

# SCRUB TYPHUS (TSUTSUGAMUSHI) IgM & IgG CARD

## Rapid Visual test for the qualitative & differential detection of IgM & IgG Antibodies to Scrub Typhus (Tsutsugamushi) 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 trobiculid 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. Scrub Typhus (tsutsugamushi) IgM & IgG card test provide accurate, easy and realiable diagnosis of Scrub Typhus, or tsutsugamushi fever in human plasma or serum.

### INTENDED USE

Scrub Typhus (Tsutsugamushi) IgM & IgG Card is a rapid solid phase immuno-chromatographic assay for the qualitative and differential detection of IgM and IgG antibodies to Scrub Typhus antibodies in human serum / plasma. This test is for in vitro diagnostic use only and is intended as an aid to early diagnosis of Scrub Typhus infection in patient with clinical symptoms.

### PRINCIPLE

Scrub Typhus (Tsutsugamushi) IgM & IgG Card test device contains three lines; “C” (Control line), “M” (IgM test line) & “G” (IgG test line). IgM test line is coated with anti-human IgM and IgG test line is coated with anti-human IgG. When a sample is added to the sample well of the device followed by two drops of buffer in the buffer well, IgM and IgG antibodies in the sample reacts with anti-human IgM and anti-human IgG antibodies coated on the membrane respectively. If the sample contain Scrub Typhus (Tsutsugamushi) antibodies, colloidal gold conjugate containing Scrub Typhus antigens is captured by the bound anti-Tsutsugamushi IgM &/or IgG on respective test bands forming a pale to dark red band at the IgM &/or IgG region of the test device. The intensity of the test bands in the device will vary depending upon the amount of antibodies present in the sample. The appearance of any pink/red colour in a specific test region should be considered as positive for that particular antibody type (IgM or IgG). A red procedural control line should always develop in the test device window to indicate that the test has been performed properly.

### KIT CONTENTS
















- |  |                       |
|--|-----------------------|
| a) Scrub Typhus (Tsutsugamushi) IgM & IgG Card | b) Sample Dropper     |
| c) Assay Buffer                                | d) Instruction Manual |

### KIT PRESENTATION


|              |              |
|--------------|--------------|
| 20 Test Pack | 50 Test Pack |
|--------------|--------------|

### 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 ISO 15223-1:2016.

|   |                                  |   |   |
|---|----------------------------------|---|---|
|  | Manufactured By                  |  | In vitro diagnostic medical device                  |
|  | No. of tests                     |  | See Instruction for use                             |
|  | Lot Number / Batch Number        |  | Temperature Limitation                              |
|  | Manufacturing Date               |  | Caution, see instruction for use                    |
|  | Expiry Date                      |  | Catalogue Number                                    |
|  | Do not use if package is damaged |  | Authorized Representative in the European Community |
|  | Single use only                  |  | Keep away from sunlight                             |
|  | Keep Dry                         |   |   |

### 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.

- The use of disposable gloves and proper biohazardous clothing is STRONGLY RECOMMENDED while running the test.
- In case there is a cut or wound in hand, DO NOT PERFORM THE TEST.
- Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.
- Tests are for in vitro diagnostic use only and should be run by competent person only.
- Do not pipette by mouth.
- All materials used in the assay and samples should be decontaminated in suitable disinfectant solution for 30-60 min. before disposal or by autoclaving at 121°C 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 and guidelines.
- 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.
- Spills should be decontaminated promptly with Sodium Hypochlorite or any other suitable disinfectant.
- 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).
- Follow standard biosafety guidelines for handling & disposal of potentially infective material.

### PRECAUTION

- Do not open or remove test card from their individually sealed pouches until immediately before their use.
- Do not reuse test cards.
- All test device, buffer and specimens must be at room temperature before running the test.
- Do not use kit beyond the stated expiry date mentioned on the kit.
- Do not mix components from different lot numbers.
- Interpret the results at the end of 20 minutes only.

### KIT STORAGE & STABILITY

Scrub Typhus (Tsutsugamushi) IgM & IgG Card test kit should be stored at 2-30°C 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. The kit should not be frozen and must be protected from exposure to humidity.

### SPECIMEN COLLECTION AND PREPARATION

- Serum / plasma samples may be used with this test.
- If serum / plasma specimens cannot be tested immediately, they should be refrigerated at 2-8°C. For storage for more than 3 days, freeze the specimen at -20°C or below.
- Repeated freezing and thawing of the specimen should be avoided.
- 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.
- The use of hemolytic, lipaemic, icteric or bacterially contaminated specimens should be avoided as it may lead to erroneous 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 on back page.

