INSTRUCTIONS FOR USE

Borrelia burgdorferi IFA
IgM Antibody Kit

Catalog Number:	BBM-120
Size:	120 test
Storage:	2-8˚C

An indirect fluorescence immunoassay for the detection of IgM class antibody against *Borrelia burgdorferi* in human serum or plasma

For in-vitro diagnostic use only

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INTENDED USE

The *Borrelia burgdorferi* IFA IgM Antibody kit is intended for the detection and semi-quantitation of IgM class human antibody to *Borrelia burgdorferi (sensu lato).*

SUMMARY AND EXPLANATION OF TEST

Lyme disease is a multisystem disorder caused by infection with the tick-borne spirochete *Borrelia burgdorferi, sensu lato.* Diagnosis is based upon clinical symptoms; exposure to ticks in an area where the disease is endemic, and, in ambiguous cases, laboratory test results. Although cultivation of the spirochete from erythema migrans lesions, blood, joint fluid, and cerebrospinal fluid has been documented, the sensitivity of these cultures in affected individuals has been low. Serologic tests for specific antibody have commonly been the more practical means of laboratory confirmation.

The IFA slides in this kit utilize washed *Borrelia burgdorferi (sensu stricto)* spirochetes. Patient and control sera are diluted to screening dilution in an IgM Sample Diluent (provided) and incubated in the individual slide wells to allow reaction of patient antibody with the organisms. The slides are then washed to remove non-reactive serum proteins, and a fluorescence-labeled anti-human IgM (Conjugate) is added, to label the antigen- antibody complexes. After further incubation, the slides are washed to remove non-reactive conjugate. The resulting reactions are visualized using standard fluorescence microscopy, where a positive reaction is seen as sharply defined apple-green fluorescent spirochetes. 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 blended (strains B31 and 297) of *Borrelia burgdorferi, sensu stricto.* Slides are fixed (inactivated) and packaged under vacuum, ready for use.

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

CONT | + — **Positive Control, 0.5 mL**
Dropper bottle with a blue cap contains reactive human serum at 1:16 screening dilution. Endpoint titer is 1:128.

CONT | – — **Negative Control, 0.5 mL**
Dropper bottle with a red cap contains non-reactive human serum at screening dilution.

MM — **Mounting Medium, 1 mL**
Dropper bottle with 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.

SAMP DIL — **IgM Sample Diluent,15 mL**
Buffer contains goat anti-human IgG antibody in PBS.

Warnings
Control sera have been screened for infectious agents by FDA-required testing. 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. 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 2-8ºC. Bring them to room temperature (20-25ºC) before opening bottles or slide envelopes.

SPECIMEN COLLECTION
Allow blood to clot and separate sera by centrifugation. Transfer sera aseptically to tightly closing sterile containers. Store at 2-8ºC. If testing will be delayed longer than 5 days, store sera at ≤ -20ºC. 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 plate for preparing dilutions
- 24 x 50 mm glass cover slips
- Fluorescence microscope with filter system for FITC (maximum excitation wavelength 490 nm, mean emission wavelength 530 nm) and 400X magnification
- 37ºC water bath or incubator
- Humidity chamber for slide incubation steps

Precautions
- Do not use components past expiration date.
- Conjugate is photosensitive. Store in the dark at 2-8ºC and return to storage immediately 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 1:16 screening dilutions (1 part patient serum with 15 parts IgM Sample Diluent) for all untested patient 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 in PBS to include one dilution above and one dilution below the stated endpoint (1:64-1:256).

3. Add 10 µL of each prepared dilution to one of the slide wells. Include the Negative Control, as supplied, and dilutions of the Positive Control prepared in step 2.

4. Place slide into a humidity chamber and incubate for 90 minutes at 37º± 0.5ºC .

5. Rinse slide wells with gentle stream of PBS from the wash bottle three (3) times, shaking PBS from the slide into a sink between each wash. Go directly to next step without allowing slide wells to dry.

6. To each slide well add 10 µL Conjugate and incubate 30 minutes at 37º± 0.5ºC, as before. Incubation should be in the dark to protect the photosensitive conjugate.

7. Wash slide as in steps 5, above, add 3-4 drops of Mounting Medium to each slide and apply cover glass.

8. Read the stained substrate slide at 400X magnification, comparing each well to the visual intensity, antigen density, and appearance of the Positive and Negative Control wells. Slide may be stored at 2-8ºC in the dark for up to 24 hours.

QUALITY CONTROL
The Negative Control (1:16) and dilutions of the Positive Control should be assayed with each daily run. 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 is considered void. Reagents and procedural steps should be rechecked, and the assay repeated from the beginning.

INTERPRETATION OF RESULTS
A positive reaction appears as bright staining (at least 1+) of characteristic spirochetes. The appearance and density of the fields 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 1:16: Supportive of the diagnosis of recent or active Lyme disease. Specimen should be titered to determine endpoint for comparison with earlier or later specimens from the same patient.

Negative at 1:16: Report as negative for *B.burgdorferi* IgM.

LIMITATIONS
The IFA procedure detects genus-specific antibody in most cases, thus a differentiation between *B. burdorferi* genogroups or other *Borrelia* species is not possible with this procedure alone. Occasional interference may also be seen from antibody to oral treponemes or *Treponema pallidum*.

Patients with infectious mononucleosis (IM) may demonstrate cross-reactive IgM-class antibody in the IFA-IgM test. Approximately 25% of acute IM sera were positive for IgM antibody to *Borrelia burgdorferi*.

EXPECTED VALUES
In patients without infection with *B. burgdorferi*, IgM titers are negative, except as noted in Limitations section, above. IgM antibody is usually detected 1-3 weeks post-onset of erythema migrans, peaking between the third and sixth week. Some patients with second and third stage disease will continue to maintain detectable, but decreased, IgM titers.

BIBLIOGRAPHY

1. Burgdorfer W, Barbour AG, Hayes SF, Benach JL, Grunwaldt E, Davis JP. Lyme disease- a tick-borne spirochetosis? Science 1982; 216:1317-1319.
2. Russell H, Sampson JS, Schmid GP, Wilkinson HW, Pilkaytis B. Enzyme-linked immunosorbent assay and indirect immunofluorescence assay for Lyme disease. J Infect Dis 1984: 149:465-470.

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