SCRUB TYPHUS IgM/ IgG/ IgA CARD

Rapid visual test for the qualitative detection of IgM/ IgG/ IgA antibodies to Scrub Typhus (Tsutsugamushi) in Human Serum/ Plasma/ Whole Blood

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.

Scrub Typhus IgM/IgG/IgA Card is a rapid solid phase immuno-chromatographic assay for the qualitative detection of IgM, IgG & IgA antibodies to Scrub Typhus in human serum / plasma / whole blood. 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.

Scrub Typhus IgM/ IgG/ IgA Card is an immunoassay based on 'sandwich' principle. The test device contains two lines; "C" (Control line), "T" (Scrub Typhus IgM/IgG/IgA antibody detection test line). Test line is coated with Scrub Typhus antigen. When a sample is added to the device, Scrub Typhus IgM/IgG/IgA antibodies if present in the sample will bind to the Scrub Typhus antigen gold colloid conjugate making antigen-antibodies complex. On addition of assay buffer, this complex migrates along the nitrocellulose membrane by capillary action to the test region and forms the visible purplish pink line at "T" as antigen-antibodyantigen gold conjugate complex. The intensity of the test band in the device will vary depending upon the amount of antibodies present in the sample. The appearance of any purplish pink/red colour in test region should be considered as reactive for, Scrub Typhus IgM/IgG/IgA antibodies. A red procedural control line should always develop in the control Region "C" to indicate that the test has been performed properly.

Scrub Typhus IgM/IgG/IgA Card Test kit contains following components:

- Scrub Typhus IgM/IgG/IgA Card
- Assay Buffer
- Sample Dropper
- Instruction Manual

KIT PRESENTATION

25 Test Pack

Scrub Typhus IqM/IqG/IqA Card test kit should be stored at 2-30°C in the cool 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.

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 IS015223-1:2021.

Manufactured By In vitro diagnostic medical device No. of tests Consult Instructions for use (li Lot Number Temperature LOT Batch Number Manufacturing Date Caution, see instruction for use Expiry Date REF Catalogue Number Contains biological Material Do not use if package **®** ~‱[√] is damaged of Animal Origin 漛 2 Single use only Keep away from sunlight Country of Manufacture Keep Dry

WARNING & PRECAUTIONS 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

- 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.
- Tests are for in vitro diagnostic use only and should be run by competent person only. 4.
- 5. 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. 8
- 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).
- Use separate sample dropper or pipette tips for each sample in order to avoid cross-contamination of samples which could cause erroneous results.
- Mark the test specimen with patient's name or identification number. Improper identification may lead to wrong result
- Follow the given test procedure and storage instructions strictly to get proper result.
- Do not open or remove test device from their individually sealed pouches until immediately before their use. 14. Do not reuse test devices.
- All test device, assay 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. 17. Interpret the results at the end of 20 minutes only. 18.
- Follow standard biosafety guidelines for handling & disposal of potentially infective material. 19.

SAMPLE / SPECIMEN COLLECTION AND STORAGE

A) Sample Collection (Venous Blood - Serum/ Plasma/ Whole Blood):

- Collect the whole blood in a clean container without anticoagulants for serum or containing anticoagulants (EDTA, citrate or fluoride) for whole blood/plasma samples by venipuncture. Fresh samples are preferred for testing as they perform best when tested immediately after collection. If samples are not immediately tested, they should be stored at 2-8°C for not more than 3 days, otherwise false / erroneous results may be obtained.
- Hemolyzed or clotted blood sample or sample with microbial contamination should not be used.
- Serum/ plasma specimens, if not tested immediately, 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.

