INSTRUCTIONS FOR USE

Bartonella henselae IFA
Human IgM Antibody Kit

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

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

For in-vitro diagnostic use only

CE

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INTENDED USE

The Bartonella henselae IFA IgM Antibody kit is intended for the detection and semi-quantitation of IgM class human antibody to *Bartonella henselae*. This kit is designed for use as an aid in the diagnosis of human infection by this pathogen.

SUMMARY AND EXPLANATION OF TEST

The IFA slides in this kit utilize Vero cells infected with *Bartonella henselae*. Test and sera are diluted to screening dilution in phosphate-buffered saline (PBS) and incubated in the individual slide wells to allow reaction of antibody with the solid-phase antigens. The slides are then washed to remove unreacted 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 again to remove unreacted Conjugate. The resulting reactions are visualized using standard fluorescence microscopy, where a positive reaction is seen as sharply defined apple-green fluorescent Bartonella within the cytoplasma of the Vero cells. 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 Vero cells infected with *Bartonella henselae*. Slides are fixed (inactivated) and packaged under vacuum.

CONJ | FITC — **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 reactive human serum, pre-treated at a 1:64 screening dilution. Endpoint titer is 1:512

CONT | – — **Negative Control, 0.5 mL**

Red cap dropper bottle contains non-reactive human serum pre-treated at a 1:64 screening dilution

IgM | DIL — **IgM Sample Diluent, 15 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.

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.

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Storage
Kit components should be stored at 2-8ºC or colder. Bring them to room temperature (20-25ºC) 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 is to be delayed longer than 5 days, store sera 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
This 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
- 12x75 mm test tubes or microtiter plate for preparing serum dilutions
- Precision pipette(s) in microliter range for making and delivering serum 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
- 37° water bath or incubator
- Humidity chamber for slide incubation steps

Precautions
- Do not use components past expiration date.
- Conjugate is photosensitive
- 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

PREPARATION OF SAMPLES AND REAGENTS

1. **Prepare Wash Buffer** by adding contents of PBS packet to 1 liter purified water and mixing thoroughly:

2. **Prepare screening dilutions** of patient sera by making an initial 1:16 dilution using IgM Sample Diluent in micro-centrifuge tubes. Mix and allow a minimum of 20 minutes for the reaction, then centrifuge at high speed to remove the aggregated IgG. Dilute 10 µL of this supernate with 30 µL Wash Buffer, resulting in a final 1:64 screening dilution.

ASSAY PROCEDURE

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

1. Prepare 1:64 screening dilutions (above) for all untested serum specimens. For sera found positive on a previous assay run, prepare serial two-fold dilutions in PBS, starting with 1:64. Acute-convalescent pairs should be compared by assaying all dilutions in parallel.

2. Prepare dilutions of the Positive Control in PBS to include one dilution above the stated endpoint and one dilution below (1:256-1:1024). Note that Controls are bottled at 1:64. Endpoint range is thus 1:4 to 1:16 of bottled material.

3. For each serum or Control dilution to be tested, add 10 µL to one slide well. For each assay, include the Negative Control, Positive Control and dilutions of the Positive Control (step 2).

4. Place slides into a humidity chamber and incubate in water bath or incubator for 90 minutes at 37°± 0.5°C.

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 the next step without allowing slide wells to dry.

6. To each slide well, add 10 µL Conjugate and return slide to the humidity chamber for 30 minutes incubation in the water bath or incubator at 37°± 0.5°C. Incubation should be in the dark to protect the photosensitive conjugate.

7. Wash slide as in step 5, above. Then add 3-4 drops Mounting Medium to each slide and apply coverglass.

8. Read the stained substrate slide at 400X magnification. Slide may be stored at 2-8°C in the dark for up to 24 hours.

QUALITY CONTROL
The Negative Control and dilutions of the Positive Control 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 (less than 1+), but uniform, greenish staining. The Positive Control wells should give an endpoint titer from 1:256 to 1:1024. The fluorescence intensity at 1:512 may be used as the cut-off level required for a test 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 should be rechecked, and the assay repeated from the beginning.

INTERPRETATION OF RESULTS
A positive reaction appears as brightly fluorescent (at least 2+) sharp, regular stained coccobacilli within the cytoplasm of the fixed Vero cells, while the Negative Control well is an example of fluorescence patterns that are to be considered negative. The size, appearance and density of the reaction must be compared with the Positive and Negative Control reactions. Patterns of reactivity different than those seen in the Positive Control must be considered non-specific.

PATIENT SPECIMENS

Positive at 1:64: Single IgM titers of 1:64 and greater are considered to reflect recent or active infection.

Negative at 1:64: Report as negative for Bartonella antibody.

LIMITATIONS
Antibody to any of the other Bartonella species may produce type-specific reactions and be seen as negative in this assay.

EXPECTED VALUES
The prevalence of antibody to Bartonella antibodies varies depending upon the geographic region and population being tested. The average prevalence of IgG seropositives in the literature is in the range of 40+%.

REFERENCES

1. Regnery RL, Olson JG, Perkins BA, Bibb W. Serological response to "Rochalimaea henselae" antigen in suspected cat scratch disease. Lancet 1992;339:1443-5.

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