### TEST PROCEDURE

- Bring the required number of Scrub Typhus (Tsutsugamushi) IgM & IgG Card foil pouches and specimen to room temperature prior to testing.
- Remove the test card from the foil pouch prior to use.
- Label the test card with patient’s name or identification number.

- Fill the lower circular part of the sample dropper with the specimen upto the mark provided on the dropper as shown in fig. (a). Then add the specimen to the sample well “S” of device as shown in fig. (b). This will add 10 µl of specimen to the device. Dispose off the dropper considering it to be biohazardous.

Alternatively, add 10 µl of sample using micropipette to the sample well of the antibody device.

### Important Note

- During addition of sample to sample window “S”, gently press the sample dropper onto the membrane of the device for 1 to 2 seconds. Ensure that the sample has been dispensed on the membrane and sample starts to flow on the membrane. This can be seen by observing the flow of the sample in device window. If the sample does not flow, again press the dropper tip gently and touch the membrane pad to remove entrapped air so that the sample starts to move and flow. Even if, still the sample does not flow, it could be that it contains particulate matter or is turbid. If so, rerun the test, after centrifuging at 10,000 rpm. for 10 minutes or more (in case clear sample is not obtained after centrifugation).**
- Do not add Assay Buffer until sample starts flow as it may lead to false positive results.**
- Never add assay buffer in the sample well “S” as it will stop the flow of conjugate. If in case the device does not show conjugate flow, it could be that the assay buffer has been added accidentally in the sample well. Repeat the test with fresh card.**
- The devices should not be opened / tampered as it will result in changes in the alignment of the membrane.**

- Add 2 drops (70 µl) of Assay Buffer to the Buffer well “B” of the device as shown in fig. (b).

**NOTE: Please ensure, no air is entrapped and full drop falls down from the nozzle tip.**

- Allow reaction to occur during the next 20 minutes.
- Read results at 20 minutes. Positive results may appear as early as 5-10 minutes. However, negative results must be confirmed after 20 minutes only.
- Discard the Scrub Typhus (tsutsugamushi) IgM & IgG Card Test immediately after reading result at 20 minutes, considering it to be potentially infectious.

### INTERPRETATION OF TEST RESULTS

#### NON-REACTIVE

Appearance of one distinct red coloured line in the control region ‘C’ only (with no line in the IgM region ‘M’ & IgG region ‘G’) indicates that the sample is non-reactive for Scrub Typhus (tsutsugamushi) antibodies.

#### IgM & IgG REACTIVE

Appearance of red coloured line in the control region ‘C’ and Test region; IgM region ‘M’ and IgG region ‘G’ indicates that the sample is reactive for Scrub Typhus (tsutsugamushi) IgM & IgG antibodies.

#### IgM REACTIVE

Appearance of red coloured line in the control region ‘C’ and Test region; IgM region ‘M’ indicates that the sample is reactive for Scrub Typhus (tsutsugamushi) IgM antibodies.

#### IgG REACTIVE

Appearance of red coloured line in the control region ‘C’ and Test region; IgG region ‘G’ indicates that the sample is reactive for Scrub Typhus (tsutsugamushi) IgG antibodies.

#### INVALID

If no control line appears after the completion of test, the test should be treated as Invalid which may be because of following reasons:

- Improper storage at temperature other than the recommended temperature.
- Wrong Procedure
- Long atmospheric exposure of the test device after opening the pouch.

**The test should be repeated using a new device and test sample.**

### LIMITATIONS OF THE TEST

- The test is for *in vitro* diagnostic use only.
- This test detects the presence of antibodies to Scrub Typhus Fever in the specimen and should not be used as the sole criteria for the diagnosis of Scrub Typhus infection.
- 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
- This is only a screening test. Therefore, other serological test like IFA , ELISA, PCR more specific alternative diagnosis method must be used in order to obtain a confirmation of Scrub Typhus infection.

### PERFORMANCE CHARACTERISTICS

The performance of the test is evaluated in- house and compared with a licensed commercially available test kit by using a known panel of Serum/ Plasma Scrub Typhus negative (50 clinical samples) ,33 cross reacting samples & 50 positive samples. The cross-reacting samples included in the evaluation are; HIV positive, HCV positive, HBV positive, Dengue positive, Chikungunya positive, Leptospira positive, Malaria positive, Typhi positive, Rheumatoid factor positive, CRP positive and ASO positive samples.

