

## INSTRUCTIONS FOR USE

### ***Anaplasma phagocytophilum* IFA Equine Antibody Kit**

Catalog Number: EEE-120

Size: 120 test

Storage: 2 – 8 °C

An Indirect immunofluorescence assay for the detection and semi-quantitative determination of equine serum antibody against *Anaplasma phagocytophilum*

**For in-vitro diagnostic use only**



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### **INTENDED USE**

The *Anaplasma phagocytophilum* Equine Antibody Detection Kit is intended for the detection and semi-quantitation of IgG class antibody to *Anaplasma phagocytophilum* in equine sera

### **SUMMARY AND EXPLANATION OF TEST**

*Anaplasma phagocytophilum* has been recognized both in the U.S. and in Europe. This IFA assay utilizes *Anaplasma phagocytophilum*-infected HL60 cells to detect antibodies induced by infection by this agent. 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 proteins, and DyLight 488-labeled anti-horse IgG (H+L) 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 (morulae) in the cytoplasm of infected cells. 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)**

10x12-well masked slides containing fixed HL60 cells infected with *Anaplasma phagocytophilum*, packaged under vacuum and ready to use..

**CONJ FITC**

#### **FITC Conjugate, 2.5 mL**

Dropper bottle with a yellow cap contains affinity-purified DyLight 488-labeled rabbit anti-equine IgG (H+L) with bovine serum albumin and Evans' blue counterstain.

**CONT +**

#### **Positive Control, 0.5 mL**

Dropper bottle with a blue cap contains reactive 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 equine serum provided at a 1:80 screening dilution.

**MM**

#### **Mounting Medium, 1 mL**

Dropper bottle with a 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. Mix well.

### **Warnings**

1. The control sera have been screened for infectious agents by USDA required testing. Since no testing can assure the absence of infectious agents, however, 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.
2. 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 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, 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 2-4 weeks 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° 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
- Liquid reagents contain thimerosal at 0.001%, which may be toxic if ingested

## 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 (or 4-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. 1:200-1:800).
3. For each serum dilution to be tested add 1 drop (10 µL) to one slide well and record the location for later reference. For each assay run include the Negative Control, Positive 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 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 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.

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 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.

The Negative Control well is an example of fluorescence patterns that are to be considered negative. If characteristic morulae are seen in this well, similar to that 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 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 infection at an undetermined time by *Anaplasma phagocytophila*. 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 *Anaplasma phagocytophilum* antibody. Further serum specimens should be drawn if the original was taken soon after onset and this etiology is still suspected.

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

## LIMITATIONS

Crossreaction with *Ehrlichia* spp. by IFA is variable from weak to strong, and can be differentiated by a variety of alternate methods including western immunoblot technique.

Original Version 5/02

**Current Version 3/2004**