Iranian Journal of Microbiology
Volume 17 Number 4 (August 2025) 600-605
DOI: http://doi.org/10.18502/ijm.v17i4.19249

ORIGINAL ARTICLE

A comparative study on diagnostic accuracy of rapid card test, IgM ELISA and real time-PCR in detecting scrub typhus infection: a cross-sectional study from tertiary care hospital

Gopinath Ramalingam, Arundadhi Muthukumar, Dhanasezhian Aridass, Krishnapriya Subramani, Sucila Thangam Ganesan*

Viral Research and Diagnostic Laboratory, Department of Microbiology, Government Theni Medical College, Theni, Tamil Nadu, India

Received: December 2024**, Accepted:** May 2025

ABSTRACT

Background and Objectives: Scrub typhus, caused by *Orientia tsutsugamushi*, is a significant zoonotic illness in the Asia-Pacific region. Timely diagnosis is crucial, but overlapping symptoms and limitations of traditional diagnostic methods pose challenges. This study evaluates the diagnostic accuracy and utility of IgM ELISA, RT-PCR, and Rapid Card test for Scrub typhus, focusing on sensitivity, specificity, and practical applicability in endemic regions.

Materials and Methods: This cross-sectional study was conducted on 192 patients with suspected Scrub typhus at a tertiary care hospital from June to November 2024. Diagnostic tests included Rapid Card, IgM ELISA, and RT-PCR. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy were calculated, along with clinical and demographic data.

Results: IgM ELISA had the highest sensitivity (96.30%) and specificity (100%), followed by Rapid Card (sensitivity: 93.55%, specificity: 99.38%) and RT-PCR (sensitivity: 92.86%, specificity: 99.44%). Common symptoms included fever (99.4%) and headache (95.8%). Positive cases were mostly males (56.7%-64.3%) and individuals aged 21-40 years.

Conclusion: IgM ELISA shows high sensitivity and specificity for Scrub typhus, while RT-PCR aids early detection. The Rapid Card offers a quick field alternative. Combining molecular and serological methods can enhance diagnostic accuracy.

Keywords: Scrub typhus; *Orientia tsutsugamushi*; Real time-polymerase chain reaction; Enzyme-linked immunosorbent assay; Diagnostic tests

INTRODUCTION

Scrub typhus, caused by the obligate intracellular bacterium *Orientia tsutsugamushi*, is a major zoonotic illness seen in rural Asia-Pacific countries. Scrub typhus, characterized by symptoms such as fever, rash, and eschar at the site of mite bites, can cause serious complications if not recognized and treated quickly (1). The clinical symptoms of dengue and typhoid fever sometimes overlap, making correct diagnosis difficult (2). Scrub typhus has traditionally been diagnosed by serological tests, such as enzyme-linked immunosorbent assays (ELISA) and indirect immunofluorescence assays (IFA), which identify antibodies against *O. tsutsugamushi*. However, the sensitivity of these tests may be limited,

*Corresponding author: Sucila Thangam Ganesan, MD, Viral Research and Diagnostic Laboratory, Department of Microbiology, Government Theni Medical College, Theni, Tamil Nadu, India. Tel: +91-9442063819 Email: drgsucilamicro@gmail.com

especially in the early stages of infection when antibodies have not yet developed (3, 4). Furthermore, serological testing may cross-react with other rickettsial illnesses, producing false positives. In recent years, reverse transcription polymerase chain reaction (RT-PCR) has emerged as a potential molecular diagnostic method for quick and precise identification of *O. tsutsugamushi* (5). RT-PCR can detect the presence of pathogen genetic material, potentially improving early diagnosis and treatment (6). Despite these advances, the comparative efficacy of RT-PCR against standard serological tests in clinical practice remains unknown (7). This study aims to evaluate the sensitivity and specificity of RT-PCR compared to serological tests for diagnosing Scrub typhus. By systematically analyzing these diagnostic methods, we aim to provide insights that could inform clinical guidelines and enhance patient outcomes in regions where Scrub typhus is endemic. The findings will contribute to understanding the most effective diagnostic strategies and ultimately support better disease management in affected populations.

MATERIALS AND METHODS

Study design. This study employed a cross-sectional design, evaluating patients with suspected Scrub typhus in a clinical setting over a defined period. It was conducted at a tertiary care hospital in Theni between June 2024 and November 2024.

