

Rapid immunochromatographic test: An evolving tool for diagnosis of scrub typhus



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ABSTRACT

Background: Scrub typhus is prevalent in many districts of South Bengal throughout the year where an average temperature of 20–35°C, which contributes to the spread of *Leptotrombidium deliense*. However, its diagnosis remains complicated by the lack of readily available and validated assays, the non-specificity of clinical symptoms on admission, and even non-availability of the pathognomonic eschar in most of the cases.

Aims and Objectives: This study was carried out to evaluate the rapid immunochromatographic test (RICT) for early detection of scrub typhus for using it as an early diagnostic tool at the field level. **Materials and Methods:** This cross-sectional study in which 181 serum samples from clinically suspected cases (after excluding dengue, malaria, Japanese encephalitis, and typhoid fever) collected over 13 months were processed for the detection of immunoglobulin M (IgM) antibodies for scrub typhus by enzyme-linked immunosorbent assay (ELISA) and rapid test. **Results:** Considering IgM ELISA for scrub typhus as the gold standard, the sensitivity, specificity, positive predictive value, and negative predictive value for RICT were found to be 100%, 86.87%, 50%, and 100%, respectively. **Conclusion:** RICT is a simple, rapid, and reliable assay for diagnosis of scrub typhus, capable of providing accurate results quickly and is highly suitable for field deployment in remote areas with limited medical support.

Key words: Rapid immunochromatographic test; Diagnosis; Scrub typhus; Evolving tool

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INTRODUCTION

Scrub typhus is an arthropod-borne zoonosis caused by *Orientia tsutsugamushi* also known as *tsutsugamushi* disease,¹ mite-borne typhus,² chigger-borne rickettsiosis,³ tropical typhus, Japanese “flood fever” (described by Baelz and Kawakami in 1878).⁴ The recent isolation of a novel species *Orientia chuto*, with “chuto” being Japanese for “middle east,” with the prototype strain of this species being strain Dubai, is a proof that the spread of scrub typhus is beyond the *tsutsugamushi* triangle.⁵ *Leptotrombidium deliense* larvae (chiggers) are the main vector of scrub typhus in Bengal⁶ and also in India.⁷ Chiggers are minute and unlike ticks, are not normally noticed.⁸ Larvae (chiggers) require habitats to have small mammals so that they will have hosts on which

to feed. Rodents such as species of *Rattus rattus*,⁷ *Apodemus*, and *Microtus* and insectivores such as shrews (*Suncus* species)⁷ and tree shrews (*Tupaia* species) are important larval hosts.⁹ Study conducted by ICMR-Vector Control Research Centre, Puducherry, India, shows that a high proportion of chiggers present in shrew, *Suncus murinus* (79.1%), and *R. rattus* (47.6%).⁷ It seems that domestic rats play little or no part in the ecology of scrub typhus.⁹ Humans are accidental hosts.¹⁰ Although scrub typhus affects mainly rural populations, presently, it is also occurring increasingly in urban settings, especially in fast-growing metropolitan areas.¹ This resurgence may be attributed to changes in human behavior unplanned urbanization, and deforestation, leading to the displacement of vectors as well as rodents from one place to another. At present,

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scrub typhus occurs throughout the year where an average temperature is between 20°C and 35°C, which is helpful to spread of vectors.¹¹ Now in India, it is present in the whole of the Shivalik ranges from Kashmir to Assam, Eastern and Western Ghats, and the Vindhya and Satpura ranges in the central part of India. There were reports of scrub typhus outbreaks in Himachal Pradesh, Sikkim, and Darjeeling.¹² For the last few years outbreaks of scrub typhus have also been reported from Andhra Pradesh,¹³ Sub-Himalayan belt, from Jammu to Nagaland, Haryana,¹⁴ Rajasthan,^{14,15} Pondichheri,¹⁶ Chennai,¹⁷ etc. Southern districts of West Bengal (Kolkata, south 24 Parganas) also reported cases of scrub typhus.^{10,18,19} The vast variability and non-specific presentation of this infection have often made it difficult to diagnose clinically.²⁰⁻²² The presence of the pathognomonic eschar is an important diagnostic clue for scrub typhus, but this sign is not always clearly visible in the Indian population, eschar and rash are seen only in <10% of cases.¹³ Untreated patients have case fatality rates as high as 30–45% with multiple organ dysfunctions if not promptly diagnosed and appropriately treated. Prompt antibiotic therapy shortens the course of the disease, lowers the risk of complications, and in turn reduces morbidity and mortality due to rickettsial diseases.^{20,23} In spite of the availability of low-cost and effective antibiotic treatment, there is a distinct need for physicians and healthcare workers at all levels to identify the cases and to confirm it by necessary laboratory investigations.²⁰ Indirect immunofluorescence is considered the gold standard, but it is not used in India as it is costly and also not available.²⁴ Laboratory diagnosis of scrub typhus infection is done by detection of antibodies to *O. tsutsugamushi*, by either immuno chromatographic test or immunoglobulin M (IgM) enzyme-linked immunosorbent assay (ELISA) which are presently available in our country. This study was carried out to evaluate the rapid immunochromatographic test (RICT) for the detection of scrub typhus for using it as an early diagnostic tool in the field level.

