

INSTRUCTIONS FOR USE

Canine Distemper IFA IgM Antibody Kit

Catalog Number: CDM-120
Size: 120 test
Storage: 2-8°C

An Indirect fluorescence immunoassay for the detection of IgM class antibody against Canine Distemper Virus in canine serum or plasma

For in-vitro diagnostic use only



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INTENDED USE

The Canine Distemper Virus IFA Antibody kit is intended for the detection and semi-quantitation of canine IgM antibody to Canine Distemper Virus.

SUMMARY AND EXPLANATION OF TEST

Substrate slides consist of teflon-masked wells containing fixed mink lung fibroblasts, approximately 7-15% of which are infected with Canine Distemper Virus and contain the characteristic cytoplasmic inclusion bodies. This assay utilizes serum pretreatment for removal of rheumatoid factor and excess specific IgG, as adverse assay factors. Canine sera are diluted in buffer, treated with antiserum against IgG-class antibody, then incubated in the individual slide wells to allow reaction of serum IgM antibody with the solid-phase antigens. Slides are then washed to remove unreacted serum proteins before DyLight 488-labeled anti-canine IgM (Conjugate) is added. After this Conjugate has reacted with the 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 inclusions within the cytoplasm of 7-15% of the cells in each field. A negative reaction is seen either as red-counterstained cells or as fluorescence unlike that seen in the Positive Control well. Positive reactions may 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 CDV-infected cells. Slides are pre-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 IgM with bovine serum albumin and Evans' blue counterstain.

IgM DIL

Serum Pretreatment Reagent, 2 mL

Bottle contains Goat anti-IgG (gamma chain-specific).

CONT +

Positive Control, 0.5 mL

Blue cap dropper bottle contains reactive canine serum, provided at a 1:20 screening dilution. Control is pre-treated and ready to use as provided. Endpoint titer is 1:160.

CONT -

Negative Control, 0.5 mL

Red cap dropper bottle contains non-reactive canine serum, provided at a 1:20 screening dilution. Control is pre-treated and ready to use as provided.

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, 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.

SPECIMENS

Store serum samples at 2-8°C. If testing is to be delayed longer than 5 days, freeze the sample at -20°C or colder. Acute specimens should be drawn at the onset of illness and convalescent specimens 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.
- Liquid reagents contain thimerosal at 0.001%, which may be toxic if ingested.

ASSAY PROCEDURE

1. Prepare 1:20 screening dilutions for all untested sera, as follows: Dilute each serum 1:10 in PBS, then mix 10 µL of this dilution with 10 µL Serum Pretreatment Reagent. For sera found positive on a previous assay run, prepare serial two-fold dilutions in PBS, starting with the pre-treated 1:20 sample.
2. Prepare dilutions of the Positive Control (in PBS) to include one dilution above the stated endpoint and one dilution below (ie. 1:80-1:320). Note that this Control is packaged at a 1:20 screening dilution.
3. For each serum dilution transfer 15 µL to a slide well and record the location for later reference. 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 **90 minutes** at 37°± 0.5°C.
5. Rinse slide wells with gentle stream of PBS from wash bottle. Shake PBS from slides into a sink and repeat this wash step 3X without allowing the wells to dry. If experience shows that slide fields contain an excess of stained precipitate, allow slides more time between washes or soak slides in a PBS bath.

6. To each slide well add 1 drop (15 µL) conjugate, and 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.

9. Read the stained substrate slides at 400X magnification, comparing each well to the visual intensity and appearance of the viral 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 1:80 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 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 inclusion bodies 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 bright inclusion bodies seen in the cytoplasm of infected cells. The size, appearance and density of the inclusions 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: IgM titers of 1:20 and greater support the diagnosis of recent or active CDV infection, unless attributable to recent vaccination. Sera positive at the 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 CDV IgM antibody.

LIMITATIONS

In the absence of vaccination within a 3-week period, a positive IgM titer can provide an accurate diagnosis for up to 3 months post onset. IgG and/or IgM class antibody induced by Distemper vaccination or past infection is a limitation in assessing antibody levels. Post-vaccination IgG levels may remain elevated for long periods. Also IgG antibody levels may be elevated and stable at presentation. CNS infection can be accurately diagnosed by elevated CSF titers, although damage to the blood-brain barrier must be taken into account and the lack of a titer does not rule out CDV.

EXPECTED VALUES

The prevalence of Canine Distemper Virus IgM-class antibody varies depending upon the geographic region and population being tested. Healthy dogs should be non-reactive at 1:20, unless recently vaccinated against CDV.