INSTRUCTIONS FOR USE

Feline Hespesvirus IFA IgG Antibody Kit

Catalog Number: FHG-120

Size: 120 test

Storage: 2 – 8 ˚C

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

For in-vitro diagnostic use only

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INTENDED USE

The Feline Herpesvirus IFA Antibody kit is intended for the detection and semi-quantitation of feline antibody to Feline Herpesvirus.

SUMMARY AND EXPLANATION OF TEST

Substrate slides consist of teflon-masked wells containing fixed feline kidney cells (Crandall), approximately 7-15% of which are infected with Feline Herpesvirus (Rhinotracheitis virus) and contain the characteristic herpesvirus cytoplasmic, membrane, and nuclear fluorescence patterns. Feline sera are diluted in buffered saline and incubated in the individual slide wells to allow reaction of antibody with the fixed antigens. Slides are then washed to remove unreacted serum proteins, and DyLight 488-labeled anti-feline IgG (conjugate) is added. This conjugate is allowed time to react with antigen-antibody complexes. After another wash step, the reactions can be visualized using standard fluorescence microscopy. A positive reaction is seen as typical apple-green fluorescence within the cytoplasm, nucleus, and cell membrane of 7-15% of the cells in each field. 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)**

10 x 12-well masked slides containing uninfected and infected feline kidney cells. Slides are pre-fixed and ready to use.

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

Dropper bottle with yellow cap contains affinity-purified DyLight 488-labeled goat anti-feline IgG (heavy and light chain) with bovine serum albumin and Evans' blue counter stain.

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

Dropper bottle with blue cap contains reactive feline serum, provided at a 1:20 screening dilution. Endpoint titer is 1:160.

CONT | – **Negative Control, 0.5 mL**

Dropper bottle with red cap contains non-reactive feline serum, provided at a 1:20 screening dilution.

MM **Mounting Medium, 1 mL**

Dropper bottle with a white cap contains 50% glycerol in PBS.

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

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

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 2-8°C. If testing is to be delayed longer than 2 weeks, store samples at -20°C or colder. Acute specimens should be drawn at the onset of illness; with convalescent specimens obtained 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 0.005%, 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. 1:80-1:320). Controls are already bottled at screening dilution.

3. For each serum dilution add 15 µL to one slide well and record the location for later reference. For each assay run includes 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. 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 (15-20 µL) conjugate, and then return slides to the humid 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, carefully removing air bubbles caught under the coverslip.

9. Read the stained substrate slides at 400X magnification, comparing each well to the visual intensity and appearance of the viral staining 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.

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 granular fluorescence 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: 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 cat.

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

EXPECTED VALUES

The prevalence of herpesvirus antibodies varies depending upon the geographic region and population being tested. Uninfected cats should not seroconvert or demonstrate a four-fold titer increase during the period of serologic testing.

New 12/2000
Initial Version