

INSTRUCTIONS FOR USE

Ehrlichia canis and Anaplasma phagocytophilum MIF Canine IgG Antibody Kit

Catalog Number: E20D-120
Size: 120 test
Storage: 2 – 8 °C

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

For in-vitro diagnostic use only



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INTENDED USE

The *Ehrlichia canis* and *Anaplasma phagocytophilum* MIF Canine IgG Antibody kit is intended for the detection and semi-quantitation of IgG class canine antibody to both *Ehrlichia canis* and *Anaplasma phagocytophilum* by micro-immunofluorescence assay.

SUMMARY AND EXPLANATION OF TEST

Both *Ehrlichia canis* and *Anaplasma phagocytophilum* are tick-borne canine pathogens. The microimmunofluorescence assay utilizes semi-purified elementary bodies and morulae from cell culture-propagated organisms. For optimal adhesion and background contrast, these antigens are dispersed in a matrix of sonicated egg yolk sac.

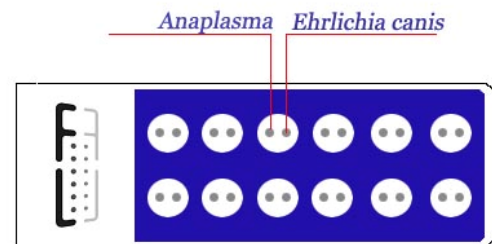
Patient sera are diluted in buffered saline and incubated in the individual slide wells to allow reaction of patient antibody with the solid-phase antigens. Slides are then washed to remove unreacted serum proteins, and DyLight 488-labeled anti-dog IgG (Conjugate) is added. This Conjugate is allowed time to react with antigen-antibody complexes. Then removed via another wash step. The resulting reactions can be visualized using standard fluorescence microscopy, where a positive reaction is seen as sharply defined apple-green fluorescent inclusions (morulae) and small cocci within a red yolk sac background. As the slides are viewed from left-to-right (frosted-end to the left), the *E. canis* antigen is viewed first. To the right of this antigen, in each slide well, is the *A. phagocytophilum* antigen dot. Positive reactions at screening dilution should be retested at higher dilutions to determine the highest reactive or endpoint dilution.

REAGENTS AND MATERIALS SUPPLIED

IFA Ag x 12

Substrate Slides (10)

10 x 12-well masked slides containing antigen dots of (LEFT) *Anaplasma phagocytophilum* and (RIGHT) *Ehrlichia canis*, both antigens dispersed in a cellular background matrix. Slides are fixed and packaged under vacuum, ready to use



CONJ FITC

IgG Conjugate, 2.5 mL

Dropper bottle with a yellow cap contains affinity-purified DyLight 488-labeled rabbit anti-dog IgG (heavy and light chains) with bovine serum albumin and Evans' blue counterstain.

CONT +

EC Positive Control, 0.5 mL

Dropper bottle with a blue cap contains *E. canis*-reactive canine serum provided at a 1:80 screening dilution. Endpoint titers are 1:640 (1:320-1:1280) to both antigens.

CONT +

AP Positive Control, 0.5 mL

Dropper bottle with a blue cap contains *A. phagocytophilum*-reactive canine serum provided at a 1:80 screening dilution. Endpoint titers are 1:640 (1:320-1:1280) to both antigens.

CONT -**Negative Control, 0.5 mL**

Dropper bottle with a red cap contains non-reactive canine 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. Since no testing can assure the absence of infectious agents, however, the Controls, 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 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 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 cover slips
- 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.
- Liquid reagents contain thimerosal at 0.01%, which may be toxic if ingested.

ASSAY PROCEDURE

Allow all reagents and sera to reach ambient temperature before starting timed assay procedure.

1. Prepare 1:80 screening dilutions (1 part patient serum with 79 parts PBS) for all 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 (i.e. 1:320-1:1280). This Control is bottled at 1:80.
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. 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 and then 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 Mounting Medium to each slide and carefully apply cover glass.
9. Read the stained substrate slides at 400X magnification, comparing each well to the visual intensity, antigen density 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 on both antigens. 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 (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.

Patient Specimens

Negative at 1:80: Report as negative for the respective antibody specificity. Further serum specimens should be drawn if the original was taken soon after onset and ehrlichiosis is still suspected.

Positive at 1:80 and greater: Serum titers at 1:80-1:320 suggest 1) titers preceding peak levels (early), 2) titers after peak levels (past exposure) or 3) titers reflecting cross-reactivity to a related organism (ie. *Ehrlichia* spp). Titers greater than 1:320 and/or IgM titers, when present, are a reliable indicator of recent infection.

Paired Sera: A four-fold increase in titer between acute and convalescent sera supports the diagnosis of recent infection by *Ehrlichia canis*, *Anaplasma phagocytophilum* or a closely related organism.

LIMITATIONS

Cross reaction between *Ehrlichia canis* and both *Ehrlichia chaffeensis* and *Ehrlichia ewingii* by IFA is variable from weak to strong, and can be differentiated by a variety of alternate methods including western immunoblot technique.

SPECIFIC PERFORMANCE CHARACTERISTICS

Anaplasma specificity was tested by 95 sera from a non-endemic region. All 95 of these sera had titers <1:80. Twelve sera from a regional public reference laboratory were also tested for concordance. All 8 positive sera were detected with titers within 1 dilution and the 4 negative sera were each <1:80. Forty sera with *E. canis* IFA titers >1:80 by standard IFA protocols were all positive in this assay, as well.

Sera from *E. canis* non-endemic regions were tested in-house, 159 from New York and 120 from Southern California. There were no positives. (100% specific). As the New York sera were from an endemic region for *Anaplasma phagocytophilum*, there were found 14 dogs (8.8%) seropositive for this related organism.

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