INSTRUCTIONS FOR USE

Anaplasma phagocytophilum IFA
Canine and Feline IgG Antibody Kit

Catalog Number: EEZ-120

Size: 120 test

Storage: 2 – 8 ˚C

An Indirect immunofluorescence assay for the detection and semi-quantitative determination of IgG class antibody against *Anaplasma phagocytophilum* in canine and feline serum or plasma

For in-vitro diagnostic use only

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INTENDED USE

The Anaplasma phagocytophilum Antibody Detection Kit is intended for the detection and semi-quantitation of IgG class canine and/or feline antibody to *Anaplasma phagocytophilum*.

SUMMARY AND EXPLANATION OF TEST

Both canine and feline granulocytic ehrlichiosis has been recognized both in the U.S. and in Europe. This IFA assay utilizes the human HL60 cell line infected with Anaplasma phagocytophilum to detect antibodies induced by this pathogen.

Canine and/or feline sera are diluted in buffered saline and incubated in the individual slide wells to allow reaction of patient antibody with the Anaplasma antigens. Slides are then washed to remove non-reactive serum proteins, and an DyLight 488-labeled anti-IgG (H+L) conjugate is added. This conjugate is allowed time to react with antigen-antibody complexes. 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 (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

Substrate Slides
10x12-well masked slides containing fixed HL60 cells infected with Anaplasma phagocytophilum and packaged under vacuum.

Conjugate, 2.5 mL
Affinity-purified FITC-labeled goat antisera to feline and canine IgG (H+L) with bovine serum albumin, 0.005% thimerosal, and Evans' blue counterstain.

Canine Positive Control, 0.5 mL
Pooled canine serum provided at a 1:50 screening dilution. Endpoint titer is 1:400.

Feline Positive Control, 0.5 mL
Pooled feline serum provided at a 1:50 screening dilution. Endpoint titer is 1:400.

Negative Control, 0.5 mL
Pooled canine serum provided at a 1:50 screening dilution.

Mounting Medium, 1 mL
Dropper bottle contains 50% glycerol in PBS, pH 7.2.

PBS, 1 liter
Add supplied powder to 1 liter purified water to produce phosphate-buffered saline at pH 7.2.

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 envelops.

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 two and 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° 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 components contain thimerosal at 0.001% as preservative, which may be toxic if ingested.

ASSAY PROCEDURE

1. Prepare 1:50 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 1:50.

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 (10-15 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 (10-15 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 3-4 drops of Mounting Medium to each slide and apply cover glass.

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 1:200 to 1:800. The fluorescence intensity at 1:400 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 clusters (morulae) 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 1:50 screening dilution: IgG titers of 1:50 and greater are considered to reflect infection at an undetermined time by Ehrlichia spp. Sera positive at the 1:50 screening dilution should be rerun to determine their endpoint titer for comparison with earlier or later specimens from the same dog.

Negative at 1:16: Report as negative for Ehrlichia equi antibody. Further serum specimens should be drawn if the original was taken soon after onset and ehrlichial 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 other Anaplasma spp. by IFA is variable from weak to strong, and can be differentiated by a variety of alternate methods including western immunoblot technique.

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