# Scrub Typhus IgG/IgM Rapid Test
## (Serum/Plasma/Whole Blood)

### INTENDED USE
The Scrub Typhus IgG/IgM Rapid Test Cassette is a lateral flow immunoassay for the qualitative detection of IgG and IgM antibodies to Rickettsia in human whole blood, serum or plasma. It provides an aid in the diagnosis of infection with Scrub Typhus.

### INTRODUCTION
Scrub typhus, also called Tsutsugamushi Disease, acute infectious diseasein humans that is caused by the parasite Rickettsia tsutsugamushi and is transmitted to humans by the bite of certain kinds of trombiculid mites, or chiggers. The causative agent of scrub typhus, the bacterium R. tsutsugamushi, is primarily a parasite of certain mites, of which two closely related species, Leptotrombidium (Trombicula) akamushi and L.deliens, arethe carriers of the disease. After being bitten by infected louse fleas or tsutsugamushi with rickettsia, the typhus rickettsiae first propagates locally, then enters the blood stream, produces rickettsiaemia, and then reaches all organs and tissues of the body, resulting in the clinical manifestations of toxaemia. The toxin released after the death of rickettsia typhus was the main cause of the disease.

### PRINCIPLE OF TEST
The Scrub Typhus IgG/IgM Rapid Test Cassette is a qualitative membrane strip based immunoassay for the detection of IgG and IgM antibodies to Rickettsia in human whole blood, serum or plasma. The test cassette consists of: 1) a burgundy colored conjugate pad containing Rickettsia recombinant envelope antigens conjugated with colloid gold (Rickettsia conjugates); 2) a nitrocellulose membrane strip containing two test lines (IgG and IgM lines) and a control line (C line). The IgM line is pre-coated with the Mouse anti-Human IgM antibody, IgG line is pre-coated with Mouse anti-Human IgG antibody. When an adequate volume of test specimen is dispensed into the sample well of the test cassette, the specimen migrates by capillary action across the cassette. IgM antibodies to Rickettsia, if present in the specimen, will bind to the Rickettsia conjugates. The immune complex is then captured by the reagent pre-coated on the IgM line, forming a burgundy colored IgM line, indicating a Rickettsia IgM positive test result. IgG antibodies to Rickettsia if present in the specimen will bind to the Rickettsia conjugates. The immune complex is then captured by the reagent pre-coated on the IgG line, forming a burgundy colored IgG line, indicating a Rickettsia IgG positive test result. Absence of both T lines (IgG and IgM) suggests a negative result. To serve as a procedural control, a colored line will always appear at the control line region indicating that proper volume of specimen has been added and membrane wicking has occurred.

### REAGENTS AND MATEREIALS PROVIDED
Scrub Typhus IgG/IgM Test cards contain the following:
- Test Device with activated silica gel
- Plastic Dropper.
- Assay Buffer Bottle
- Package Insert (Instruction for use)

### MATERIALS MAY BE REQUIRED AND NOT PROVIDED
- Positive Control
- Negative Control

### MATERIALS REQUIRED BUT NOT PROVIDE
- Timer
- Digital Clock
- Specimen collection container Tube
- Pipette

### WARNINGS AND PRECAUTIONS:
***For in Vitro Diagnostic Use***
- This package insert must be read completely before performing the test.
- The test device should remain in the sealed pouch until use
- Do not use expired devices.
- Do not use the kit if the cassette package is damaged or the seal is broken.
- Bring all reagent to room tempreature (15-30°C) before use.
- Do not use hemolyzed blood specimens for testing.
- Wear personal protective equipment,such as gloves and lab coats when handling kit reagents.wash hands thoroughly after performing the test.
- Do not smoke,drink or eat in areas where specimens or kit reagents are being handled.
- Dispose of all specimens and material used to performe the test as bio-hazardous waste.