Study population: inclusion criteria. Patients aged 18 years and older presenting with acute febrile illness (fever >38°C) and at least one of the following: rash, eschar, lymphadenopathy, or history of exposure in endemic areas.

Exclusion criteria. Lysed or turbid blood samples; Prior antibiotic treatment for Scrub typhus within 14 days; Other infectious diseases that could affect test results; Significant comorbidities (e.g., severe immunocompromised states, chronic illnesses); Pregnancy (due to potential immune response alterations) and Recent rickettsial vaccinations or infections that could cause cross-reactivity.

Clinical samples. Each patient provided detailed demographic information, a comprehensive medical history, and underwent a clinical examination. The study included 192 clinically suspected individuals presented to the hospital with relevant symptoms. From each participant, 5 mL of blood was collected within the first week of illness onset. Samples were processed for both RT-PCR and serological testing.

Diagnostic methods: rapid diagnostic tests (RDT). It is a solid phase immunochromatographic test (SD Biosensor, Republic of Korea) designed for the fast and qualitative detection of *O. tsutsugamushi*-specific IgG/IgM antibodies in human serum, plasma, and whole blood. The Scrub typhus IgG/IgM Rapid card was conducted using commercially available kits, following the manufacturer's recommendations.

Serological testing. The presence of immunoglobulin M (IgM) antibodies against Scrub typhus was detected in all samples using an Enzyme-Linked Immunosorbent Assay (ELISA). The Scrub typhus IgM ELISA was performed using commercially available ELISA kits according to the manufacturer's instructions (InBios International Inc. kit from Seattle, WA, USA). The optical density (OD) was measured at 450 nm and interpreted in accordance with the manufacturer's guidelines.

RT-PCR. Total RNA was extracted from 200 µL of blood samples using a commercial RNA extraction kit following the manufacturer's protocol (QIAamp RNA Blood Mini Kit). Commercially available HELINI Scrub typhus Real-time PCR diagnostic kits were used following the manufacturer's protocol (HELINI Biomolecules, Chennai, INDIA). Positive and negative controls were included in each run to validate the results. All the samples were run on a ABI Biosystems 7500 PCR machine and the BioRad CFX96 PCR machine.

Data analysis. The Statistical analysis was performed using SPSS software version 21.0 (IBM Corp. IBM SPSS Statistics v21). Sensitivity was calculated as the proportion of true positive cases identified by each test. Specificity was determined as the proportion of true negative cases. The Sensitivity, specificity, positive predictive value and negative predictive value of the Scrub typhus RT PCR were evaluated by using online MedCalc (MedCalc Software bvba, Belgium; version 18.9). The significance of the p-value is taken as $p < 0.05$.

Ethical considerations. The Ethics Committee at GTMC, Theni, approved the study after it was conducted in accordance with the Declaration of Helsinki (Ref No: 1515/MEIII/2024). The study comprised patients of all ages and genders who reported an acute onset of high fever, body aches, convulsions, and a change in mental status.

RESULTS

To identify Scrub typhus, 192 clinically suspected patient samples were collected between June 2024 and November 2024. Samples suspected of containing Scrub typhus IgM ELISA antibodies were used for the test. Scrub typhus diagnostic tests were performed on 192 suspected cases using three different approaches (Table 1). The Rapid Card test identified 30 positive cases, with a positivity rate of 15.63%. The IgM ELISA test detected 29 positive cases (15.10%), while the RT-PCR test identified 14 positive cases (7.29%). The higher positivity rates in the Rapid Card and IgM tests compared to RT-PCR are likely due to the different diagnostic windows of these tests. While serological tests detect antibodies that develop later in infection, RT-PCR identifies the pathogen directly and is more effective during the early phase of illness.

Table 1. Total No. of serologically tested positive cases out of the total suspected subjects

S. No	Test Name	Total	Positive (%)
1	Scrub typhus Rapid card	192	30
2	Scrub typhus IgM	192	29
3	Scrub typhus RT-PCR	192	14

The clinical features of the 192 symptomatic patients (114 males and 78 females) are detailed in Table 2. Fever was the most commonly reported symptom, observed in 99.4% of patients, with no significant gender difference. Headache was also prevalent, affecting 95.8% of the cohort. Notable gender-based variations were observed for several symptoms. Cough was more frequently reported in males (69.2%) compared to females (44.8%), whereas chills were more common in females (62.8%) than males (33.3%). Similarly, vomiting, rash, abdominal pain, body pain, and myalgia were reported more frequently among female patients.

