INSTRUCTIONS FOR USE

Canine Brucellosis IFA
IgG Antibody Kit

Catalog Number:	BCG-120
Size:	120 test
Storage:	2-8˚C

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

For in-vitro diagnostic use only

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INTENDED USE

The Canine Brucellosis IFA IgG Antibody kit is intended for the detection and semi-quantitation of IgG class canine antibody to *Brucella canis*. This kit is designed for use as an aid in the diagnosis of canine infection by this pathogen.

SUMMARY AND EXPLANATION OF TEST

The IFA slides in this kit utilize fixed brucellae within a matrix of egg yolk sac sonicate. Canine patient and control sera are diluted to screening dilution in phosphate-buffered saline (PBS) and incubated in the individual slide wells to allow reaction of patient antibody with the solid-phase antigens. The slides are then washed to remove unreacted serum proteins, and Dylight 488-labeled anti-canine IgG (Conjugate) is added to label the antigen-antibody complexes. After further incubation, 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 brucellae (coccobacilli) against a contrasting red background of the matrix sonicate. A negative reaction is seen as either red-counterstained yolk sac alone 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 chemically-killed *Brucella ovis* within a background matrix sonicate. Slides are fixed (inactivated) and packaged under vacuum.

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.

Storage

Kit components should be stored at 2-8°C or colder. Bring them to room temperature (20-25°C) before opening bottles or slide envelopes.

Warnings

Since no testing can assure the absence of infectious agents 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.

SPECIMEN COLLECTION
Allow blood sample 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, storage 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
This kit supplies sufficient reagents and materials for 120 determinations.

Materials Required But Not Supplied

- Purified (distilled or deionized) water
- Clean 250 or 500 mL wash bottle for PBS
- 12x75 mm test tubes or microtiter plate for preparing serum dilutions
- Precision pipette(s) in microliter range for making and delivering serum dilutions
- 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
- Humidity 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 at 2-8°C and return to storage immediately after use.
- Conjugate contains Evans' Blue dye, which may be carcinogenic. Avoid contact with skin.

ASSAY PROCEDURE

1. Prepare 1:50 screening dilutions (1 part patient serum with 49 parts PBS) for all untested serum specimens. For sera found positive on a previous assay run, prepare serial two-fold dilutions in PBS, starting with 1:50. Acute-convalescent pairs should be compared by assaying all dilutions in parallel.

2. Prepare dilutions of the Positive Control in PBS to include one dilution above the stated endpoint and one dilution below the stated endpoint (1:200-1:800). Note that control have been bottled at 1:50 diltuions.

3. For each serum or Control dilution to be tested, add 10 µL to one slide well. For each assay, include the Negative Control, Positive Control and dilutions of the Positive Control (step 2).

4. Place slides into a humidity chamber and incubate in water bath or incubator for 30 minutes at 37°± 0.5°C.

5. Rinse slide wells with gentle stream of PBS from the wash bottle three (3) times, shaking PBS from the slide into a sink between each wash. Go directly to the next step without allowing slide wells to dry.

6. To each slide well, add 10 µL Conjugate then return slide to the humidity chamber for 30 minutes incubation in the humidity chamber at 37°± 0.5°C. Incubation should be in the dark to protect the photosensitive conjugate.

7. Wash slide as in step 5, above. Then add 2-3 drops Mounting Medium to each slide and apply coverglass.

8. Read the stained substrate slide at 400X magnification. Slide may be stored at 2-8°C in the dark for up to 24 hours.

QUALITY CONTROL

The Negative Control and dilutions of the Positive Control 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 (less than 1+), but uniform, greenish staining. The Positive Control wells should give an endpoint titer from 1:100 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 considered void. Reagent components and procedural steps should be rechecked, and the assay repeated from the beginning.

The Negative Control well is an example of fluorescence patterns that are to be considered negative. If bright staining is 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 brightly fluorescent (at least 1+) sharp, regular stained coccobacilli evenly distributed in a matrix of red-counterstained egg yolk sac sonicate, while the Negative Control well is an example of fluorescence patterns that are to be considered negative. The Positive Control wells should give an endpoint titer of 1:00 to 1:800. The fluorescence intensity at 1:400, however, may be used as the cut-off level required for a patient reaction to be called positive.

The size, appearance and density of the brucellae must be compared with the Positive and Negative Control reactions. Patterns of reactivity different than those seen in the Positive Control must be considered non-specific.

Canine brucellosis is characterized by a prompt rise in IFA titer in both IgG and IgM fractions. The IgG titer is most often specifically measured since the IgM antibody is quite crossreactive and is also the persisting form of antibody. The IgG antibody titer, in contrast, is specific and will decline to undetectable levels with successful treatment of the infection.

PATIENT SPECIMENS

Positive at 1:50: Single IgG titers of 1:50 and greater are considered to reflect recent or active infection.

Negative at 1:50: Report as negative for Brucella antibody.

LIMITATIONS
Titers in the lower ranges (1:50-1:200) are often below the detection limits of the agar gel diffusion test used for confirmation. Anecdotal evidence from the attending veterinarians in such cases has supported the IFA result in all cases investigated to date.

EXPECTED VALUES
The prevalence of canine brucella antibodies varies depending upon the geographic region and population being tested. The average prevalence of seropositives by microagglutination titer is generally less than 1%. Random sera (n=50) submitted to a veterinary testing laboratory in southern California were all found to be <1:16 (<1% seroprevalence). Positive sera generally fall into the range 1:500-1:2000.

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