INSTRUCTIONS FOR USE

Leptospirosis IFA
IgM Antibody Kit

Catalog Number: LEM-120

Size: 120 test

Storage: 2-8˚C

An Indirect fluorescence immunoassay for the detection of IgM class antibody against *Leptospira interrogans* in human serum or plasma

For in-vitro diagnostic use only

CE

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INTENDED USE
The Leptospirosis IFA IgM Antibody Kit is intended for the detection and semi-quantitation of IgM class human antibody to *Leptospira spp.*, to be used as an aid in the diagnosis of human Leptospirosis.

SUMMARY AND EXPLANATION OF TEST
Leptospirosis is an acute, febrile, septicemic disease caused by pathogenic spirochetes of the species *Leptospira interrogans.* These organisms are found worldwide in feral and domestic mammals, infecting humans through contact with infected animals or indirectly via infected urine. The immune response to infection is detectable by the first week of symptomatic disease and the serologic detection of specific antibody may be used as an indirect indication of acute Leptospirosis.

The IFA slides in this kit utilize a non-pathogenic culture-propagated Leptospire as the substrate antigen. Patient sera are initially diluted 1:200 in IgM Serum Prep, containing anti-human IgG antibody. The treated sera are then transferred to the individual slide wells to allow reaction of patient antibody with the leptospires. The slides are then washed to remove unreacted serum proteins, and an Alexafluor 488-labeled anti-human IgM (Conjugate) is added, to label the antigen-antibody complexes. After further incubation, 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 leptospires. A negative reaction is seen as either no fluorescence 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 acetone-fixed (non-pathogenic) leptopires (Patoc I), packaged under vacuum.

CONJ | FITC — **IgM Conjugate, 2.5 mL**
Yellow cap dropper bottle contains affinity-purified Alexafluor 488-labeled goat anti-human IgM (heavy chain) with bovine serum albumin and Evans' blue counterstain.

CONT | + — **Positive Control, 0.5 mL**
Blue cap dropper bottle contains pre-treated human serum at a 1:200 screening dilution. Endpoint titer is 1:1600

CONT | – — **Negative Control, 0.5 mL**
Red cap dropper bottle contains pre-treated human serum at a 1:200 screening dilution

IgM | DIL — **IgM Serum Prep, 10 mL**
Buffer contains goat anti-human IgG antibody in PBS.

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 (phosphate-buffered saline) at pH 7.2.

Warnings
The control sera have been screened for infectious agents by USFDA required testing. 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.

SPECIMEN COLLECTION
Allow blood sample to clot and separate sera by centrifugation. Transfer sera aseptically to tightly closing sterile containers. Store at 2-8°C. If testing will be delayed longer than 5 days, freeze samples at -20°C or colder. Acute specimens are 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
- Purified (distilled or deionized) water
- Clean 250 or 500 mL wash bottle for PBS
- Test tubes or microtiter plates for serum dilutions
- Precision pipettor(s) for microliter range
- 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 is photosensitive. Store Conjugate in the dark.
- 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

PREPARATION OF SAMPLES

Prepare 1:100 dilutions of patient sera, as follows: Prepare an initial 1:10 dilution of serum in IgM Serum Prep. Mix well and allow 30 minutes for precipitin aggregates for form. Centrifuge mixture to remove aggregates and dilute the supernate 1:20 further in PBS to achieve a final 1:100 serum dilution.

ASSAY PROCEDURE

Allow all reagents and sera to reach ambient temperature before starting timed assay procedure.

1. The Positive and Negative Controls are supplied at screening dilution (1:200). Prepare dilutions of the Positive Control in PBS through 1:16.

2. For each serum dilution, apply 10 µL to one slide well. For each assay include the Positive Control dilutions. Add 1 drop of Negative Control to one well.

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

4. Rinse slide wells with gentle stream of PBS from washbottle, then shake beaded PBS into waste. Repeat this wash step 2X, then add 1 drop Conjugate.

5. Return slides to humidity chamber for 30 minutes incubation at 37°± 0.5°C. Incubation should be in the dark to protect the photosensitive Conjugate.

6. Wash slides as in step 4, add 2-3 drops of Mounting Medium to each slide and apply cover slip.

7. 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 no distinct leptospires staining. The Positive Control wells should give an endpoint titer from 1:800 to 1:3200. The fluorescence intensity at 1:1600 may be used as the cut-off level required for a reaction to be called positive. If either of the Controls does not react as specified, the assay should be considered void, reagent components and procedural steps should be rechecked, and the assay repeated from the beginning.

INTERPRETATION OF RESULTS
A positive reaction appears as bright uniform staining (at least 1+) of typical leptospires. Patterns of reactivity different than that seen in the Positive Control must be considered non-specific.

Acute infection is characterized by a prompt rise in both IgG and IgM class antibody by IFA testing. IgM antibody levels peak approximately 3-4 weeks post onset of symptoms and remain detectable for 2-3 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 screening dilution: IgM titers of 1:200 and greater reflect recent infection. Positive sera should be rerun to determine their endpoint titer for comparison with earlier or later specimens from the same patient.

Negative at screening dilution: Report as negative for Leptospirosis antibody.

LIMITATIONS
- Due to the genus-specific nature of the IFA response, the infecting serovar cannot be determined. Urine cultures in appropriate Leptospira isolation medium should be attempted.

- Tests with high titered sera from cases of Lyme disease and syphilis suggest no detectable crossreactivity in this procedure with other pathogenic spirochetes.

- The specific immune response to acute Leptospirosis is dominated by IgM antibody. Some patients make a much weaker or delayed IgG response in comparison to the IgM response.

EXPECTED VALUES
The prevalence of specific antibodies varies depending upon the geographic region and population being tested. Specific IgM antibody titers of 1:100 and higher are unusual and were detected in less than 1% of sera submitted to a reference laboratory in California.

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