Table 2. Clinical profiles of symptomatic enrolled patients

Clinical Presentation	n = 192 (%)	Males (n=114) (%)	Females (n=78) (%)
Fever	191 (99.4%)	113 (99.1%)	78 (100%)
Cough	114 (59.3%)	79 (69.2%)	35 (44.8%)
Chills	87 (45.4%)	38 (33.3%)	49 (62.8%)
Skin Rash	34 (17.7%)	16 (14.0%)	18 (23.0%)
Headache	184 (95.8%)	109 (95.6%)	75 (96.1%)
Seizures	75 (39.0%)	42 (36.8%)	33 (42.3%)
Vomiting	67 (34.8%)	28 (24.5%)	39 (50%)
Abdominal pain	54 (28.1%)	26 (22.8%)	28 (35.8%)
Sore throat	112 (58.3%)	68 (59.6%)	44 (56.4%)
Body pain	134 (69.7%)	75 (65.7%)	59 (75.6%)
Myalgia	74 (38.5%)	36 (31.5%)	38 (48.7%)

Table 3 presents the distribution of Scrub typhus-positive cases by gender and age group. Males accounted for the majority of positive cases across all diagnostic methods, with the highest proportion found in RT-PCR-positive individuals (64.3%). The age group 21–40 years had the highest number of positive cases in all three testing methods: 56.7% for Rapid Card, 62.1% for IgM ELISA, and 64.3% for RT-PCR. In contrast, the 0-20 years age group had the fewest positive cases, particularly in RT-PCR, where no cases were detected. The 41-60 years group contributed a moderate number of cases, while individuals above 60 years had the lowest representation overall. These results suggest that young adults, particularly males aged 21-40, are more frequently affected by Scrub typhus in this setting.

Diagnostic performance of Scrub typhus detection methods. The diagnostic performance of the three Scrub typhus detection methods—Rapid Card, IgM ELISA, and RT-PCR—was assessed based on sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and overall accuracy (Table 4). The Scrub typhus Rapid Card test demonstrated a sensitivity of 93.55% (95% CI: 78.58%-99.21%) and a specificity of 99.38% (95% CI: 96.59%-99.98%). It showed a PPV of 96.67% (95% CI: 80.40%-99.51%) and an NPV of 98.77% (95% CI: 95.44%-99.67%), with an overall diagnostic accuracy of 98.44% (95% CI: 95.50%-99.68%). The Scrub typhus IgM ELISA test exhibited the highest sensitivity among the three at 96.30% (95% CI: 81.03%-99.91%), with a perfect

Table 3. Distribution of serologically positive cases based on gender and age

S. No	Test Name	Number of Positive	Male (%)	Female (%)	0-20 Years	21-40 years	41-60 Years	>60 Years
1	Scrub typhus Rapid card	30	17	13	2	17	8	3
2	Scrub typhus IgM	29	16	13	1	18	8	2
3	Scrub typhus RT-PCR	14	9	5	0	9	4	1

Table 4. Comparative evaluation of Scrub typhus for their sensitivity, specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV) and CI=Confidence Interval

Group	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	Accuracy (95% CI)
Scrub typhus Rapid card	93.55% (78.58% to 99.21%)	99.38% (96.59% to 99.98%)	96.67% (80.40% to 99.51%)	98.77% (95.44% to 99.67%)	98.44% (95.50% to 99.68%)
Scrub typhus IgM	96.30% (81.03% to 99.91%)	100.00% (97.75% to 100.00%)	100.00% (86.77% to 100.00%)	99.39% (95.95% to 99.91%)	99.47% (97.09% to 99.99%)
Scrub typhus RT-PCR	92.86% (66.13% to 99.82%)	99.44% (96.91% to 99.99%)	92.86% (64.68% to 98.93%)	99.44% (96.40% to 99.91%)	98.96% (96.29% to 99.87%