The results obtained are as follows:

|                           |                           |
|---------------------------|---------------------------|
| <b>Sensitivity : 100%</b> | <b>Specificity : 100%</b> |
|---------------------------|---------------------------|

**Precision:** Within run (Intra assay) & between run (Interassay) precision have been determined by testing 10 replicates of 4 samples - 2 negative samples & 2 Scrub Typhus positive samples. The results of all the four samples were within the defined acceptance criteria.

### 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.

### BIBLIOGRAPHY

- Kinetics of IgM and IgG abtibodies after scrub typhus infection and the clinical implications. G.M.Varghese, V.Manikandan Rajagopal etal. International Journal of Infectious diseases 71 (2018) 53-55.
- Diagnostic performance of serological test to detect antibodies against scrub typhus infection in central. K. Pote, R. Narang & P. Deshmukh. India. Ind J Med. Micro. 2018, Vol. 36, No.1,108-112
- Scrub Typhus: risks, diagnostic issues and management challenges. Research and Reports in Tropical Medicine. John Antony Jude Prakash. 2017:8, 73-83

REFER THE BACK PAGE OF THE INSTRUCTION MANUAL FOR PICTORIAL REPRESENTATION & BETTER CLARITY OF THE TEST PROCEDURE AND RESULT INTERPRETATION

IN-VITRO DIAGNOSTIC REAGENT, NOT FOR MEDICINAL USE

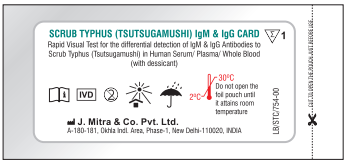
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# SCRUB TYPHUS (TSUTSUGAMUSHI) IgM & IgG CARD

Rapid Visual test for the qualitative & differential detection of IgM & IgG Antibodies to Scrub Typhus (Tsutsugamushi) in Human Serum / Plasma

## MATERIAL PROVIDED

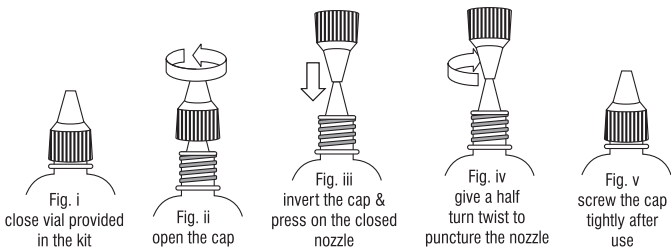


Test Device foil pouched with a desiccant. Lot No., Mfg. & Exp. date are printed on back of the pouch.



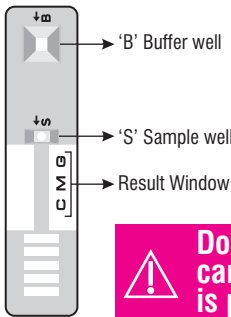
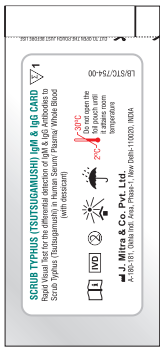
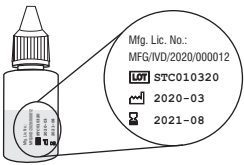
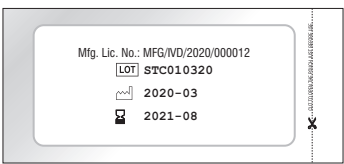
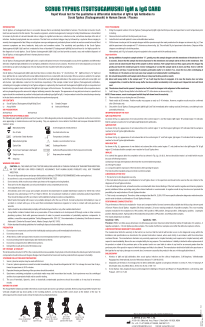
Instruction Manual

## BEFORE YOU START



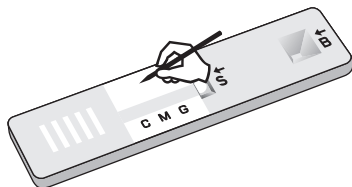
## PREPARATION FOR THE TEST

- 1) Carefully read the instruction manual for using the Scrub Typhus Rapid Test
- 2) Check the expiry date at the back of cassette pouch and Assay Buffer label. Use fresh kit if expiry date has passed.
- 3) Cut open the card foil pouch and check the color of the dessicant pouch, it should be blue in color.  
**Do not use the card if dessicant is pink in color.**
- 4) Bring the required number of Card, Assay Buffer and specimen to room temperature prior to testing.
- 5) Label the test card with patient's name or identification number.



