TRUSTline Scrub typhus IgG/IgM Rapid Test – Cassette

for the detection and differentiation of IgG and IgM antibodies for scrub typhus in human serum/ plasma/ Whole blood specimen.

INTENDED USE

The TRUSTline Scrub typhus IgG/IgM Rapid Test is a lateral flow chromatographic immunoassay for the simultaneous detection and differentiation of IgG and IgM antibodies for *Orientia tsutsugamushi* (Scrub typhus) in Human Serum/ Plasma/ Whole blood specimen. This device is intended to be used as a screening test and as an aid in the diagnosis of infection with Scrub typhus. Any reactive specimen with the TRUSTline Scrub typhus IgG/IgM Rapid Test must be confirmed with alternative testing method(s) and clinical findings.

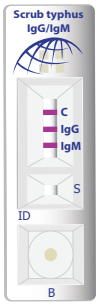
SUMMARY AND EXPLANATION OF THE TEST

Scrub typhus (ST) is an infectious disease that is caused by Orientia tsutsugamushi (formerly Rickettsia), a tiny parasite about the size of bacteria that belongs to the family Rickettsiaceae. A bite from the larval trombiculid mite, a parasite of rodents, will transmit the disease[1]. An ulcer of the skin is characteristic of a bite from a trombiculid mite, followed by symptoms including fever, a spotted rash on the torso, and swelling of the lymph glands. After the onset of symptoms, the IgM antibody titers increased gradually over 2–3 weeks, peaked at about 4 weeks, and started to decrease rapidly between 4 and 5 weeks. Over the first 2 weeks, IgG antibody titers increases sharply, peaked at about 4 weeks and decreased rather gradually thereafter[3].

Scrub typhus generally occurs after exposure to areas with secondary (Scrub) vegetation, which is where its name is derived from. However, the disease can also be prevalent in sandy, mountainous, and tropical areas. Scrub Typhus is a worldwide illness, but particular to South East Asia and the Western Pacific. It accounts for approximately 20% of fever in some regions in South East Asia, where it is endemic[2]. Illness lasts for a period of 10 to 12 days after the initial bite. With therapy, the fever will break within 36 hours, but if left untreated, complications or death may occur

The TRUSTline Scrub typhus IgG/IgM Rapid Test is developed for solving these obstacles. It utilizes specific antibodies (IgG or IgM) to simultaneously detect and differentiate infection with *Orientia tsutsugamushi* (Scrub typhus). The test can be performed by untrained or minimally skilled personnel, without laboratory equipment.

TEST PRINCIPLE



The TRUSTline Scrub typhus IgG/IgM Rapid Test is a lateral flow chromatographic immunoassay. The strip test components consist of: 1) a burgundy colored conjugate pad containing recombinant Orientia tsutsugamushi antigens conjugated with colloidal gold (antigen conjugates) and a control antibody conjugated with colloidal gold, and 2) a nitrocellulose membrane strip containing a IgG line (IgG line), IgM line (IgM Line) and a control line (C line). The IgG line is pre-coated with Anti-human IgG antibodies, The IgM line is Pre-coated with Anti-human IgM antibodies and the C line is pre-coated with a control line antibody.

During the assay, when an adequate volume of test specimen is dispensed into the specimen well (S) of the test cassette and a sample diluent is added to the buffer well (B), the specimen migrates by capillary action across the strip held in the cassette. Specific antibody IgG of Orientia tsutsugamushi, if present in the specimen will bind to the recombinant Orientia tsutsugamushi antigen colloidal gold conjugates. The immunocomplex is then captured on the membrane by the pre-coated Anti-human IgG antibodies, forming a burgundy colored IgG band, indicating a Scrub typhus IgG antibody positive test result.

Alternatively, IgM if present in the specimen will bind to the recombinant Orientia tsutsugamushi antigen colloidal gold conjugates. The immunocomplex is then captured on the membrane by the pre-coated Anti-human IgM antibodies, forming a burgundy colored IgM band, indicating a Scrub typhus IgM positive test result.

The test contains an internal control (C line), which should exhibit a burgundy colored line of the immunocomplex of the control antibodies regardless of color development on the test lines. If the C line does not develop, the test result is invalid and the specimen must be retested with another device.