Aims and objectives

The present study was conducted to evaluate the Rapid Immunochromatographic Test (RICT) for detection of scrub typhus befitting as an early diagnostic tool in field level.

MATERIALS AND METHODS

This institution-based cross-sectional study was conducted for a period from April 2019 to April 2020. Clinically suspected pyrexia of unknown origin cases (negative for malaria [rapid diagnostic test (RDT)/blood smears], Japanese encephalitis and dengue ELISA, enteric fever [blood culture/typhoid/Widal test]) who attended and/or

were admitted, respectively, in the out-patient department and/or inpatient departments of department of general medicine and pediatric medicine of Bankura Sammilani Medical College and Hospital, were included as study subjects. Immune-compromised patients like those suffering from acquired immunodeficiency syndrome/lymphomas/malignancy/bleeding disorders/diabetes, fever of more than 4 weeks duration, and patients on chemotherapy or long-term steroids were excluded using exclusion criteria. IgM antibody was detected using InBios Scrub Typhus Detect™ IgM ELISA Kit. Whereas SD BIOLINE *tsutsugamushi* (18FK10, 18FK11) was used as rapid detection kit. The ethical clearance was obtained from the Institutional Ethics Committee of Bankura Sammilani Medical College. Ethical clearance number was BSMC/Aca/172, dated 09.01.2019. Data collection was started after obtaining permission from concerned authority of Bankura Sammilani Medical College and Hospital. After collection data were entered into MS Excel sheet and it was checked twice to detect any erroneous entry. After organizing and presenting the data in the forms of tables and diagrams they were analyzed by applying the principles of descriptive statistics. Statistical Package for the Social Sciences version 20 was used to analyze the data. An appropriate statistical test was done.

RESULTS

Among the 181 samples tested, 129 were from males and 52 from females. The age and sex distribution of patients and their seropositivity is shown in Table 1. Of the 181 samples, only 21 samples were positive by ELISA, and 42 samples were positive by RICT. Hence, both are positive in 21 samples. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of RICT were 100%, 86.87%, 50% and 100% respectively (Table 2).

100% of the study subjects presented with fever before sample collection. Eschar was found to be present in 2 male patients (Figures 1 and 2).

Table 1: Age and gender-wise distribution of serology-positive cases

Age (in completed years)	Gender		Number of seropositive cases
	Female	Male	
≤10	5	1	6
21–30	5	4	9
31–40	3	4	7
41–50	1	5	6
51–60	1	6	7
≥61	0	7	7
Total	15	27	42

Table 2 : Rapid immunochromatographic test as an early diagnostic test of scrub typhus (n=181)

RICT	Scrub typhus		Total
	Positive	Negative	
Positive	21	21	42
Negative	0	139	139
Total	21	160	181

Sensitivity= $\frac{21}{(21+0)} \times 100$ % = 100%, Specificity= $\frac{139}{(139+21)} \times 100$ % = 86.87%, Positive predictive value= $\frac{21}{(21+21)} \times 100$ % = 50%, Negative predictive value= $\frac{139}{(139+0)} \times 100$ % = 100%, R ICT: Rapid immunochromatographic test