### REAGENT PREPARATION AND STORAGE
**INSTRUCTIONS:** All reagents are ready to use as supplied. Store unused test device unopened at 2-30 °C, ensure that the test device brought to room temperature before opening. The test device is stable through the expiration date printed on the sealed pouch. Do not freeze the kit or expose the kit to temperature 30°C.

**SPECIMEN COLLECTION, STORAGE AND HANDLING**
Consider any materials of human origin as infectious and handle them using standard bio-safety procedures.

**1. Plasma**
- **Step 1:** Collect blood specimen into a lavender, blue or green top collection tube (containing EDTA, citrate or heparin, respectively, in Vacutainer®) by venipuncture.
- **Step 2:** Separate the plasma by centrifugation.
- **Step 3:** Carefully withdraw the plasma into a new pre-labeled tube.

**2. Serum**
- **Step 1:** Collect blood specimen into a red top collection tube (containing no anti coagulants in Vacutainer®) by venipuncture.
- **Step 2:** Allow the blood to clot.
- **Step 3:** Separate the serum by centrifugation.
- **Step 4:** Carefully withdraw the serum into a new pre-labeled tube.

Test specimens as soon as possible after collecting. Store Specimens at 2-8°C if not tested immediately, for up to 5 days. The specimens should be frozen at -20°C for longer storage.

**3. Whole Blood**
- Drops of whole blood can be obtained by either finger tip puncture or venipuncture. Collect blood specimen into a lavender, blue or green top collection tube (containing EDTA, citrate or heparin, respectively in Vacutainer®). Do not use hemolyzed blood for testing.
  Whole blood specimens should be stored in refrigeration (2-8°C),if not tested immediately. The specimens must be tested within 24 hours of collection.

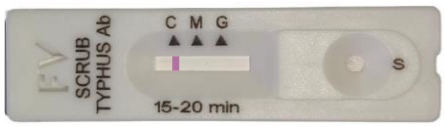
### DIRECTIONS FOR USE
Allow test cassette, specimen, and/or controls to equilibrate to room temperature (15-30°C) prior to testing.
1. Bring the pouch to room temperature before opening it.
2. Remove the test cassette from the sealed pouch and place it on flat dry surface.
3. **For Serum or Plasma Specimen**: Hold the dropper vertically and transfer **1 Drop** of serum or plasma (**approximately 10µl**) to the specimen area, then add **2 Drops** of buffer **(approximately 80µl),** and start the timer, see illustration below.

   **.For Whole Blood Specimen:** Hold the dropper vertically and transfer **2 Drops** of whole blood (**approximately 20µl**) to the specimen area, then add **2 Drops** of buffer (**approximately 80µl**), and start the timer. See illustration below.

4. Wait for the colored line(s) to appear. The test result should be read at 15-20 minutes.
   **Note.** ***Do not interpret the result after 20 minutes*.**

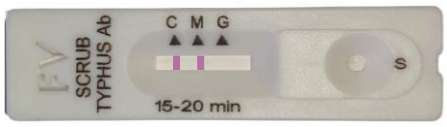
## INTERPRETATION OF RESULTS

**Negative:** If only the C line is developed, the test indicates that the antibodies IgG or IgM to Scrub typhus in the specimen are undetectable. The result is negative.



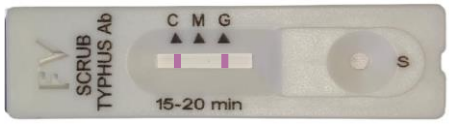

**Negative**

**IgM Positive:** In addition to the control band, a pink-purple band also appears under the test region marked 'M'. This is positive for IgM antibodies.



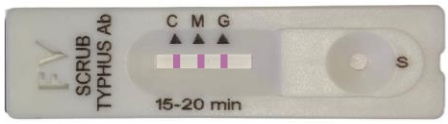

**IgM Positive**

**IgG Positive:** In addition to the control band, a pink-purple band also appears under the test region marked 'G'. This is positive for IgG antibodies.