specificity of 100.00% (95% CI: 97.75%-100.00%). The test yielded a PPV of 100.00% (95% CI: 86.77%-100.00%) and an NPV of 99.39% (95% CI: 95.95%-99.91%), achieving the highest overall accuracy of 99.47% (95% CI: 97.09%-99.99%). The Scrub typhus RT-PCR test showed a sensitivity of 92.86% (95% CI: 66.13%-99.82%) and a specificity of 99.44% (95% CI: 96.91%-99.99%). It had a PPV of 92.86% (95% CI: 64.68%-98.93%), an NPV of 99.44% (95% CI: 96.40%-99.91%), and an overall accuracy of 98.96% (95% CI: 96.29%-99.87%). These results suggest that all three diagnostic methods exhibit high accuracy and reliability. However, the IgM ELISA marginally outperforms the others in terms of sensitivity, specificity, and overall diagnostic precision.

DISCUSSION

This study highlights the diagnostic strengths and limitations of Rapid Card, IgM ELISA, and RT-PCR in identifying Scrub typhus across different stages of infection. Among the methods evaluated, IgM ELISA demonstrated the highest sensitivity and specificity, supporting its role as a reliable tool for diagnosing Scrub typhus in both early and later stages of illness. Its performance aligns with previous reports by Ramlingam et al. and Blacksell et al., who also documented high diagnostic accuracy using the In-Bios Scrub typhus Detect ELISA (3, 8). Comparable results were reported by Jang et al. and Prakash et al., with findings indicating sensitivity and specificity levels similar to those of the gold standard immunofluorescence assay (IFA) (9, 10). Unlike the findings from Prakash et al., this study did not observe significant cross-reactivity, suggesting a lower false-positive rate in this population (11). This contrasts with the Kumaon region study, which emphasized the need for carefully calibrated cutoff values to minimize false positives in IgM-based assays and recommended RT-PCR as a confirmatory tool, especially after day 8 of the illness (6). These observations reinforce the complementary nature of serological and molecular diagnostics in Scrub typhus. RT-PCR was more successful in the early stages of infection, indicating higher bacterial loads, but serological techniques, which detect antibodies, were more reliable later. These differences highlight the complimentary nature of these diagnostic methods, implying that combining RT-PCR and serological assays could improve diagnosis accuracy.

In contrast, RT-PCR exhibited high specificity and moderately lower sensitivity, reflecting its effectiveness primarily during the early phase of infection when bacterial loads are higher. This method is instrumental in confirming infection before seroconversion occurs and is especially useful in distinguishing Scrub typhus from other rickettsial

infections, where serological tests may suffer from cross-reactivity. These findings are consistent with those reported by Anitharaj et al. (JIPMER), who confirmed over half of ELISA-positive cases using RT-PCR. Moreover, studies by Singhsilarak et al. and Bakshi et al., validated RT-PCR by targeting the 56-kDa gene with positivity rates between 62.9% and 73% (12-14). The gender and age distribution of positive cases showed that males, possibly due to increased outdoor exposure, and people aged 21 to 40 were the most impacted, which corresponded to occupational and environmental risk factors. While fever and headache were uniformly reported, other symptoms, such as rash and stomach pain were more common in females, indicating that clinical presentation may vary by gender.

The timing of sample collection had a significant impact on diagnostic yield. RT-PCR performed best within the first week of illness, while IgM ELISA was more reliable from the second week onward, consistent with findings from Saisongkorh et al., who reported PCR positivity extending to day 22 in some cases (15). This reinforces the utility of a dual diagnostic approach, employing molecular methods early and serological assays in later stages, to maximize detection accuracy. In addition to laboratory findings, the demographic and clinical profiles observed provide important epidemiological insights. Males, particularly those aged 21 to 40, were more frequently affected, possibly due to occupational and environmental exposures. Gender-based symptom differences were also evident—rash, vomiting, and abdominal pain were more prevalent among females—highlighting the potential for variable clinical presentations between sexes. Despite the strengths of each method, practical limitations still remain. RT-PCR, though highly specific, requires specialized equipment and trained personnel, making it less feasible in many endemic areas. Serological assays, though more accessible, may yield false positives in co-endemic settings without confirmatory molecular testing. Therefore, diagnostic strategies must consider both resource availability and the stage of illness.