**Figure 1:** Eschar over the abdomen in the left iliac fossa region**Figure 2:** Eschar over the left chest

DISCUSSION

Scrub typhus can mimic other acute febrile illnesses common in the tropics, especially when pathognomonic eschars are absent. Therefore, laboratory tests become mandatory for confirmation of the diagnosis. Weil–Felix test was used in the past, but its results are archaic and misleading²⁵ because the test is neither sensitive nor specific in the serodiagnosis of rickettsial diseases and hence has largely been replaced by other assays.²⁶ Indirect

immunofluorescence is not used in India as it is costly²⁴ along with non-availability of fluorescence microscopes everywhere. The indirect immunoperoxidase (IIP) test (a modification of the IFA) is currently not commercially available.²⁵ The development of ELISAs using either culture-derived *O. tsutsugamushi* antigens or recombinant proteins to detect *Orientia*-specific antibodies has contributed to improved diagnostic accuracy and enabled higher throughput, over the often cumbersome IFAs and IIP test.¹ In India, serological testing using IgM ELISA continues to be the mainstay in the laboratory diagnosis of scrub typhus²⁷ but its availability is limited only in few major private laboratories or in tertiary care hospitals such as medical colleges. Again results of this test may not be available on the same day as samples need to be pooled for testing thus causing delayed diagnosis and treatment, which at times may be fatal.²⁴ Hence, serological tests which are easy to perform at the point of care demand time and soon will be the mainstay of diagnosis. The procedure for RICT takes <15 min to finish and is much simpler to perform than the commercial dip-stick assay reported previously. RICT is suitable for rural health setup²⁸ and doctors' clinics where advanced medical support is limited.²⁹ The higher sensitivity of RICT is suitable for the earlier diagnoses of scrub typhus during the acute phase, which can avoid delayed treatment and death due to multi-organ failure³⁰ and can replace other diagnostic tests and is applicable for generalized diagnosis of scrub typhus by the doctors delivering service at a rural hospital or block primary health center or primary health centers in India. The results of the SD BIOLINE *tsutsugamushi* assay in our study were positive, with all cases found to be positive in IgM ELISA assay. In the present study, 21/181 show false positive result. Moreover, the 100% sensitivity of the RICT result was due to the detection of IgM, IgG, and IgA antibodies in comparison to other RICT tests, which only detects either IgM or IgG antibody against *O. tsutsugamushi*.²³ Whereas, a study conducted by Blacksell *et al*, shows that the scrub typhus RICT, sensitivities ranged from 74% to 96% and specificities ranged from 86% to 99%. Moreover, in that study, scrub typhus RICT shows poor overall sensitivity results.³¹ The reason behind the poor sensitivity of the RICT in the said study may be due to the detection of only IgM antibody against *O. tsutsugamushi*. Whereas in a cross-sectional seroprevalence study of scrub typhus at a tertiary care hospital in Andhra Pradesh where 100 samples were tested and 97% correlation between ELISA and rapid method has been found.²⁴

Two studies conducted in China stated that the specificity of RDT was 100% for both IgM and IgG tests. Considering IgM and IgG together, the sensitivity was 100%.^{32,33} Again, in a study conducted by Silpasakorn *et al*,²³ the sensitivity and specificity of the ICT tests for the detection

of IgM, IgG, and IgA antibodies against *O. tsutsugamushi* were 66.7% and 98.4%, respectively.²³ Hence, to the best of our knowledge, it can be concluded that scrub typhus RICT, not only facilitates prompt and early detection in resource-poor health-care settings but also may help prevent and manage complications due to scrub typhus by initiation of affordable early treatment with azithromycin or doxycycline.

Limitations of the study

Antigen or nucleic acid-based detection which is useful in diagnosing scrub typhus in the early course of illness cannot be evaluated in this study due to their non-availability. Hence, serotype or genotype of the etiologic organisms was not detected and/or confirmed.

CONCLUSION

To exclude scrub typhus as a cause of fever of unknown origin, RICT is a simple, rapid, and reliable assay for diagnosis of scrub typhus, capable of providing accurate results quickly, and is highly suitable for field deployment in remote areas with limited medical support.