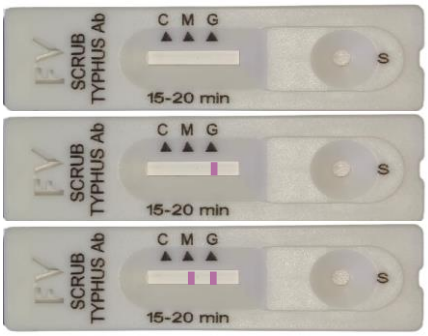

**IgG Positive**

**IgG and IgM Positive**: In addition to the control band, two pink-purple bands also appear under the test region marked 'G' and 'M'. This is positive for both IgM and IgG antibodies.




**IgG & IgM Positive**

**Invalid:** The test should be considered invalid if no bands appear on the device. The test should also be considered Invalid if only test bands appear and no control bands appear. Repeat the test with a new device ensuring that the test procedure has been followed accurately.




**Invalid**

## QUALITY CONTROL

A procedural control is included in the test. A colored line appearing in the control region (C) is considered an internal procedural control. It confirms sufficient specimen volume, adequate membrane wicking and correct procedural technique.

Control standards are not supplied with this kit. However, it is recommended that positive and negative controls be tested as good laboratory practice to confirm the test procedure and to verify proper test performance.

## PERFORMANCE CHARACTERISTIC

### Accuracy

A side-by-side comparison was conducted using the Scrub Typhus IgG/IgM Rapid Test and commercially ELISA. 233 clinical specimens from three Professional Point of Care sites were evaluated. The following results are tabulated from these clinical studies:

**Rickettsia-IgG:**

| Rickettsia-IgG | Commercially ELISA | | Total |
|---|---|---|---|
| | Positive | Negative | |
| Positive | 71 | 6 | 77 |
| Negative | 3 | 153 | 156 |
| Total | 74 | 159 | 233 |

A statistical comparison was made between the results yielding.
**Sensitivity:** 95.95%
**Specificity:** 96.23%
**Accuracy :** 96.14%

**Rickettsia-IgM:**

| Rickettsia-IgM | Commercially ELISA | | Total |
|---|---|---|---|
| | Positive | Negative | |
| Positive | 80 | 7 | 87 |
| Negative | 4 | 142 | 146 |
| Total | 84 | 149 | 233 |

**Sensitivity:** 95.95%
**Specificity:** 96.23%
**Accuracy:** 96.14%

### Cross-Reactivity and Interference

Other common causative agents of infectious diseases were evaluated for cross-reactivity with the test. Some positive specimens of other common infectious diseases were spiked into the Rickettsia positive and negative specimens and tested separately. No cross-reactivity was observed with specimens from patients infected with HIV, HAV, HBsAg, HCV, HTLV, CMV and TP.

## LIMITED EXPRESS WARRANTY DISCLAIMER

Bioline Diagnostics LLP. products are warranted to meet the applicable product specifications described. Notice of non-conforming products should be made to Bioline Diagnostics LLP. for which liability is limited to either replacement of the product and in no case liable to for claim of any kind for an amount greater than the purchase price of the goods in which damages are likely to be claimed. Bioline Diagnostics LLP. disclaims any and all responsibility for any injury or damage or legal implications which may be caused by the fault of the user or buyer in accordance with the limitations and specifications herein. Due to continuous development, the manufacturer reserves the right to improve /change any specification /components without prior information /notice to the buyer.

## REFERENCES

1. Walker DH (1996). Baron S; et al., eds.Rickettsiae. In: Barron's Medical Microbiology(4th ed.). Univ of Texas Medical Branch. ISBN 0- 9631172-1-1.
2. Un sworth NB, Stenos J, Graves SR, et al. (April 2007). "Flinders Island spotted fever rickettsioses caused by "marmionii" strain of Rickettsia honei, Eastern Australia". Emerging Infectious Diseases. 13 (4): 566-73