# INSTRUCTIONS FOR USE

**Flea-borne Typhus**
**IFA IgM Antibody Kit**

Catalog Number: FBTM-120

Size: 120 test

Storage: 2-8˚C

An Indirect fluorescence immunoassay for the detection and quantitative determination of IgM class antibody against both *Rickettsia typhi* and *Rickettsia felis* in human serum or plasma

**For in-vitro diagnostic use only**

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**INTENDED USE**

The Flea-borne typhus IFA IgM Antibody kit is intended for the simultaneous detection and semi-quantitation of IgM class human antibody to both typhus group Rickettsia and Rickettsia felis, to be used as an aid in the diagnosis of human infection by these pathogens.

**SUMMARY AND EXPLANATION OF TEST**

Spotted Fever Group Rickettsia are found worldwide and are usually mediated by ticks whose bite transfers an infection. ***Rickettsia felis*** is, however, an unusual spotted fever Rickettsia that is transmitted by fleas and is also found throughout the world.

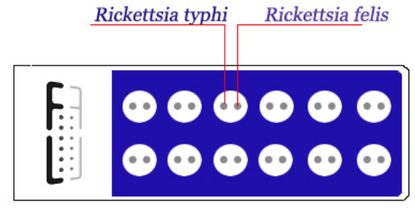
Typhus Group Rickettsia are transmitted by infected fleas and lice. This group includes ***Rickettsia typhi*** (endemic typhus) and ***Rickettsia prowazekii*** (epidemic typhus), both species found worldwide. The ensuing infections induce a specific antibody response, which may be detected and used as an indirect means of identifying an infected human.

The IFA slides in this kit utilize purified ***Rickettsia typhi*** and ***Rickettsia felis*** as individual substrate antigens within each slide well. Patient sera are diluted at least 1:64 in an adsorbent suspension and incubated in the individual slide wells to allow reaction of serum antibody with the rickettsia. The slides are then washed to remove non-reactive serum proteins, and a fluorescence conjugate is added to label the antigen-antibody complexes. After further incubation, the slides are washed again to remove non-reactive Conjugate. The resulting reactions can be visualized using standard fluorescence microscopy, where a positive reaction is seen as small sharply defined fluorescent rod forms dispersed within a red-counterstained background matrix. A negative reaction is seen as either counterstained (red) background or fluorescence different from that seen in the Positive Control wells. 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 x 12-well masked slides containing 2 acetone-fixed antigen spots in each well. Please note: As the slides are viewed from left-to-right (frosted-end to the left), the R. typhi antigen is viewed first. To the right of this antigen, in each slide well, is the R. felis antigen dot. Be aware of the inversion produced by the lens optics.




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

Yellow-cap dropper bottle contains ready to use affinity-purified and Alexafluor 488-labeled goat anti-human IgM (heavy chain) with anti-mouse IgG, bovine serum albumin and Evans' blue counterstain.

SAMP DIL **IgM Sample Diluent, 15 mL**

Buffer contains goat antihuman IgG serum in PBS with 3% sonicated background matrix as adsorbent. Mix by inversion before use.

CONT + **Positive Control, 0.5 mL**

Blue-cap dropper bottle contains serum reactive against ***Rickettsia felis*** and is bottled at a 1:64 screening dilution. Endpoint titer is 1:512.

CONT + **Positive Control, 0.5 mL**

Green-cap dropper bottle contains serum reactive against ***Rickettsia typhi*** and is bottled at a 1:64 screening dilution. Endpoint titer is 1:512.

CONT – **Negative Control, 0.5 mL**

Red-cap vial contains serum non-reactive against both Rickettsia and is bottled at a 1:64 screening dilution.

MM **Mounting Medium, 1 mL**

White-cap dropper bottle 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.

**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**

Store kit components refrigerated at 2-8ºC. Bring them to ambient temperature (20-25ºC) before opening bottles or slide envelops.