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REFERENCES

- Paris DH. *Orientia tsutsugamushi* (Scrub typhus). In: Bennett JE, Dolin R and Blaser MJ, editors. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases. 9th ed. Philadelphia, PA: Elsevier; 2020. p. 2377-2381.
- Megaw JW. Scrub typhus as a war disease. Br Med J. 1945;2(4412):109-112.
<https://doi.org/10.1136/bmj.2.4412.109>
- Traub R and Wissernan CL Jr. The ecology of chigger-borne rickettsiosis (scrub typhus). J Med Entomol. 1974;11(3): 237-303.
<https://doi.org/10.1093/jmedent/11.3.237>
- Luce-Fedrow A, Lehman ML, Kelly DJ, Mullins K, Maina AN, Stewart RL, et al. A review of scrub typhus (*Orientia tsutsugamushi* and related organisms): Then, now, and tomorrow. Trop Med Infect Dis. 2018;3(1):8.
<https://doi.org/10.3390/tropicalmed3010008>
- Izzard L, Fuller A, Blacksell SD, Paris DH, Richards AL, Aukkanit N, et al. Isolation of a novel *Orientia* species (*O. chuto* sp. nov.) from a patient infected in Dubai. J Clin Microbiol. 2010;48(12):4404-4409.
<https://doi.org/10.1128/JCM.01526-10>
- Vector of scrub typhus in Bengal. Nature. 1948;162(4133):107.
- Candasamy S, Ayyanar E, Paily K, Karthikeyan PA, Sundararajan A, Purushothaman J, et al. Abundance and distribution of trombiculid mites and *Orientia tsutsugamushi*, the vectors and pathogen of scrub typhus in rodents and shrews collected from Puducherry and Tamil Nadu, India. Indian J Med Res. 2016;144(6):893-900.
https://doi.org/10.4103/ijmr.IJMR_1390_15
- Newton PN and Day NP. Scrub typhus. In: Ryan ET, Hill DR, Solomon T, Aronson NE and Endy TP, editors. Hunter's Tropical Medicine and Emerging Infectious Diseases, Part 3 Bacterial Infections. 10th ed. Edinburgh: Elsevier Inc.; 2020. p. 583-586.
- Service M. Medical Entomology for Students. Scrub Typhus Mites (*Trombiculidae*). 5th ed., Ch. 19. United Kingdom: Cambridge University Press; 2012. p. 260-267.
- Sarma N and Chakraborty S. Scrub typhus in Southern districts of West Bengal. Indian J Dermatol. 2017;62(5):512-514.
https://doi.org/10.4103/ijd.IJD_382_17
- Mahajan SK, Rolain JM, Kashyap R, Bakshi D, Sharma V, Prasher BS, et al. Scrub typhus in himalayas. Emerg Infect Dis. 2006;12(10):1590-1592.
<https://doi.org/10.3201/eid1210.051697>
- Chakraborty S and Sarma N. Scrub typhus: An emerging threat. Indian J Dermatol. 2017;62(5):478-485.
https://doi.org/10.4103/ijd.IJ_388_17
- Usha K, Kumar E, Kalawat U, Kumar SB, Chaudhury A and Sai Gopal DV. Molecular characterization of *Orientia tsutsugamushi* serotypes causing scrub typhus outbreak in southern region of Andhra Pradesh, India. Indian J Med Res. 2016;144(4):597-603.
<https://doi.org/10.4103/0971-5916.200886>
- Chaudhry D and Goyal S. Scrub typhus-resurgence of a forgotten killer. Indian J Anaesth. 2013;57(2):135-136.
<https://doi.org/10.4103/0019-5049.111836>
- Takhar RP, Bunkar ML, Arya S, Mirdha N and Mohd A. Scrub typhus: A prospective, observational study during an outbreak in Rajasthan, India. Natl Med J India. 2017;30(2):69-72.
- Vivekanandan M, Mani A, Priya YS, Singh AP, Jayakumar S and Purty S. Outbreak of scrub typhus in Pondicherry. J Assoc Physicians India. 2010;58:24-48.
- Srinivasan S and Menon T. Molecular detection of *Orientia tsutsugamushi* from suspected scrub typhus cases. Indian J Pathol Microbiol. 2017;60(1):71-73.
<https://doi.org/10.4103/0377-4929.200043>
- Saha B, Chatterji S, Mitra K, Ghosh S, Naskar A, Ghosh MK, et al. Socio-demographic and clinico-epidemiological study of scrub typhus in two tertiary care hospitals of Kolkata. J Assoc Physicians India. 2018;66(5):22-25.
<https://doi.org/10.18535/jmscr/v7i6.24>
- Varghese GM, Trowbridge P, Janardhanan J, Thomas K, Peter JV, Mathews P, et al. Clinical profile and improving mortality trend of scrub typhus in South India. Int J Infect Dis. 2014;23:39-43.
<https://doi.org/10.1016/j.ijid.2014.02.009>
- Rahi M, Gupte MD, Bhargava A, Varghese GM and Arora R. DHR-ICMR Guidelines for diagnosis and management of Rickettsial diseases in India. Indian J Med Res. 2015;141(4):417-422.
<https://doi.org/10.4103/0971-5916.159279>
- Kingston WF, Blacksell SD, Tanganuchitcharnchai A, Laongnualpanich A, Basnyat B, Day NP, et al. Comparative accuracy of the InBios scrub typhus detect IgM rapid test for the detection of IgM antibodies by using conventional serology. Clin Vaccine Immunol. 2015;22(10):1130-1132.
<https://doi.org/10.1128/cvi.00390-15>
- Kim YJ, Yeo SJ, Park SJ, Woo YJ, Kim MW, Kim SH, et al. Improvement of the diagnostic sensitivity of scrub typhus using a mixture of recombinant antigens derived from *Orientia*

