INSTRUCTIONS FOR USE

Trypanosoma cruzi IFA
Canine IgG Antibody Kit

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

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

For in-vitro diagnostic use only

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INTENDED USE
The Trypanosoma IFA Antibody Kit is intended for the detection and semi-quantitation of canine antibody to Trypanosoma.

SUMMARY AND EXPLANATION OF TEST
Substrate slides consist of teflon-masked wells containing fixed ***Trypanosoma cruzi*** epimastigotes. Canine sera are diluted in buffered saline and incubated in the individual slide wells to allow reaction of test antibody with the fixed 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 peripheral apple-green fluorescent epimastigotes. A negative reaction is seen either as red-counterstained cells or fluorescence unlike that seen in the positive control wells. 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 Teflon-masked slides containing *Trypanosoma cruzi* epimastigotes. Slides are pre-fixed and ready to use.

CONJ | FITC — **Conjugate, 2.5 mL**
Yellow cap dropper bottle contains affinity-purified Alexafluor 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 1:20 screening dilution. Endpoint titer is 1:160

CONT | – — **Negative Control, 0.5 mL**
Red cap dropper bottle contains non-reactive canine serum at a 1:20 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
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.

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 2-8ºC. If testing is to be delayed longer than 5 days, store samples at -20ºC or colder. Acute specimens should be drawn at the onset of illness and convalescent specimens 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 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

1. Prepare 1:20 screening dilutions for all untested sera. For sera found positive on a previous assay run, prepare serial two-fold dilutions in PBS, starting with 1:20.

2. Prepare dilutions of the Positive Control to include 1 dilution above the stated endpoint and one dilution below (ie. 1:40-1:160).

3. For each serum dilution apply 10 µL to one slide well.. For each assay 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, 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 of Mounting Medium to each slide and apply coverglass, carefully removing air bubbles caught under the coverglass.

9. Read the stained substrate slides at 400X magnification, comparing each well to the visual intensity and appearance of that 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 1:80 to 1:320. The fluorescence intensity at 1:160 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 rechecked, and the assay repeated from step #1.

INTERPRETATION OF RESULTS
A positive reaction appears as bright, sharp, and regular staining of the epimastigote outer membrane. The size, appearance and density of the fluorescence 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 1:20 screening dilution: IgG titers of 1:20 and greater are considered to reflect infection at an undetermined time. Sera positive at the 1:20 screening dilution should be rerun to determine their endpoint titer for comparison with earlier or later specimens from the same dog.

Negative at 1:20: Report as negative for Trypanosoma antibody.

LIMITATIONS
Trypanosoma cruzi is antigenically related and crossreactive with other *Trypanosoma spp.* by IFA methods, as well as Leishmania spp. Thus, differentiation at the species level by IFA or ELISA titers, without isolation of the organism or PCR detection, is problematic.

EXPECTED VALUES
The prevalence of Trypanosoma antibodies varies depending upon the geographic region and population being tested. Uninfected dogs should be non-reactive at 1:20.

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