REAGENTS AND MATERIALS PROVIDED

1. Individually sealed foil pouches containing:
   a. One cassette device
   b. One desiccant
2. Specimen transfer device
3. Sample diluent (5 mL/bottle)
4. Package insert (instruction for use)

MATERIALS REQUIRED BUT NOT PROVIDED

1. Clock or Timer
2. Lancing device for whole blood test
3. Alcohol swab
4. Disposable gloves

WARNINGS AND PRECAUTIONS

For in Vitro Diagnostic Use

1. This package insert must be read completely before performing the test. Failure to follow the insert may lead to inaccurate test results.
2. Do not open the sealed pouch unless ready to conduct the assay.
3. Do not use the test device if pouch is not intact.
4. Do not use expired devices or components.
5. Bring all reagents to room temperature (15-30°C) before use.
6. Do not use the components of different lots and of any other type of test kit as a substitute for the components in this kit.
7. Do not use hemolyzed blood for testing.
8. Wear protective clothing and disposable gloves while handling the kit reagents and clinical specimens. Wash hands thoroughly after performing the test.
9. Users of this test should follow the US CDC Universal Precautions for prevention of transmission of HIV, HBV and other blood-borne pathogens.
10. Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.
11. Dispose of all specimens and materials used to perform the test as bio-hazardous waste.
12. Handle the negative and positive controls in the same manner as the patient specimens.
13. The test result should be read 20 minutes after a specimen is applied to the sample well of the device. Reading the test result after 30 minutes may give erroneous results.
14. Do not perform the test in a room with strong air flow, i.e. an electric fan or strong air conditioning.
15. Clean up spills thoroughly using appropriate disinfectant.

REAGENT PREPARATION AND STORAGE INSTRUCTIONS

All reagents are ready to use as supplied. Store test kit at 1-30°C. If stored at 2-8°C, ensure that all reagents are brought to room temperature before opening. The sample diluent (opened and unopened) and unopened test device is stable through the expiration date printed on the label, when stored at recommended temperature. Do not freeze the kit or expose the kit to temperatures above 30°C. The test device is sensitive to humidity and heat. Perform the test immediately after removing the test device from the foil pouch.

SPECIMEN COLLECTION AND HANDLING

Consider any materials of human origin as infectious and handle them using standard bio-safety procedures.

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.

Serum

Step 1: Collect blood specimen into a red top collection tube (containing no anticoagulants 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. The specimens can be stored at 2-8°C for up to 5 days. The specimens should be frozen at -20°C for longer storage.

Avoid multiple freeze-thaw cycles. Prior to testing, bring frozen specimens to room temperature slowly and mix gently. Specimens containing visible particulate matter should be clarified by centrifugation before testing.

Do not use samples demonstrating gross lipemia, gross hemolysis or turbidity in order to avoid interference with result interpretation.

Blood

Drops of whole blood can be obtained by either fingertip 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. Capillary blood (fingertip puncture) can be used directly without anticoagulant. Collect blood with sample pipette and transfer it to sample well of device.

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.

ASSAY PROCEDURE

Step 1: Bring the specimen and test components to room temperature if refrigerated or frozen. Once thawed, mix the specimen well prior to performing the assay.

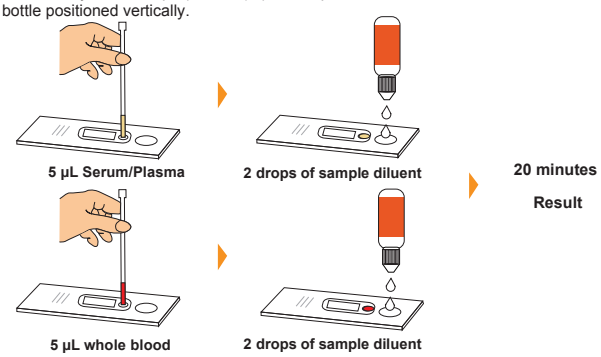
Step 2: When ready to test, open the pouch at the notch and remove device. Place the test device on a clean, flat surface.

Step 3: Be sure to label the device with specimen's ID number.

