INSTRUCTIONS FOR USE

***Neorickettsia risticii* IFA**
Equine IgG Antibody Kit

Catalog Number: ERE-120

Size: 120 test

Storage: 2-8˚C

An Indirect fluorescence immunoassay for the detection of IgG class antibody against *Neorickettsia risticii* in equine serum or plasma

For in-vitro diagnostic use only

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INTENDED USE

The *Neorickettsia risticii* IFA Equine IgG Antibody Kit is intended for the detection and semi-quantitation of IgG class equine antibody to *Neorickettsia risticii*.

SUMMARY AND EXPLANATION OF TEST

Substrate slides consist of teflon-masked wells containing fixed canine macrophage cells (DH82 cell line), approximately 25-35% of which are infected with *Neorickettsia risticii* and contain the characteristic cytoplasmic morulae. Equine sera are diluted in buffered saline and incubated in the individual slide wells to allow reaction of serum antibody with the solid phase antigens. Slides are then washed to remove unreacted serum and DyLight 488-labeled anti-equine 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 are visualized using standard fluorescence microscopy, where a positive reaction is seen as sharply defined apple-green fluorescent morulae within the cytoplasm of the infected cells in each field. A negative reaction is seen as either red-counterstained cells or fluorescence different than 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 x 12-well masked slides containing fixed *Neorickettsia risticii*-infected DH82 cells. Slides are fixed, packaged under vacuum and ready for use.

CONJ | FITC **Conjugate, 2.5 mL**
Yellow cap dropper bottle contains affinity-purified DyLight 488-labeled rabbit anti-equine IgG (heavy and light chain) with bovine serum albumin and Evans' blue counter stain.

CONT | + **Positive Control, 0.5 mL**
Blue cap dropper bottle contains pooled equine serum at a 1:80 screening dilution. Endpoint titer is 1:640.

CONT | – **Negative Control, 0.5 mL**
Red cap dropper bottle contains non-reactive equine serum at a 1:80 screening dilution.

MM **Mounting Medium, 1 mL**
White cap dropper bottle 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.

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 samples to clot and separate serum by centrifugation. Transfer serum aseptically to a tightly closing sterile container. Store at 2-8°C. If testing is to be delayed longer than 5 days, store sample ≤ -20°C. Acute specimens should be drawn at the onset of illness, with convalescent specimens obtained at intervals to check for titer changes.

PROCEDURE
The kit supplies sufficient reagents and 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° waterbath 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.

ASSAY PROCEDURE
1. Prepare 1:80 screening dilutions for all untested sera. For sera found positive on a previous assay run, prepare serial two-fold 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. 1:320-1:1280).

3. For each serum dilution apply 10 µL to one slide well and record the location for later reference. For each assay includes 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 washbottle. 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 10 µL (1 drop) Conjugate and 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 2-3 drops of Mounting Medium to each slide and apply coverslip.

9. Read the stained substrate slides at 400X magnification, comparing each well to the visual intensity and appearance of the Neorickettsia inclusions seen in the 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 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 one or more distinct apple-green phagosomes (morula) within the cytoplasm of infected cells. The size, appearance and density of the reaction must be compared with the Positive and Negative Control reactions.

Clinical Specimens

Positive at 1:80 screening dilution: IgG titers of 1:80 and greater are considered to reflect exposure infection at an undetermined time by *Neorickettsia risticii*. 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 *Neorickettsia risticii* antibody.

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

LIMITATIONS
Neorickettsia risticii is antigenically varied, with strain differences sufficient to produce titer variations depending upon the infecting and test strains. Antibody induced by vaccination is not distinguishable from naturally-aquired infection.

EXPECTED VALUES
The prevalence of *Neorickettsia risticii* antibodies varies depending upon the geographic region and population being tested.

REFERENCE

Ristic, M., C.J. Kolland, J.E. Dawson, J. Sessions, and J. Palmer. 1986. Diagnosis of equine monocytic ehrlichiosis (Potomac horse fever) by indirect immunofluorescence. J. Am. Vet. Med. Assoc. 189:39-46.

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