B) Finger Prick Sample Collection:

- Wipe the complete fingertip with the alcohol swab. Wait until the finger has completely dried (minimum 30 seconds).
- 2) Take the lancet and prick the side of the pulp (ball of the finger) with the lancet, perpendicular to the lines of the fingerprint.
- Make sure a well formed drop of blood is present on the tip of the finger. 3)
- Take the sample dropper/micropipette and collect $10\mu l$ of blood by dipping the tip of the sample dropper/micropipette into the blood drop as shown in Fig and immediately place the tip of the sample dropper in the sample well "\$". (Care should be taken that the blood sample does not clot & the transfer to the sample pad is immediate)

BEFORE YOU START

The Assay Buffer 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

STEP-1 PREPARATION FOR THE TEST

- $Carefully \ read\ the\ instruction\ manual\ for\ using\ the\ Scrub\ Typhus\ IgM/IgG/IgA\ Card.$
- B) Bring the required number of Scrub Typhus IgM/IgG/IgA card foil pouches and specimen to room temperature prior to testing.
- C) Remove the test card from the foil pouch prior to use and place it on flat and dry surface.
- D) Label the test card with patient's name and identification number.

STEP-2 RUNNING THE TEST ON DEVICE

Take 10μ I whole blood/Serum/Plasma using the sample dropper/micropipette and add the sample to sample well.

Important Note:

- Press the tip of the dropper onto the sample pad in the sample well to ensure that the complete volume of specimen has
- B) Addition of incorrect volume of sample/specimen may give invalid or false result.
- C) Sample dropper should be discarded immediately considering it to be bio-hazardous.
- Add 1 drop of assay buffer into the sample well. Ensure FREE FALLING OF DROP on the membrane, holding the vial/dropper vertically for proper volume. Screw cap the vial after use

Note: Addition of incorrect no. of drops of Assay Buffer may give invalid or false result.

- Allow reaction to occur for 20 minutes.
- Read results at 20 minutes. Positive results may appear as early as 2-10 minutes. However negative results must be confirmed at 20 minutes. Do not read results after 30 minutes.
- Discard the Scrub Typhus IqM/ IqG/ IqA card immediately after reading results at 20 minutes considering it to be potentially infectious. Follow the local regulations for disposal of potential biohazardous material

INTERPRETATION OF THE RESULTS

REACTIVE

Appearance of two purplish pink colored lines, one each at Control region 'C' & Test region 'T' indicates the presence of Scrub Typhus antibodies in the specimen.

NON-REACTIVE

Appearance of only one purplish pink colored line in the control region 'C' and no line on test region indicates that the specimen is non-reactive for Scrub Typhus antibodies.

If neither control line nor test line appears or only test line appears, the test should be treated as Invalid. Repeat the test again with new card. Invalid test may be because of following reasons:

- (a) Kit is stored in high humidity and temperature
- (b) Wrong test procedure.
- (c) Long atmospheric exposure of the test device after opening the pouch

(d) Use of turbid or lapaemic sample

Centrifuge the sample at 10,000 rpm for 15 minutes and repeat the test using a new device.

LIMITATIONS AND INTERFERENCES

- The test is for in vitro diagnostic use only.
- This test detects the presence of antibodies to Scrub Typhus in the human serum/ plasma/ whole blood 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 i.e more specific alternative diagnosis method like IFA, ELISA, PCR must be used in order to obtain a confirmation of Scrub Typhus infection.

PERFORMANCE CHARACTERISTICS OF SCRUB TYPHUS IgM/IgG/IgA CARD

The performance of the test is evaluated in- house and compared with a licensed commercially available rapid test kit by using a known panel of Serum, Plasma, and Whole blood samples ;Scrub Typhus negative (240 clinical samples) ,33 cross reacting samples & 10 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:

Sensitivity: 100% Specificity: 100%

External Evaluation:

The performance of the kit has also been evaluated with clinical samples; scrub typhus positive (35), scrub typhus negative (95) and 6 cross-reacing; 2 each of dengue, chikungunya and typhoid positive samples in comparision to commercially available (Inbios) ELISA. The results obtained are as follows

Sensitivity: 100% Specificity: 100%

(iii) Precision:

- Intra-Assay: Within run (Intra assay) precision have been determined by testing 10 replicate of 2 scrub typhus positive samples (1 weak & 1 medium) and 2 scrub Typhus negative samples. The CV (%) for all samples is ≤10%
- 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 samples (1 weak and 1 medium) and 2 Scrub Typhus negative samples. The CV (%) for all samples is $\leq 10\%$.

