Scrubcheck™

Rapid test for detection of IgM & IgG antibodies to *O. tsutsugamushi* in human serum/plasma

DEVICE

INTENDED USE

Scrubcheck™ is a rapid, qualitative immunochromatographic test for the detection of IgM and IgG antibodies to *Orientia tsutsugamushi (Scrub typhus)* in human serum or plasma. It can be used as a screening test and as an aid for early diagnosis of infection of Scrub typhus and differential diagnosis of past infection.

SUMMARY

Scrub typhus is caused by *Orientia tsutsugamushi* (also known as Rickettsia). Scrub typhus is a mite-borne infectious disease caused primarily by the obligate intracellular bacteria *Orientia tsutsugamushi*, which is transmitted by chigger mites. During their larval stage, these mites acquire the infection from wild rodents or other small animals. The infection is passed to humans when a mite larva bites a person. Scrub typhus is transmitted by some species of trombiculid mites ("chiggers", particularly *Leptotrombidium deliense*), which are found in areas of heavy scrub vegetation. The mites feed on infected rodent hosts and subsequently transmit the parasite to other rodents and humans. Approximately one million cases are known to occur annually in Asia Pacific countries including India, Arabian Peninsula, Chile and possibly Kenya also. Diagnosis is often difficult since most of the clinical manifestations, such as fever, headache, nausea, myalgia, abdominal pain, lymphadenopathy and maculopapular rash. Complications of scrub typhus such as jaundice, renal failure, pneumonitis, ARDS, septic shock, myocarditis, and meningoencephalitis usually develop after the first week of illness. Serological detection of O. tsutsugamushi specific IgM & IgG antibodies is the primary method for diagnosis of Scrub typhus infection. After the onset of symptoms, IgM antibody titres increases gradually over 2-3 weeks, peaks at about 4 weeks, and starts to decrease rapidly between 4 and 5 weeks. Over the first 2 weeks, IgG antibody titres increases sharply, peaks at about 4 weeks and decreases rather gradually thereafter.

PRINCIPLE

Scrubcheck™ is based on the principle of agglutination of antibodies / antisera with respective antigen in immuno-chromatography format along with use of nano gold particles as agglutination revealing agent. The specific Agglutinating sera for human IgM and specific Agglutinating sera for human IgG are immobilized on the nitrocellulose membrane as two individual test bands (IgM and IgG) at region 'M' and 'G' respectively.

As the test sample flows through the membrane assembly within the test device, the recombinant *O. tsutsugamushi* antigen colloidal gold conjugate complexes with specific antibodies (IgM and/ or IgG) to *O. tsutsugamushi*, if present in the sample. This complex moves further on the membrane to the test region where it is immobilized by the specific Agglutinating sera for human IgM and/or Agglutinating sera for human IgG coated on the membrane leading to formation of a colored band/s which confirms a positive test result. Absence of these colored bands in the test region indicates a negative test result for IgM and IgG antibodies to *O. tsutsugamushi*.

A built-in control band in the control area marked 'C' appears when the test has been performed correctly, regardless of the presence or absence of the *O. tsutsugamushi* antibodies in the specimen. It serves to validate the test performance of each device.

REAGENTS AND MATERIALS SUPPLIED

Scrubcheck™ kit contains:

A. Individual pouches, each containing:
   1. DEVICE : Membrane assembly pre-dispensed with O. tsutsugamushi specific recombinant antigen colloidal gold conjugate, streptavidin gold conjugate, Agglutinating sera for Human IgM at test region 'M', Agglutinating sera for Human IgG at test region 'G' and Biotinylated BSA at control region 'C'.
   2. Desiccant pouch.

B. BUF : Sample running buffer in a dropper bottle.

C. Package insert

REF	501120010	501120025
	10 T	25 T

OPTIONAL MATERIAL REQUIRED BUT NOT PROVIDED

Calibrated micropipette capable of delivering 5µl specimen accurately. Stop watch.

STORAGE AND STABILITY

The sealed pouches in the test kit and kit components may be stored at 4°C to 30°C for the duration of the shelf life as indicated on the pouch/carton. After first opening of the sample running buffer bottle, the buffer is stable until the expiry date mentioned on the buffer label, if kept at 4°C to 30°C for the duration of its shelf life. DO NOT FREEZE.

NOTES

1. For in vitro diagnostic use and professional use only. NOT FOR MEDICINAL USE.
2. Do not use the kit beyond expiry date and do not re-use the test device.
3. Read the instructions carefully before performing the test.
4. Do not intermix the reagent or devices from different lots.
5. Any modification to the test procedure and / or use of other reagents will invalidate the test procedure.
6. Contact with the contents of desiccant pouch containing, among other substances, cobalt chloride (CAS# 7646-79-9) should be kept to a minimum. Inhalation / swallowing may cause harm.
7. Handle all specimens as if potentially infectious. Follow standard biosafety guidelines for handling and disposal of potentially infectious material.
8. If desiccant colour at the point of opening the pouch has turned from blue to pink or colourless, another test device must be run.
9. Sample running buffer contains Sodium Azide (0.1%), avoid skin contact with this reagent. Azide may react with lead and copper in the plumbing and form highly explosive metal oxides. Flush with large volumes of water to prevent azide build up in the plumbing.

