# Scrub Typhus IgM Quanti Card

***Fluorescence immunoassay for qualitative detection of Scrub Typhus IgM antibodies in Human Serum/ Plasma***

## INTRODUCTION

Scrub Typhus, or tsutsugamushi fever, is a zoonotic disease that is accidentally transmitted to humans. The infection is found in South East Asia and western Pacific islands. The causative organism, Orientia tsutsugamushi, belongs to family Rickettsiaceae is transmitted to humans by the bite of a larval trombiculid mite or chigger. A cigratte burn-like sore, called an eschar, sometimes develops at the site of infection. Swollen lymph glands also are common. The bite from an infected chigger may by followed by a systemic illness ranging in severity from inapparent to fatal. Many scrub typhus cases go undiagnosed, particularly those in which an eschar cannot be found. Most common symptoms are fever, headache, body ache and sometime rashes. As very few health facilities have accessible accurate diagnostic tests, the diagnosis of scrub fever must be based on clinical features. However, this is difficult because the clinical symptoms and signs are similar to those of many other febrile diseases, such as murine typhus, leptospirosis, and dengue virus infection. The diagnosis of scrub typhus infection has relied on the detection of O. tsutsugamushi antibodies during the acute phase of the disease.

## INTENDED USE

Scrub Typhus IgM Quanti Card is a sensitive immuno- chromatographic test for the qualitative detection of Scrub Typhus IgM antibodies in human Serum/ Plasma with iQuant Analyzer. This test is for in vitro diagnostic use only and is intended as an aid in the diagnosis of Scrub Typhus infection.

## PRINCIPLE (ANTIGEN-ANTIBODY REACTION)

Scrub Typhus IgM Quanti Card is a fluorescence immunoassay. The test area is coated with Scrub Typhus antigen . When a sample is added to the device, Scrub Typhus IgM antibodies in the sample react with anti-human IgM antibodies conjugated to flourescent dye. On addition of Assay Buffer, this complex migrates along the nitrocellulose membrane to the test region and forms an antibody-antigen-antibody fluorescence immunocomplex. The result will be displayed by iQuant Analyzer.

## MATERIALS PROVIDED

Scrub Typhus IgM Quanti Card kit contains following components to perform the assay:
1. Scrub Typhus IgM Quanti Card Device (1 Tests) 2. Assay Buffer
3. Instruction Manual

## MaTERIAL rEQUIRED, bUT nOT pROVIDED

iQuant Analyzer Micropipette & Microtips
Stop Watch

## KIT PRESENTATION

10 Test Pack 25 Test Pack

## STORAGE AND STABILITY

The kit should be stored at 2-8ºC in the coolest and driest area available. Expiry date on the kit indicates the date beyond which kit and its components should not be used. Scrub Typhus IgM Quanti Card should not be frozen and must be protected from exposure to humidity.

## DESCRIPTION OF SYMBOLS USED

The following are graphical symbols used in or found on J. Mitra diagnostic products and packing. These symbols are the most common ones appearing on medical devices and their packing. They are explained in more detail in the European Standard EN ISO15223-1:2021.

| | | | |
|---|---|---|---|
| | Manufactured By | IVD | In vitro diagnostic medical device |
| Σ | No. of tests | | See Instruction for use |
| LOT | Lot Number Batch Number | 2°C 8°C | Temperature Limitation |
| | Manufacturing Date | | Caution, see instruction for use |
| | Expiry Date | REF | Catalogue Number |
| | Do not use if package is damaged | BIO | Contains biological Material of Animal Origin |
| 2 | Single use only | | Keep away from sunlight |
| | Keep Dry | IN | Country of Manufacture |

## WARNING FOR USERS

⚠ **CAUTION:** ALL THE SAMPLES TO BE TESTED SHOULD BE HANDLED AS THOUGH CAPABLE OF TRANSMITTING INFECTION. NO TEST METHOD CAN OFFER COMPLETE ASSURANCE THAT HUMAN BLOOD PRODUCTS WILL NOT TRANSMIT INFECTION.