Step 4: Fill the specimen transfer device with specimen (about 5 µL) not to exceed the specimen line as shown in the images below. **For better precision, transfer specimen using a pipette capable of delivering a 5 µL volume.**

Holding the specimen transfer device vertically, dispense the entire specimen into the center of the specimen well making sure that there are no air bubbles.

Immediately add 2 drops (50-100 µL) of sample diluent to the Buffer well with the bottle positioned vertically.



Step 5: Set up timer.

Step 6: Result should be read in 20 minutes.

Do not read result after 30 minutes. To avoid confusion, discard the test device after interpreting the result.

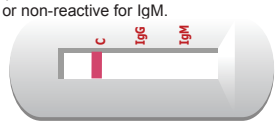
QUALITY CONTROL

1. **Internal Control:** This test contains a built-in control feature, the C line. The C line develops after adding specimen and sample diluent. If the C line does not develop, review the entire procedure and repeat the test with a new device.

2. **External Control:** Good Laboratory Practice recommends using external controls, positive and negative, to assure the proper performance of the assay, particularly under the following circumstances:
   a. A new operator uses the kit, prior to performing testing of specimens.
   b. A new lot of test kits is used.
   c. A new shipment of test kits is used.
   d. The temperature used during storage of the kit falls outside of 1-30°C.
   e. The temperature of the test area falls outside of 15-30°C.
   f. To verify a higher than expected frequency of positive or negative results.
   g. To investigate the cause of repeated invalid results.

INTERPRETATION OF ASSAY RESULT

1. **NEGATIVE RESULT**:
If only the C line is developed, the test indicates that the antibodies IgG or IgM to Scrub typhus in the specimen is undetectable. The result is negative or non-reactive for IgG and negative or non-reactive for IgM.



2. **POSITIVE RESULT**:
2.1 If both the C line and IgM line are developed, the test indicates that the specimen contains antibodies to IgM of Scrub typhus. The result is IgM positive or reactive.

2.2 If both the C line and IgG line are developed, the test indicates that the specimen contains IgG antibodies to Scrub typhus. The result is IgG positive or reactive.

2.3 If the C line and both test lines (IgG and IgM) are developed, the test indicates that the specimen contains IgG and IgM antibodies to Scrub typhus. The result is IgG positive or reactive and IgM positive or reactive.

Samples with reactive results should be confirmed with alternative testing method(s) such as ELISA and clinical findings before a final diagnostic decision is made.

3. **INVALID:**
If no C line is developed, the assay is invalid regardless of color development in the test lines as indicated below. Repeat the assay with a new device.

PERFORMANCE CHARACTERISTICS

1. **Clinical Performance for IgG Antibody Test**
A total of 808 samples from susceptible subjects were tested with the TRUSTline Scrub typhus IgG/IgM Rapid Test in Comparison with ELISA test. Comparisons for all subjects is shown in the following table.

	TRUSTline Scrub typhus IgG/IgM Rapid Test		
EIA	**IgG Positive**	**Negative**	**Total**
Positive	21	1	22
Negative	25	761	786
Total	46	762	808

Relative Sensitivity: 95.45%, Relative Specificity: 96.81%,

Overall Agreement:96.78%

2. **Clinical Performance for IgM Antibody Test**
A total of 808 samples from susceptible subjects were tested with the TRUSTline Scrub typhus IgG/IgM Rapid Test in Comparison with ELISA test. Comparisons for all subjects is shown in the following table.

	TRUSTline Scrub typhus IgG/IgM Rapid Test		
EIA	**IgM Positive**	**Negative**	**Total**
Positive	25	1	26
Negative	18	764	782
Total	43	765	808

Relative Sensitivity: 96.15%, Relative Specificity: 97.69%,

Overall Agreement: 97.64%

3. **Cross-Reactivity**
Cross-reactivity with specimens from other infectious diseases:

Specimen	Sample Size	IgG Reactivity	IgM Reactivity
Dengue Positive Serum	10	Negative	Negative
HAV Positive Serum	10	Negative	Negative
HCV Positive Serum	10	Negative	Negative
HIV Positive serum	10	Negative	Negative
HBsAg Positive Serum	10	Negative	Negative
Malaria Positive Whole Blood	10	Negative	Negative
Typhoid Positive Serum	5	Negative	Negative
ANA Positive Serum	5	Negative	Negative
RF positive Serum (≤2,500 IU/ml)	5	Negative	Negative

4. **Interference**
Common substances (such as pain and fever medication and blood components) may affect the performance of the TRUSTline Scrub typhus IgG/IgM Rapid Test. This was studied by spiking these substances into three levels of IgG and IgM Ab standard controls. The results are presented in the following table and demonstrate that at the concentrations tested, the substances studied do not affect the performance of the TRUSTline Scrub typhus Rapid Test.

