INSTRUCTIONS FOR USE

Francisella tularensis IFA

IgG Antibody Kit

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

An indirect fluorescence immunoassay for the detection of IgG class antibody against *Francisella tularensis* in human serum or plasma

For in-vitro diagnostic use only

FULLER LABORATORIES

1135 E. Truslow Avenue
Fullerton, California 92831 USA
Phone: +1-714-525-7660
Fax +1-714-525-7614
Email:info@fullerlabs.com
URL: www.fullerlabs.com

EC REP

MediMark Europe Sarl
11, rue Émile Zola – BP 2332
F-38033 Grenoble Cedex 2 – France

INTENDED USE

The *Francisella tularensis* IFA IgG Antibody Kit is intended for the detection and semi-quantitation of IgG class human antibody to *Francisella tularensis*, to be used as an aid in the diagnosis of human infection by this pathogen.

SUMMARY AND EXPLANATION OF TEST

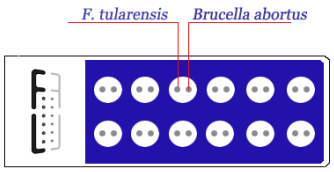
Tularemia is a zoonotic disease found throughout much of the northern hemisphere, associated with a wide range of mammalian, avian and insect reservoir hosts. Diagnosis is based upon clinical symptoms, exposure to reservoir hosts and, in ambiguous cases, laboratory test results.

The IFA slides in this kit utilize both *Francisella tularensis* (subspecies tularensis) and *Brucella abortus*, each within a contrasting background matrix. Brucella abortus is placed in a separate spot, to the right of the Francisella antigen, as a control antigen. Patient and control sera are diluted to screening dilution in PBS and incubated in the individual slide wells to allow reaction of serum antibody with the organisms. The slides are then washed to remove unreacted serum proteins, and an DyLight 488-labeled anti-human IgG (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 coccobacilli. A negative reaction is seen as the absence of sharply defined organisms, like that seen in the Negative 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 avirulent strain B-38 *Francisella tularensis* and *Brucella abortus* in separate spots (see below), each within a background matrix. Slides are fixed (inactivated) and packaged under vacuum, ready for use.



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

Dropper bottle with a yellow cap contains affinity-purified DyLight 488-labeled goat anti-human IgG (heavy chain) and goat anti-rabbit IgG (heavy chain) with bovine serum albumin and Evans' blue counterstain.

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

Dropper bottle with a blue cap contains reactive rabbit serum, provided at a 1:64 screening dilution. Endpoint titer is 1:512.

CONT | – **Negative Control, 0.5 mL**

Dropper bottle with a red cap contains non-reactive human serum at a 1:50 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.

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, but 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 samples at -20ºC 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

- 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
- 37ºC water bath or incubator
- Humidity chamber for slide incubation steps

Precautions

- Do not use components past expiration date.
- Liquid reagents contain thimerosal at 0.001%, which may be toxic if ingested

ASSAY PROCEDURE

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

1. Prepare 1:64 screening dilutions (1 part patient serum with 63 parts PBS) for all untested serum specimens. For sera found positive on a previous assay run, prepare serial dilutions in PBS, starting with 1:64.

2. Prepare dilutions of the Positive Control in PBS to include one dilution above the stated endpoint and one dilution below the stated endpoint (1:4-1:16 of bottled Control).

3. For each serum dilution, add 10 µL to one slide well., Include dilutions of the Positive Control prepared in step 2, along with the Negative Control, as supplied.

4. Place slides into a humid chamber and incubate 30 minutes at 37º± 0.5ºC .

5. Rinse slide wells with gentle stream of PBS from the wash bottle three (3) times and go directly to next step.

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

7. Wash slide as in steps 5, above, add 2-3 drops of Mounting Medium to each slide and apply cover slip.

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 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, not demonstrating any of the specific fluorescence or antigen density seen in the Positive Control. 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 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 staining (at least 1+) of characteristic coccobacilli. The size, 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 1:64: Sera positive at 1:64 should be titered to endpoint. Endpoint titers between 1:64 and 1:128 may be considered borderline positive and warrant testing for IgM antibody.

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

LIMITATIONS

The IFA procedure detects genus-specific antibody in most cases, thus a differentiation between *Francisella tularensis* and other *Francisella spp.* is not possible with this procedure alone.

EXPECTED VALUES

The prevalence, sensitivity and specificity varies with the geographic region and population being tested. When a series of normal blood donor sera (n=96 from Texas) were tested with this kit, the highest titer was 1:64 (1 serum).

Another group of sera were drawn from landscapers on Martha's Vineyard in Massachusetts, an endemic area for tularemia[2]. These sera had been tested previously by the micro-agglutination test (MAT). Of the 213 sera 17 were MAT positive at a cutoff of 1:128, while 23 were positive by IFA. The IFA+/MAT- sera were in the range 1:64-1:256 and all IgM-negative, suggesting past infection.

Of the 17 MAT positive sera, 16 were IFA-IgG positive and 13 also IFA-IgM positive. The single MAT+/IFA- was negative for both IgG and IgM by IFA.

BIBLIOGRAPHY

1. Porsch-Öscürümez M, Kischel N, Priebe H, Spettstösser W, Finke E, Grunow R. Comparison of Enzyme-Linked Immunosorbent Assay, Western Blotting, Microagglutination, Indirect Immunofluoresccence Assay, and Flow Cytometry for Serological Diagnosis of Tularemia. Clin Diag Lab Immunol. 2004 **11**: 1008-1015.

2. Feldman, K. A., D. Stiles-nos, K. Julian, B. T. Matyas, S. R. Telford III, M. C. Chu, L. R. Petersen, and E. B. Hayes. 2003. Tularemia on Martha's Vineyard: seroprevalence and occupational risk. Emerg Infect. Dis. **9**:350-354.

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