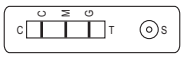
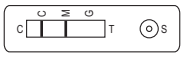
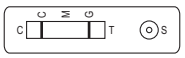
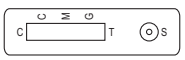
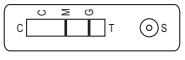
SPECIMEN COLLECTION AND PREPARATION

1. No special preparation of the patient is necessary prior to specimen collection by approved techniques.
2. Though fresh serum/plasma is preferable, serum/plasma specimen may be stored at 2°C to 8°C for upto 72 hours, in case of delay in testing. Refrigerated specimens must be brought to room temperature prior to testing.
3. Do not use haemolysed or contaminated specimens.
4. Turbid specimens should be centrifuged or allowed to settle and only the clear supernatant should be used for testing.
5. Repeated freezing, thawing of the specimen should be avoided.

TESTING PROCEDURE

1. Bring the **Scrubcheck**™ test kit components to room temperature prior to testing.
2. Open the foil pouch by tearing along the "notch" and remove the test device.
3. Check the colour of the desiccant pouch. It should be blue. If the desiccant has turned colorless or pink, discard the test device and use another device. **Once opened, the device must be used immediately.**
4. Label the device with specimen identity.
5. Place the device on a flat horizontal surface.
6. Tighten the cap of the buffer bottle in the clockwise direction to pierce the dropper bottle nozzle.
7. Using precision micropipette carefully add **5µl** serum or plasma specimen into the specimen port (S).
8. Add **two drops** of sample running buffer into the same specimen port (S) by holding the buffer bottle vertically and immediately start the stopwatch.
9. Read the final result at the end of **15 minutes**.

INTERPRETATION OF RESULTS

	**Negative Result** The presence of only single pink-purple coloured band in the control area marked 'C', indicates the absence of specific antibodies against *O. tsutsugamushi* or that the amount of antibodies is below the detection limit of the test.
  	**Positive Result** In addition to the control band in the control area marked 'C', appearance of two pink- purple colored bands in the test region 'M' and region 'G', indicates the presence of *O. tsutsugamushi* specific IgM and IgG antibodies. In addition to the control band in the control area marked 'C', appearance of a pink-purple coloured band in the test region 'M', indicates the presence of *O. tsutsugamushi* specific IgM antibodies. In addition to the control band in the control area marked 'C', the appearance of a pink-purple colored band in the test region 'G', indicates the presence of *O. tsutsugamushi* specific IgG antibodies.
 	**Invalid Result** The test result is invalid if no bands appear on the device. The test should also be considered invalid if only the test band appears and no control band appears. In such cases, verify the test procedure and repeat the test with a new device.

PERFORMANCE CHARACTERISTICS

Internal Evaluation

In an in-house evaluation study, 100% co-relation in results were obtained when **Scrubcheck™ (Device)** was evaluated in comparison with Commercial Rapid test kit for detection of IgM & IgG antibodies to *Orientia tsutsugamushi* (Scrub typhus). The results are summarised as follows:

Specimens	Nos. Tested	Scrubcheck™ (Device)	Commercial Rapid test kit	Scrubcheck™ (Device)	
				Specificity (95% Confidence Interval)	Sensitivity (95% Confidence Interval)
Scrub typhus Negative	185	185	185	100% (98.03% to 100.00%)	-
Scrub typhus Positive	15	15	15	-	100% (78.20% to 100.00%)

External Evaluation

In a NABL accredited reputed reference laboratory in India, **Scrubcheck™ (Device)** was tested with 22 nos. Clinically suspected positive specimens and 68 nos. Healthy individuals in comparison with another licensed commercial rapid kit for Scrub typhus antibodies.
Co-relating results were obtained as below:

Specimens	Nos. Tested	Scrubcheck™ (Device)	Commercial Rapid test kit
Scrub typhus Negative	68	68	68
Scrub typhus IgM Positive	12	12	12
Scrub typhus IgG Positive	8	8	8
Scrub typhus IgM & IgG Positive	2	2	2

Based on this evaluation:
Specificity of **Scrubcheck™ (Device)** : 100%
Sensitivity of **Scrubcheck™ (Device)** : 100%

LIMITATIONS OF THE TEST

1. **Scrubcheck™** detects the presence or absence of IgM and /or IgG antibodies to *O. tsutsugamushi* in the human serum/plasma specimen. It should be used as sole criteria for the diagnosis of Scrub typhus infection.
2. As with all diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single test but should rather be made by a clinician after all clinical findings have been evaluated.
3. A negative test result indicates that IgM and IgG antibodies to *O. tsutsugamushi* are either not present or at levels undetectable by the test.
4. **Scrubcheck™** has not been validated on specimens from neonates.
5. Some specimens containing unusually high titers of heterophile antibodies or rheumatoid factor may affect expected results.
6. Do not interpret the test results beyond 30 minutes.

WARRANTY

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

BIBLIOGRAPHY

1. Scrub typhus seroprevalence from an eastern state of India: Findings from the state-wide serosurvey. Debapasad Parai, Matrujyoti Pattnaik et al. Oxford University Press on behalf of Royal Society of Tropical Medicine and Hygiene.
2. A serosurvey of Orientia tsutsugamushi from patients with scrub typhus. D M Kim, Y-M Lee et al. "Clin Microbiol Infect"[jour].
3. Data on file. Zephyr Biomedicals. Tulip Diagnostics (P) Ltd.

SYMBOL KEYS

	Temperature Limitation		Manufacturer	DEVICE	Device	Xn NaN, R22, S23-46-61	Harmful if swallowed. Do not breathe vapour. If swallowed, seek medical advice immediately and show this container or label. Avoid release to the environment. Refer to special instructions.
	Use by		Consult Instructions for use	BUF	Sample Running Buffer		
	Date of Manufacture	REF	Catalogue Number		This side up		
LOT	Batch Number / Lot Number	IVD	*In vitro* Diagnostic Medical Device		Do not use if package is damaged		
Σ	Contains sufficient for <n> tests		Do not reuse				

Manufactured by:

Zephyr Biomedicals

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