

INSTRUCTIONS FOR USE

Orientia tsutsugamushi IFA IgM Antibody Kit

Catalog Number: OTM-100

Size: 100 test

Storage: 2-8°C

An Indirect fluorescence immunoassay for the detection and semi-quantitative determination of IgM class antibody against *Orientia tsutsugamushi* in human serum or plasma

For in-vitro diagnostic use only



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INTENDED USE

The *Orientia tsutsugamushi* IFA IgM Antibody kit is intended for the simultaneous detection and semi-quantitation of IgM class human antibody to *Orientia tsutsugamushi*, to be used as an aid in the diagnosis of human infection by this pathogen.

SUMMARY AND EXPLANATION OF TEST

Scrub typhus is caused by the bite of trombiculid mite larvae (chiggers) harboring *Orientia tsutsugamushi*. This acute febrile disease is found throughout much of eastern Asia, Australia and the South Pacific Islands. There are many strains described and sufficient variation throughout the wide range of strains to make vaccination strategies difficult.

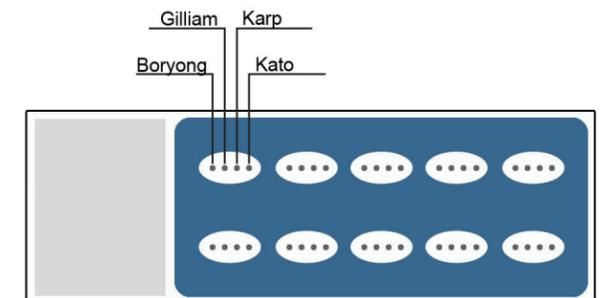
The IFA slides in this kit utilize 4 strains (Boryong, Gilliam, Karp and Kato) propagated in L292 cells *in vitro* and presented in a linear array of infected cells within each slide well. Patient sera are diluted at least 1:64 in IgM Serum Diluent and incubated in the individual slide wells to allow reaction of serum antibody with the *Orientia*. 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 each antigen spot, both intracellular (cytoplasmic) and extracellular. A negative reaction is seen as counterstained (red) cells alone 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 10-well masked slides containing 4 acetone-fixed antigen strains (Boryong, Gilliam, Karp, and Kato) in each well.



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 bovine serum albumin and Evans' blue counterstain.

CONT +

Positive Control, 0.5 mL

Blue-cap dropper bottle contains human serum reactive against *Orientia* and is bottled at a 1:64 screening dilution. Endpoint titers are as follows:

Kato 1:512
Karp 1:512
Boryong 1:256
Gilliam ≥1:1024

SAMP DIL**IgM Serum Diluent, 10 mL**

Buffer contains goat anti-human IgG antibody.

CONT –**Negative Control, 0.5 mL**

Red-cap vial contains human serum non-reactive against Orientia and 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 heat-inactivated antigens. However, the slides should be considered potentially infectious and handled accordingly.

Storage and Handling

Store kit components at 2-8°C. Bring them to ambient room temperature (20-25°C) before opening bottles or slide envelopes.

SPECIMEN COLLECTION

Allow blood samples 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 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 100 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 20 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
- 37°C 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 1:64 dilutions** of patient sera by first diluting the sera 1:16 in IgM Serum Diluent, then diluting 1:4 further using the Wash Buffer. Mix well and allow this mixture to incubate at least 20 minutes at ambient temperature. Centrifuge these treated samples and use the supernatant serum for testing.
3. This 1:64 dilution is referred to as the screening dilution. For further dilutions of positive sera, dilute the screening dilution with Wash Buffer (PBS).

ASSAY PROCEDURE

1. Prepare serial 2-fold dilutions in Wash Buffer of the Positive Controls 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 20 µL to one slide well and record the location for later reference. For each assay 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 37°± 0.5°C.
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.
5. To each slide well add 1 drop (20 µL) IgM Conjugate, then return slides to humidity chamber for a 30 minute incubation at 37°± 0.5°C. Incubation should be in the dark to protect the photosensitive conjugate.
6. Wash slides as in steps 5-6, above.
7. Add 3-4 drops of Mounting Medium to each slide and apply coverglass.
8. 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 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 Orientia. The Positive Control wells should give endpoint titers from 1:256 to 1:1024 (4-16-fold beyond the bottled screening dilution). Antigen-specific endpoints are listed under Positive Control description. The fluorescence intensity at listed endpoints 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 in any of the 4 antigen areas. 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 suggest recent infection and 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

- Varied cross-reactivity is seen in the MIF procedure between strains used in this assay and strains seen in various regions. This assay is not intended to determine strain-specificity of antibody reactivity, rather to improve test sensitivity by using 4 different strains.

Original Version 4/2010
10-well linear array: 09/2016