INSTRUCTIONS FOR USE

Toxoplasma gondii IFA

Feline IgG Antibody Kit

Catalog Number: TXF-120

Size: 120 test

Storage: 2 – 8 ˚C

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

For in-vitro diagnostic use only

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INTENDED USE

The Toxoplasmosis IFA-IgG Antibody kit is intended for the detection and semi-quantitation of IgG class feline antibody to *Toxoplasma gondii*, to be used as an aid in the diagnosis of felinetoxoplasmosis.

SUMMARY AND EXPLANATION OF TEST

Toxoplasma gondii is an obligate intracellular coccidian parasite that infects most warm-blooded animals. Cats, both domestic and wild, serve as the primary host. The immune response to infection is detectable within the first 2-3 weeks post infection and the serologic detection of specific antibody may be used as an indirect indication of toxoplasmosis.

The IFA slides in this kit utilize killed *T. gondii* tachyzoites as the substrate antigen. Feline sera are diluted in buffered saline and incubated in the individual slide wells to allow reaction of patient antibody with the antigen. The slides are then washed to remove unbound serum proteins, and a fluorescence-labeled anti-feline IgG (Conjugate) is added, to react with and tag the antigen-antibody complexes. After further incubation, the slides are washed to remove unbound Conjugate. The resulting reactions can be visualized using standard fluorescence microscopy, where a positive reaction is seen as sharply defined apple-green fluorescent tachyzoite membranes. A negative reaction is seen as either no reactivity or reactivity limited to the broad end of the organism (polar cap) only. 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 fixed *Toxoplasma gondii* tachyzoites. Slides are pre-fixed and ready to use.

CONJ | FITC **Conjugate, 2.5 mL**

Affinity-purified DyLight 488-labeled rabbit anti-cat IgG (H+L) with bovine serum albumin and Evans' blue.

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

Dropper bottle with blue cap contains reactive serum, provided at a 1:16 screening dilution. Endpoint titer is 1:128.

CONT | – **Negative Control, 0.5 mL**

Dropper bottle with red cap contains non-reactive serum, provided at a 1:16 screening dilution.

MM **Mounting Medium, 1 mL**

Dropper bottle with white cap contains 50% glycerol in PBS, pH 7.2.

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

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

Warnings

- Since no testing can assure the absence of infectious agents, the Control sera, 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. Although the substrate slides are prepared with chemically inactivated antigens, they 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.

SPECIMENS
Allow blood samples to clot and separate the 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, store samples at -20°C or colder. 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 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
- Test tubes or microtiter plates for preparing serum dilutions
- Precision pipettor for making and delivering dilutions
- 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°C waterbath or incubator
- Humidity chamber for slide incubation steps

Precautions
- Do not use components past expiration date.
- 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 1:16 screening dilutions in PBS for test sera. For sera found positive on a previous assay run, prepare serial two-fold dilutions in PBS, starting with 1:16.

2. Prepare dilutions of the Positive Control to include 1 dilution above the stated endpoint and one dilution below the stated endpoint (8-fold from bottled Control).

3. For each serum dilution, add 10 µL to one slide well and record the location for later reference. For each assay run, include the Negative Control (as provided), Positive Control and dilutions of the Positive Control prepared in step 2.

4. Place slides into a humidity chamber and incubate for 30 minutes at 37°± 0.5°C.

5. Rinse slide wells with gentle stream of PBS from washbottle, then shake beaded wash buffer into a sink or waste. Repeat this wash step twice more, then add 1 drop (~10 µL) Conjugate to each well.

6. Return slides to humidity 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 2-3 drops of Mounting Medium to each slide and apply coverslip.

9. Read the stained substrate slides at 400X magnification, comparing each well to the visual intensity and appearance of 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. The Positive Control wells should give an endpoint titer between the 1:4 and 1:16 dilution. The fluorescence intensity at an 8-fold dilution of the Positive Control may be used as the cut-off level required for a test 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 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 bright uniform staining (at least 1+) of the entire membrane. Fluorescence limited to the polar cap of the tachyzoite is related to Fc receptors and is not considered specific reactivity. Patterns of reactivity different than that seen in the Positive Control must also be considered non-specific.

Acute infection is characterized by a prompt rise in IgM antibody, followed in 3-4 weeks by a rise in IgG class antibody by IFA testing. IgM antibody levels peak approximately 3-4 weeks post onset of symptoms and remain detectable for 2-4 months. IgG class antibody peaks in 7-12 weeks, but declines much more slowly than IgM antibody levels and remains elevated for over 9-12 months.

PATIENT SPECIMENS
Positive at 1:16 screening dilution: IgG titers of 1:16 and greater reflect infection at an undetermined time (seropositive). Positive sera should be rerun to determine their endpoint titer for comparison with earlier or later specimens from the same cat.

Negative at 1:16: Report as negative for *Toxoplasma* antibody. Further serum specimens should be drawn, if the original was taken immediately post onset of symptoms.

Paired Sera: A four-fold increase in titer between acute and convalescent serum specimens is considered strong evidence supporting the diagnosis of recent infection.

LIMITATIONS
1. Elevated titers, even at 1:1024, may be due to past exposure or reactivation. Recent infection is demonstrated more accurately by the presence of IgM class antibody or a 4-fold increase in IgG or total antibody within a 2-3 week period. For the latter criterion, the acute and convalescent sera should be assayed concurrently.

2. The early immune response to acute toxoplasmosis is dominated by IgM antibody for 2-3 weeks, followed by an increase in IgG class.

EXPECTED VALUES
The prevalence of specific antibodies varies depending upon the geographic region and age of the population being tested.

New Version (2/01)