INSTRUCTIONS FOR USE

Neospora caninum IFA
Canine IgG Antibody Kit

Catalog Number: NCD-120

Size: 120 test

Storage: 2 – 8 ˚C

An Indirect immunofluorescence assay for the detection and semi-quantitative determination of IgG class antibody against *Neospora caninum* in canine serum or plasma

For in-vitro diagnostic use only

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INTENDED USE

The *Neospora caninum* IFA IgG Antibody kit is intended for the detection and semi-quantitation of canine antibody to *Neospora caninum*.

SUMMARY AND EXPLANATION OF TEST

Substrate slides consist of teflon-masked wells containing fixed tachyzoites of *Neospora caninum.* Canine sera are diluted in buffered saline and incubated in the individual slide wells to allow reaction of patient antibody with the Neospora 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 tachyzoite membranes. 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)**

10x12-well masked slides containing *Neospora caninum*-tachyzoites. Slides are pre-fixed and ready to use.

CONJ | FITC **IgG Conjugate, 2.5 mL**

Dropper bottle with a yellow cap contains affinity-purified DyLight 488-labeled rabbit anti-canine IgG (H+L) with bovine serum albumin and Evans' blue counter stain.

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

Dropper bottle with a blue cap contains reactive canine serum, provided at a 1:16 screening dilution with 0.005% thimerosal as preservative. Endpoint titer is 1:128.

CONTROL | – **Negative Control, 0.5 mL**

Dropper bottle contains non-reactive canine serum, provided at screening dilution with 0.005% thimerosal.

MM **Mounting Medium, 1 mL**

Dropper bottle contains 50% glycerol in PBS, pH 7.2, containing 0.005% thimerosal.

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, 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 envelops.

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 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.

ASSAY PROCEDURE

1. Prepare 1:16 screening dilutions for all untested sera. For sera found positive on a previous assay run, prepare serial dilutions in PBS, starting with 1:16.
2. Prepare dilutions of the Positive Control to include one dilution above the stated endpoint and one dilution below (ie. 1:64-256 final dilution from the 1:16 in bottle).
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 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 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 another 30 minute 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 cover glass.
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, with either uniform red counterstain or slight, but uniform greenish staining. The Positive Control wells should give an endpoint titer from 1:64 to 1:256. The fluorescence intensity at 1:128 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 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 bright and distinct tachyzoite membranes 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 complete, bright, sharp, regular stained tachyzoite membranes. Disregard staining restricted to the polar cap only. The size, appearance and density of the reaction must be compared with the Positive and Negative Control reactions. Patterns of reactivity different from that seen in the Positive Control must be considered non-specific.

Patient Specimens

Positive at 1:16 screening dilution: IgG titers of 1:16 and greater are considered to reflect infection at an undetermined time. Sera positive at the 1:16 screening dilution should be rerun to determine their endpoint titer for comparison with earlier or later specimens from the same dog.

Negative at 1:16: Report as negative for *Neospora caninum* antibody.

EXPECTED VALUES

The prevalence of *Neospora caninum* antibodies varies depending upon the geographic region and population being tested. A random sample of sera submitted to a veterinary diagnostic laboratory in New York State produced a 3% seropositive rate (titer 1:16 or higher).

REFERENCE

Dubey, JP, Hattel AL, Lindsay DS, et. al. Neonatal *Neospora caninum* infection in dogs: Isolation of the causative agent and experimental transmission. *J Am Vet Med Assoc* 1988; 193: 1259-1263.

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