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 OF SUGGESTED READING

- Kinetics of IgM and IgG antibodies 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

SCRUB TYPHUS IgM/ IgG/ IgA CARD

Rapid visual test for the qualitative detection of IgM/ IgG/ IgA antibodies to Scrub Typhus (Tsutsugamushi) in Human Serum/ Plasma/ Whole Blood





Individually foil pouched test device with a dessicant.
Lot No., Mfg. & Exp. date are printed on back of the pouch.

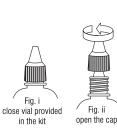


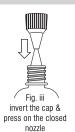
Sample Dropper

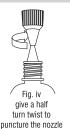


Instruction Manual

BEFORE YOU START









PREPARATION FOR THE TEST

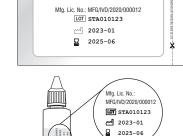
1) Carefully read the instruction manual for using the Scrub Typhus IgM/ IgG/ IgA Card Test



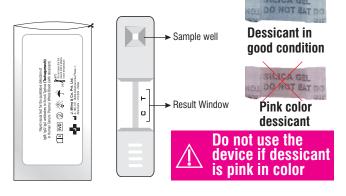
2) Bring the required number of test devices, Assay Buffer and specimen to room temperature prior to testing.



3) Check the expiry date at the back of test device foil pouch and Assay Buffer label. **Use fresh kit if expiry date has passed**.



4) Cut open the device foil pouch and check the color of the dessicant pouch, it should be blue in color. **Do not use the device if dessicant is pink in color.**



5) Label the test device with patient's name or identification number.

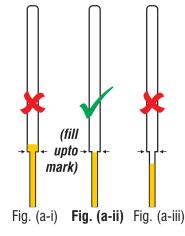


TEST PROCEDURE

1) Specimen collection

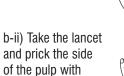
a) Venous Blood:

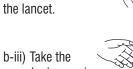
Fill the lower circular part of the sample dropper with the specimen upto the mark provided on the dropper as shown in fig. (a-ii).

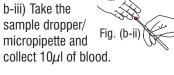


b) Finger Prick Sample Collection: b-i) Wipe the complete

fingertip with the alcohol swab.





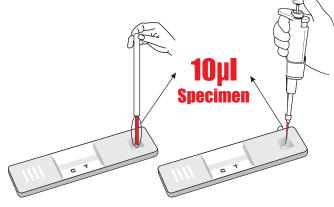


2) Specimen Addition

Add the specimen to the sample well of device.



Add $10 \mu l$ of specimen



Note: Ensure tip of the sample dropper/ pipette touches the membrane of the device and sample starts to flow.

3) Assay Buffer Addition

Add 1 drop (35 μ I)of Assay Buffer to the sample well of the device.



4) Reading Time

Read result at 20 Minutes



Do not read results
after 30 minutes

RESULT INTERPRETATION

REACTIVE for scrub typhus antibodies



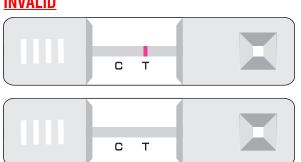
Appearance of two purplish pink colored lines, one each at Control region 'C' & Test region 'T' indicates the presence of Scrub Typhus antibodies in the specimen.

NON-REACTIVE for scrub typhus antibodies



Appearance of only one purplish pink colored line in the control region 'C' and no line on test region indicates that the specimen is non-reactive for Scrub Typhus antibodies.

INVALID



If neither control line nor test line appears or only test line appears, the test should be treated as Invalid.