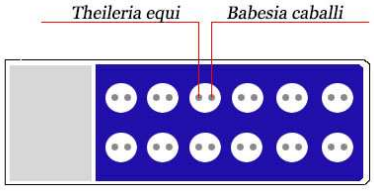
Equine piroplasmosis is caused by the protozoans **Theileria equi** (previously Babesia equi) and **Babesia caballi,** which are transmitted by infected ticks. Historically, diagnosis is made by the demonstration of characteristic intra-erythrocytic inclusions in thin-smear preparations of peripheral blood. The serologic response of these horses is specific for ***Babesia caballi*** and/or ***Theileria equi*** (see Limitations). This micro-IFA assay utilizes infected horse erythrocytes, propagated *in vitro,* as the 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 caballi* and *Theileria equi* antigen dots. Slides are then washed to remove non-reactive serum proteins, and fluorescence-labeled anti-horse IgG (conjugate) is added to each well. This conjugate is allowed time to react with bound serum antibody before the slides are washed again to remove non-reactive conjugate. The resulting reactions can be visualized using standard fluorescence microscopy, where a positive reaction is seen as sharply defined apple-green fluorescent inclusions. A negative reaction is seen as either red-counterstained background 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 merozoites in lysed equine erythrocytes infected with *Theileria equi* and *Babesia caballi,* as pictured below.



CONJ | FITC **Conjugate, 2.5 mL**

Dropper bottle with yellow cap contains affinity-purified Alexafluor 488-labelled goat anti-equine IgG (heavy chain) with bovine serum albumin and Evans' blue counterstain.

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

Dropper bottle with blue cap contains reactive horse serum and is bottled at screening dilution (1:80). Endpoint titer is 1:640.

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

Dropper bottle with blue cap contains reactive horse serum and is bottled at screening dilution (1:80). Endpoint titer is 1:640.

CONT | – **Negative Control, 0.5 mL**

Dropper bottle with red cap contains non-reactive horse serum at a 1:80 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, freeze the sample at -20°C or colder. 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 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

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

3. For each serum dilution carefully add 10 µL to a slide well and record the location for later reference. Add samples at top-center of the wells to avoid damaging the antigens. 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. 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 apply coverslip, carefully dislodging air bubbles from wells.

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 endpoint titers 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.

INTERPRETATION OF RESULTS

A positive reactions appear as small (*Theileria equi*) or large merozoites (*Babesia caballi*) distributed within the appropriate antigen dots. 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 1:80 and greater are considered to reflect exposure to *Babesia caballi* and/or *Theileria equi*.

Negative at 1:80: Report as negative for *Babesia caballi* and/or *Theileria equi* antibody.

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

LIMITATIONS

Crossreaction between antigens has been documented, although titer differential is sizable.

REFERENCE

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

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