- tsutsugamushi* serotypes. Korean Med Sci. 2013;28(5):672-679. <https://doi.org/10.3346/jkms.2013.28.5.672>
23. Silpasakorn S, Waywa D, Hoontrakul S, Suttinont C, Losuwanaluk K and Suputtamongkol Y. Performance of SD bioline *tsutsugamushi* assays for the diagnosis of scrub typhus in Thailand. J Med Assoc Thai. 2012;95(Suppl 2):S18-S22.
 24. Ramyasree A, Kalawat U, Rani ND and Chaudhury A. Seroprevalence of scrub typhus at a tertiary care hospital in Andhra Pradesh. Indian J Med Microbiol. 2015;33(1):68-72. <https://doi.org/10.4103/0255-0857.148381>
 25. Newby JG. The weil-felix test is archaic and misleading. J Am Med Assoc. 1986;255(8):1020. <https://doi.org/10.1001/jama.1986.03370080042021>
 26. Coleman RE, Sangkasuwan V, Suwanabun N, Eamsila C, Mungviriya S, 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(5):497-503. <https://doi.org/10.4269/ajtmh.2002.67.497>
 27. Varghese GM, Mathew A, Kumar S, Abraham OC, Trowbridge P and Mathai E. Differential diagnosis of scrub typhus meningitis from bacterial meningitis using clinical and laboratory features. Neurol India. 2013;61(1):17-20. <https://doi.org/10.4103/0028-3886.107919>
 28. Lijuan Z, Si H, Yuming J, Liang L, Xuemei L, Lianying L, et al. A rapid, sensitive and reliable diagnostic test for scrub typhus in China. Indian J Med Microbiol. 2011;29(4):368-371. <https://doi.org/10.4103/0255-0857.90166>
 29. Ching WM, Rowland D, Zhang Z, Bourgeois AL, Kelly D, Dasch GA, et al. Early diagnosis of scrub typhus with a rapid flow assay using recombinant major outer membrane protein antigen (r56) of *Orientia tsutsugamushi*. Clin Diagn Lab Immunol. 2001;8(2):409-414. <https://doi.org/10.1128/cdli.8.2.409-414.2001>
 30. Zhang L, He S, Wang S, Yu H, Li X, Zhang D, et al. Comparison of a rapid diagnostic test and microimmunofluorescence assay for detecting antibody to *Orientia tsutsugamushi* in scrub typhus patients in China. Asian Pacific J Trop Med. 2011;4(8):666-668. [https://doi.org/10.1016/s1995-7645\(11\)60169-7](https://doi.org/10.1016/s1995-7645(11)60169-7)
 31. Blacksell SD, Jenjaroen K, Phetsouvanh R, Tanganuchitcharnchai A, Phouminh P, Phongmany S, et al. Accuracy of rapid IgM-based immunochromatographic and immunoblot assays for diagnosis of acute scrub typhus and murine typhus infections in Laos. Am J Trop Med Hyg. 2010;83(2):365-369. <https://doi.org/10.4269/ajtmh.2010.09-0534>
 32. Walter DE, Lindquist EE, Smith IM, Cook DR and Krantz GW. Order trombidiformis. In: Krantz GW and Walter DE, editors. A Manual of Acarology. 3rd ed. USA: Texas Tech University Press; 2009. p. 233-420.
 33. Park K, editor. Park's Text book of Preventive and Social Medicine. Rickettsial Zoonosis (Rickettsial diseases). 23rd ed. Jabalpur: Banarisidas Bhanot; 2015. p. 300.

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SS, SKP, and **MP**- Concept and design of the study, prepared first draft of manuscript; **SS, JBD,** and **TG**- Reviewed the literature, and manuscript preparation; **SKP, JBD,** and **SC**- Concept, coordination, statistical analysis, and interpretation, Interpreted the results; **MP, TG,** and **SC**- Revision of the manuscript.

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