1. The use of disposable gloves and proper biohazardous clothing is STRONGLY RECOMMENDED while running the test.
2. In case there is a cut or wound in hand, DO NOT PERFORM THE TEST.
3. Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.
4. Tests are for in vitro diagnostic use only and should be run by competent person only.
5. Mark the test specimen with patient's name or identification number. Improper identification may lead to wrong result reporting.
6. Do not pipette by mouth.
7. All materials used in the assay and samples should be decontaminated in 5% sodium hypochlorite solution for 30-60 min. before disposal or by autoclaving at 121ºC at 15psi for 60 min. Do not autoclave materials or solution containing sodium hypochlorite. They should be disposed off in accordance with established safety procedures.
8. Wash hands thoroughly with soap or any suitable detergent, after the use of the kit. Consult a physician immediately in case of accident or contact with eyes, in the event that contaminated material are ingested or come in contact with skin puncture or wounds.
9. Spills should be decontaminated promptly with Sodium Hypochlorite or any other suitable disinfectant.
10. Assay Buffer contains Sodium Azide as a preservative. If these material are to be disposed off through a sink or other common plumbing systems, flush with generous amounts of water to prevent accumulation of potentially explosive compounds. In addition, consult the manual guideline "Safety Management No. CDC-22", Decontamination of Laboratory Sink Drains to remove Azide salts" (Centre for Disease Control, Atlanta, Georgia, April 30, 1976).
11. Follow standard biosafety guidelines for handling & disposal of potentially infective material.

## PRECAUTIONS

In order to obtain reproducible results, the following instructions must be followed:

1. Do not use the kit beyond the expiry date.
2. Do not mix reagents from different batches.
3. Do not open the foil pouch until it attains room temperature.
4. Do not re-use the test cartridge.
5. Follow the given test procedure and storage instructions strictly.
6. Do not temper / paste any sticker or write anything on the QR-Code as this will lead to erroneous result.
7. Do not touch the membrane with the pipette tip.

***Important Note: Scrub Typhus IgM Quanti Card is only operational in conjection with iQuant Analyzer.***

## SAMPLE / SPECIMEN COLLECTION AND STORAGE

1. Serum/ Plasma samples may be used with this test.
2. For plasma, collect the whole blood in a clean container (containing EDTA, sodium flouride or heparin) by venipuncture. Fresh samples are preferred for testing as they perform best when tested immediately after collection. If specimens cannot be tested immediately, they should be refrigerated at 2-8ºC. For storage for more than 3 days, freeze the specimen at -20ºC or below.
3. Repeated freezing and thawing of the specimen should be avoided.
4. Specimens containing precipitate or particulate matter may yield inconsistent test results. Such specimens must be centrifuged and the clear supernatant should only be used for testing.
5. The use of hemolytic, lipaemic, icteric or bacterially contaminated specimens should be avoided as it may lead to erratic results.

## BEFORE YOU START

The Assay Buffer Solution provided in the kit has closed nozzle and screw cap with pin (outside). Before using Assay Buffer, keep the vial vertically straight and tap down gently on the working platform, so that Assay Buffer comes down at the bottom of the vial. To orifice/puncture the closed nozzle, follow the instruction as illustrated below:

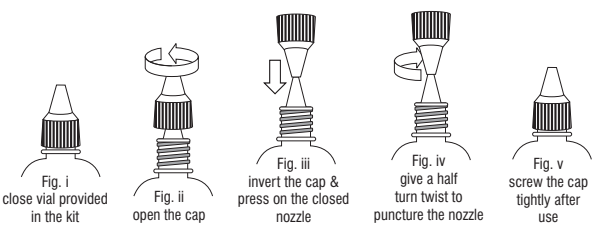

Fig. i close vial provided in the kit
Fig. ii open the cap
Fig. iii invert the cap & press on the closed nozzle
Fig. iv give a half turn twist to puncture the nozzle
Fig. v screw the cap tightly after use

## TEST PROCEDURE

1. Bring the complete kit and specimen to be tested to room temperature prior to testing.
2. Remove the test cartridge from the foil pouch prior to use and place it on a flat and dry surface. The test should be performed immediately after removing the test card from the foil pouch.
3. Label the test cartridge with patient's name or identification number as shown in Fig. (a). Do not write on QR code.

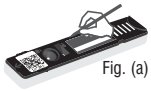
Fig. (a)

4. Add 10$\mu$l Serum/ Plasma sample using micropipette onto the sample pad in the sample well 'S'. Care should be taken to avoid any spillage on the QR Code.

***NOTE: Make sure that the sample from the micropippette has been completely transferred to the sample pad.***

5. Add 3 drops of the Assay Buffer in the buffer well 'B'.
6. Allow the reaction to occur for 20 minutes.
7. Insert the test cartridge into the i-Quant Analyzer with arrow (←) marked side on the top of cartridge facing towards the analyzer and press RUN icon. Note down the value displayed on the screen of i-Quant Analyzer & interpret the result as mentioned below.
8. Discard the Scrub Typhus IgM Quanti Card immediately after reading results at 20 minutes considering it to be potentially infectious.