**SPECIMEN COLLECTION**

Allow blood samples to clot and separate sera by centrifugation. Transfer sera aseptically to tightly closing sterile containers. Store at 2-8ºC. If testing is to be delayed longer than 5 days, sera should be stored at -20ºC or colder. Acute sera should be drawn at the onset of illness and convalescent sera at two and four week intervals to check for titer changes.

**PROCEDURE**

**Material Supplied**

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
- Wash bath with slide rack
- Test tubes or microtiter equivalent for manual dilutions
- Precision pipette(s) for making dilutions and delivering exactly 10 µL per slide well
- 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 and is packaged in opaque plastic for protection. Store in the dark and return to storage immediately after use
- Liquid components contain 0.001% thimerosal as preservative. Do not ingest..
- Conjugate contains Evans' Blue dye, which may be carcinogenic. Avoid contact with skin.

**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 Sample Diluent in micro-centrifuge tubes. Mix and allow a minimum of 5 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**

1. Prepare serial 2-fold dilutions in Wash Buffer of the Positive Control to include 1 dilution above and 1 dilution below the stated endpoint (1:512). All controls are pre-diluted and bottled at 1:64

2. For each serum dilution, add 10 µL to one slide well and record the location for later reference. For each run include the Positive Control and the serial dilutions of the Positive Control prepared in step 1. Also add 1 drop of Negative Control to one well. Samples should be applied to the top or bottom edge of the well, avoiding the mid-section containing the antigen microdots.

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

4. Remove humidity chamber from incubator or water bath. Also remove conjugate from storage. Rinse slide wells with gentle stream of PBS from wash bottle, washing 1 row of wells at a time to avoid mixing of specimens. Then immerse slides in a container filled with fresh PBS for at least 5 minutes.

5. Dip slides briefly in distilled water and allow them to air dry.

6. To each slide well add 1 drop (10 µL) IgM Conjugate, then return slides to humidity chamber for a 30 minute incubation at 37º± 0.5ºC. Incubation should be in the dark to protect the photosensitive conjugate.

7. Wash slides as in steps 5-6, above.

8. Add 3-4 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 Positive and Negative Control wells. Slides may be stored at 2-8ºC in the dark for up to 24 hours.

**QUALITY CONTROL**

The Negative Control serum and dilutions of the Positive Controls must be assayed with each daily run. The Negative Control well is an example of a non-reactive serum, with no distinct and characteristic staining of the rickettsia. The Positive Control wells should give endpoint titers from 1:256 to 1:1024 (4-16-fold beyond the bottled screening dilution). The fluorescence intensity at 1:512 may be used as the cut-off level required for test reactions to be called positive. If any of the Controls do not react as specified, the assay run is 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 staining (at least 1+) of short pleomorphic rod forms. The size, appearance, and density of each field must be compared with the Positive and Negative Control reactions. Patterns of reactivity different from the Positive Controls must be considered non-specific.

Primary (initial) infection is characterized by a prompt rise in both IgG and IgM class antibody by IFA. IgM levels peak approximately 3 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 approximately 12 months.

**PATIENT SPECIMENS**

**Positive at 1:64:** IgM titers of 1:64 and greater reflect infection within the recent past. Positive sera should be titered to determine their endpoint for comparison with earlier or later specimens from the same patient.

**Negative at 1:64**: Report as negative. Further sera should be drawn if the original was taken immediately post onset of symptoms, especially if antibiotic therapy was instituted**.**

**Paired Sera**: A four-fold increase in titer, between acute and convalescent serum specimens, is considered strong evidence supporting the diagnosis of recent infection**.**

**LIMITATIONS**

- In attempting to support the diagnosis of rickettsial infection in newborns the IgM class antibody should be tested for, as any IgG class antibody detected may be maternal in origin.

- A low-level of cross-reactivity is seen with the R. felis antigen, mostly with R. akari and R. australis. Cross-reactivity within the typhus group, which includes the species *typhi* and *prowazekii*, is very strong using the IFA technique.

- Cross-reactivity between spotted fever group and typhus fever group reactivity is much less evident, and titers 8-32-fold lower than those to the infecting antigenic group are observed.

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Revision New