Note: -: Negative; +: Weak Positive; +++: Strong Positive

Potential Interfering Substances Spiked	IgG Reactivity			IgM Reactivity		
	Negative	Weak Positive	Strong Positive	Negative	Weak Positive	Strong Positive
Control	-	+	+++	-	+	+++
Bilirubin 15 mg/dL	-	+	+++	-	+	+++
Creatinine 5 mg/dL	-	+	+++	-	+	+++
Glucose 120 mg/dL	-	+	+++	-	+	+++
Albumin 5 g/L	-	+	+++	-	+	+++
Salicylic Acid 4.34 mmol/L	-	+	+++	-	+	+++
Urea 40 mg/dL	-	+	+++	-	+	+++
EDTA 3.4 µmol/L	-	+	+++	-	+	+++

LIMITATIONS OF TEST

1. The Assay Procedure and Interpretation of Assay Result sections must be followed closely when testing for the presence of IgG and IgM antibodies to Scrub typhus in serum, plasma or whole blood from individual subjects. Failure to follow the procedure may lead to inaccurate test results.
2. The TRUSTline Scrub typhus IgG/IgM Rapid Test is limited to the qualitative detection of IgG and IgM antibodies to *Orientia tsutsugamushi* (Scrub typhus) in human serum, plasma or whole blood. The intensity of the test line does not correlate with the antibody titer of the specimen.
3. If the test result is negative and clinical symptoms persists, additional testing using other clinical methods is recommended. A negative result does not at any time conclude the possibility of Scrub typhus infection.
4. Hemolytic samples may give reddish background even after end of the test time.
5. Unusually high titers of heterophile antibodies or rheumatoid factor in specimens may affect expected results.
6. The results obtained with this test should only be interpreted in conjunction with other diagnostic procedures and clinical findings.

REFERENCES

1. George M. Varghese, Veera Manikandan Rajagopal, Paul Trowbridge, Divya Purushothaman, and Martin. Kinetics of IgM and IgG antibodies after infection and the implications. Int J Infect Dis. 2018 Jun; 71: 53–55.
2. Coleman RE, Sangkasuwan V, Suwanabun N, Eamsila C, Mungviriya S, Devine P, et al. Comparative evaluation of selected diagnostic assays for the detection of IgG and IgM antibody to Orientia tsutsugamushi in Thailand.Am J Trop Med Hyg 2002;67:497-503.
3. Kim DM, Lee YM, Back JH, Yang TY, Lee JH, Song HJ, Shim SK, Hwang KJ, Park MY.A serosurvey of Orientia tsutsugamushi from patients with Scrub typhus. Clin Microbiol Infect. 2010 May;16(5):447-451.

Index of Symbols

Consult instructions for use	REF	Catalogue number		Use-by date
IVD For *in vitro* diagnostic use only	LOT	Batch code		Tests per kit
1°C–30°C Temperature limit 1-30 °C		Do not re-use		Keep dry
Manufacturer		Date of manufacture	CE	European Conformity
NON STERILE If device is non-sterile		Warnings / Precautions	EC REP	Authorized Representative

Do not use if package is damaged

Keep away from sunlight

Athenese-Dx Pvt. Ltd.
Module No. 407 & 408, 4th Floor,
TICEL Bio Park II, No. 5, CSIR Road.
Taramani, Chennai-600113, India
Tel: +91-44-22541131
E-mail: info@athenesedx.com
Website: www.athenesedx.com

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English version

EC REP Obelis s.a.
Bd General Wahis 53
1030 Brussels
Belgium
Tel: +(32)2 732-59-54
E-mail:mail@obelis.net