INSTRUCTIONS FOR USE

Ehrlichia canis IFA
Canine IgG Antibody Kit

Catalog Number: ECG-120

Size: 120 test

Storage: 2-8˚C

An Indirect fluorescence immunoassay for the detection of IgG class antibody against *Ehrlichia canis* in canine serum or plasma

For in-vitro diagnostic use only

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INTENDED USE

The *Ehrlichia canis* IFA Canine IgG Antibody Kit is intended for the detection and semi-quantitation of IgG class canine antibody to *Ehrlichia canis*.

SUMMARY AND EXPLANATION OF TEST

Substrate slides consist of teflon-masked wells containing fixed canine macrophage cells (DH82 cell line), approximately 20-30% of which are infected with *Ehrlichia canis* and contain the characteristic cytoplasmic morulae. Canine sera are diluted in buffered saline and incubated in the individual slide wells to allow reaction of patient antibody with the Ehrlichial antigens. Slides are then washed to remove unreacted serum proteins, and fluorescence-labeled anti-canine 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 can be visualized using standard fluorescence microscopy, where a positive reaction is seen as sharply defined apple-green fluorescent morulae within the cytoplasm of 20-30% of the cells in each field. A negative reaction is seen either as 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)**

10 x 12-well masked slides containing *Ehrlichia canis*-infected canine DH82 cells. Slides are fixed, packaged under vacuum and ready to use.

CONJ | FITC **Conjugate, 2.5 mL**

Yellow cap dropper bottle contains affinity-purified DyLight 488-labeled rabbit anti-canine IgG (heavy chain) with bovine serum albumin and Evans' blue counterstain.

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

Blue cap dropper bottle contains reactive canine serum at a 1:50 screening dilution. Endpoint titer is 1:400

CONT | – **Negative Control, 0.5 mL**

Red cap dropper bottle contains non-reactive canine serum at a 1:50 screening dilution

MM **Mounting Medium, 1 mL**

White cap dropper bottle contains glycerol (50% v/v) in PBS

BUF | WASH | PBS **PBS, 1 liter**

Add supplied powder to 1 liter purified water to produce PBS.

Warnings

The control sera have been screened for infectious agents by USFDA 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. 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, freeze samples ≤ -20°C. Acute specimens are obtained 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. Store in the dark.
- 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

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

1. Prepare 1:50 screening dilutions in PBS for all untested sera. For sera found positive on a previous assay run, prepare serial two-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 10 µL to one 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. 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. 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 place coverslip.

9. Read the stained substrate slides at 400X magnification, comparing each well to the visual intensity and appearance of the Ehrlichia 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 1:200 to 1:800. The fluorescence intensity at 1:800 may be used as the cut-off level required for a patient reaction to be called positive. If either of the Controls do not react as specified, the assay run should considered void, reagent components and procedural steps rechecked, and the assay repeated from step #1.

INTERPRETATION OF RESULTS
A positive reaction appears as bright, sharp, regularly stained inclusion bodies seen in the cytoplasm of infected cells. The size, appearance and density of the inclusions (morulae) must be compared with the Positive and Negative Control reactions. Patterns of reactivity different than that seen in the Positive Control must be considered non-specific.

Patient Specimens

Positive at 1:50 screening dilution: IgG titers of 1:50 and greater are considered to reflect infection at an undetermined time. 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 1:50: Report as negative for *E. canis* antibody.

LIMITATIONS
Ehrlichia canis is antigenically related to *Ehrlichia ewingii* and *Ehrlichia chaffeensis*, both of which are canine pathogens. Thus, differentiation of these agents by IFA or ELISA titers, without isolation of the organism in cell culture or PCR detection, is problematic. Relatedness to other Ehrlichia species is sometimes detected by IFA, but titers are an order of magnitude lower than against the homologous genogroup antigens.

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

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