***Important Note: Do not read results after 20 minutes.***

***Discard the Scrub Typhus IgM Quanti Card immediately after reading results considering it to be potentially infectious.***

## INTERPRETATION OF RESULTS

The iQuant Analyzer will display results as Reactive, Equivocal or Non-Reactive as follows:

**Reactive:** >1.1 U and above: interpret the result as Scrub Typhus IgM Antibody reactive.

**Equivocal:** >0.9 to ≤1.1 U: Interpret the result as equivocal. Repeat the test after centrifuging the sample at 5000 rpm for 20 minutes. Even after repeating the test, if result comes equivocal, further test the sample with alternative method or collect another sample.

**Non-Reactive:** Below ≤0.9 U: Interpret the result as Scrub Typhus IgM Antibody non-reactive.

## PERFORMANCE CHARACTERISTICS OF SCRUB TYPHUS IgM QUANTI CARD

**(i) In-House Evaluation:**

(A) The performance of the test is evaluated in-house and compared with a licensed commercially available test kit by using a known panel of Scrub Typhus negative & Positive clinical samples .The Evaluation includes 160 negative samples ,10 positive samples & 10 cross reacting samples. The cross-reacting samples included in the evaluation are; Malaria positive, Dengue positive, Typhi positive, CRP, Leptospira positive, HIV positive, HBV positive, HCV positive, Chikungunya positive, and RA positive samples. The results obtained are as follows:

Sensitivity : **100%** Specificity : **100%**

**(B) Precision**

**a) Intra-Assay:** Within run (Intra assay) precision have been determined by testing 10 replicates of 2 Scrub Typhus positive panel samples (1 weak & 1 Medium) and 2 Scrub Typhus negative panel samples. The C.V. (%) for all samples is ≤10%.

**b) Inter-Assay (Reproducibility):** Between run (Inter assay) precision have been determined by testing 10 replicates in 10 different runs for 10 sequential days by the same analyst of 2 Scrub Typhus positive panel samples (1 weak & 1 Medium) and 2 Scrub Typhus negative panel samples. The C.V. (%) for all samples is ≤10%.

**(ii) External Evaluation**

The performance of Scrub Typhus IgM Quanti card has been evaluated by Referral Government Medical College with clinical patient samples (fresh and frozen) ; Scrub Typhus Positive and negative samples in comparison with standard reference kit ; Scrub TyphusTM In-Bios Elisa . The evaluation included 69 Scrub typhus negative & 15 positive samples . The results obtained on 3 different lots are as follows:

Sensitivity : **100%** Specificity: **98.55%**

## LIMITATIONS AND INTERFERENCES

1. The test is for in vitro diagnostic use only.
2. This test detects the presence of Scrub Typhus IgM antibodies to Scrub Typhus fever in the specimen and should not be used as the sole criteria for the diagnosis of Scrub Typhus infection.
3. As with all diagnostic tests, all results must be correlated with other clinical findings. If the test result is negative and clinical symptoms persist, additional follow-up testing using other clinical methods is recommended. A negative result at any time does not preclude the possibility of an early infection of Scrub Typhus infection.
4. This is only a screening test. Therefore, other serological test like IFA, ELISA and PCR more specific alternative diagnosis method must be used in order to obtain a confirmation of Scrub Typhus infection.

## LIMITED EXPRESSED WARRANTY DISCLAIMER

The manufacturer limits the warranty to the test kit, as much as that the test kit will function as an in vitro diagnostic assay within the limitations and specifications as described in the product instruction-manual, when used strictly in accordance with the instructions contained therein. The manufacturer disclaims any warranty expressed or implied including such expressed or implied warranty with respect to merchantability, fitness for use or implied utility for any purpose. The manufacturer's liability is limited to either replacement of the product or refund of the purchase price of the product and in no case liable to claim of any kind for an amount greater than the purchase price of the goods in respect of which damages are likely to be claimed. The manufacturer shall not be liable to the purchaser or third parties for any injury, damage or economic loss, howsoever caused by the product in the use or in the application there of.

## TROUBLE SHOOTING

| | PROBLEM | POSSIBLE CAUSE | SOLUTION |
|---|---|---|---|
| 1. | False negative Test Result | a) Low sample volume along with less volume of Assay Buffer used. | Repeat the test with proper volume of samples and/or Assay Buffer. |
| 2. | False positive Test Result | a) Less volume of Assay Buffer added/used. | Repeat the test with proper volume of Assay Buffer. |

## REFERENCES


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in vitro diagnostic reagent, not for medicinal use


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