**Do not use the card if dessicant is pink in color**

**RT**  
**20-30°C**



## TEST PROCEDURE

### 1) Specimen collection

Fill the lower circular part of the sample dropper with the specimen upto the mark provided on the dropper as shown in fig. (a).

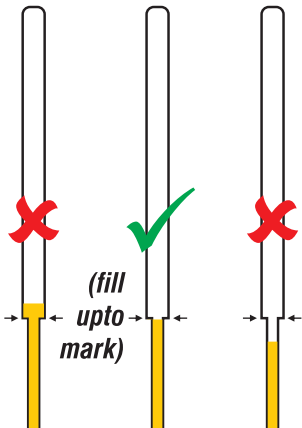


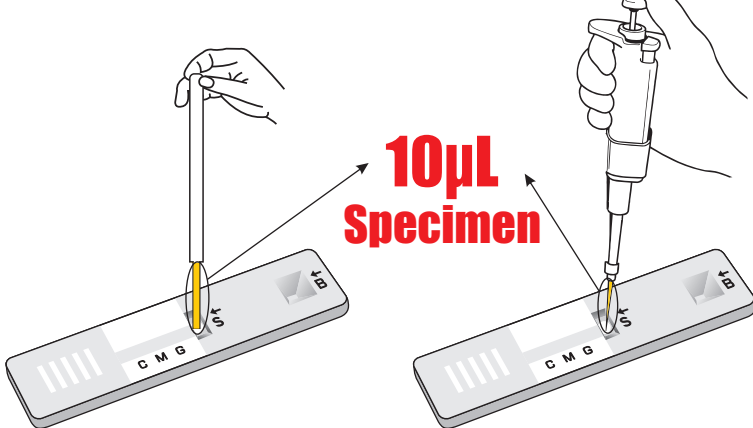
Fig. (a-i) Fig. (a-ii) Fig. (a-iii)

### 2) Specimen Addition

Add the specimen to the sample well "S" of device as shown in fig. (b).

**OR**

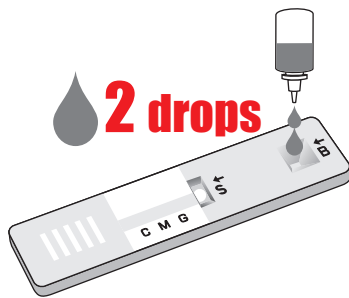
Add 10  $\mu$ L of sample using micropipette to the sample well of the antibody device.



**Note: Ensure tip of the sample dropper/ pipette touches the membrane of the device**

### 3) Assay Buffer Addition

Add 2 drops (70  $\mu$ L) of Assay Buffer to the Buffer well "B" of the device.



### 4) Reading Time

Read result after 20 Minutes



**20 minutes**

**Do not read results after 30 minutes**

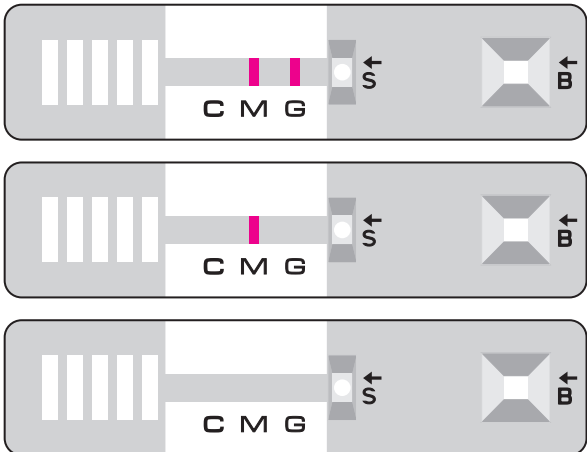
## RESULT INTERPRETATION

### NON-REACTIVE



Only 1 red color band in the control region 'C' indicates that the sample is non-reactive for Scrub Typhus (tsutsugamushi) IgM & IgG antibodies.

### INVALID



If no control line 'C' appears after the completion of test, the test should be treated as Invalid. Repeat the test using new card.

### REACTIVE for IgM & IgG



### REACTIVE for IgM



### REACTIVE for IgG



Appearance of red coloured line in the control region 'C' and Test region; IgM region 'M' and / or IgG region 'G' indicates that the sample is reactive for Scrub Typhus (tsutsugamushi) IgM & / or IgG antibodies.