CONCLUSION

This study underscores the importance of selecting appropriate diagnostic tools for Scrub typhus based on both clinical context and resource availability. IgM ELISA emerged as the most accurate and reliable method across disease phases, offering high sensitivity and specificity. The Rapid Card test, while slightly less precise, remains valuable for its rapid results and ease of use in field settings. RT-PCR plays a crucial role in early diagnosis and in distinguishing Scrub typhus from other febrile illnesses, particularly in the pre-seroconversion window. The findings support an integrated diagnostic approach that combines serological and molecular techniques to enhance accuracy—particularly in endemic regions where multiple febrile illnesses may overlap. The use of RT-PCR in the early phase, followed by IgM ELISA in the later phase, can significantly improve case detection and treatment outcomes. Finally, targeted interventions should focus on high-risk groups—especially males aged 21 to 40—to enable early identification and management, reducing the burden of complications and improving public health outcomes in endemic settings.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge all the patients who participated in this study. They also wish to extend their sincere thanks to the Department of Health Research (DHR), the Ministry of Health and Family Welfare, and the ICMR for the Viral Research and Diagnostic Laboratory initiative (Ref. No: VIR/66/2013/ECD-I;30.09.2014).

REFERENCES

1. Gitau MS, Asiago OH, Rogers OO, Reddy KS, PradeepKumar B, Sudheer A, et al. A Comprehensive review on Scrub typhus: Epidemiology, Transmission, Diagnosis and Management. *Uttar Pradesh J Zool* 2024; 45: 93-105.
2. Xu G, Walker DH, Jupiter D, Melby PC, Arcari CM. A review of the global epidemiology of Scrub typhus. *PLoS Negl Trop Dis* 2017; 11(11): e0006062.
3. Ramlingam G, Muthukumar A, Aridass D, Ganesan ST. Seroprevalence and clinical Profile of Leptospirosis and Scrub typhus in clinically Suspected Patients at a Tertiary care center: A Retrospective study from Theni, Tamil Nadu. *Biomed Biotechnol Res J* 2024; 8: 382-386.

4. Gautam R, Parajuli K, Tshokey T, Stenos J, Sherchand JB. Diagnostic evaluation of IgM ELISA and IgM Immunofluorescence assay for the diagnosis of Acute Scrub typhus in central Nepal. *BMC Infect Dis* 2020; 20: 138.
5. Kannan K, John R, Kundu D, Dayanand D, Abhilash KP, Mathuram AJ, et al. Performance of molecular and serologic tests for the diagnosis of Scrub typhus. *PLoS Negl Trop Dis* 2020; 14(11): e0008747.
6. Rawat V, Singh RK, Kumar A, Saxena SR, Varshney U, Kumar M. Epidemiological, clinical and laboratory profile of Scrub typhus cases detected by serology and RT-PCR in Kumaon, Uttarakhand: a hospital-based study. *Trop Doct* 2018; 48: 103-106.
7. Peniche-Lara G, Moo-Salazar I, Dzul-Rosado K. A Multiplex PCR assay for a differential diagnostic of rickettsiosis, Lyme disease and Scrub typhus. *J Vector Borne Dis* 2022; 59: 178-181.
8. Blacksell SD, Tanganuchitcharnchai A, Nawtaisong P, Kantipong P, Laongnualpanich A, Day NP, et al. Diagnostic accuracy of the InBios Scrub typhus detect enzyme-linked immunoassay for the detection of IgM antibodies in northern Thailand. *Clin Vaccine Immunol* 2015; 23: 148-154.
9. Jang WJ, Huh MS, Park KH, Choi MS, Kim IS. Evaluation of an immunoglobulin M capture enzyme-linked immunosorbent assay for diagnosis of *Orientia tsutsugamushi* infection. *Clin Diagn Lab Immunol* 2003; 10: 394-398.
10. Prakash JA, Kavitha ML, Mathai E. Nested polymerase chain reaction on blood clots for gene encoding 56 kDa antigen and serology for the diagnosis of Scrub typhus. *Indian J Med Microbiol* 2011; 29: 47-50.
11. Prakash JA, Abraham OC, Mathai E. Evaluation of tests for serological diagnosis of Scrub typhus. *Trop Doct* 2006; 36: 212-213.
12. Anitharaj V, Stephen S, Pradeep J, Park S, Kim SH, Kim YJ, et al. Serological diagnosis of acute Scrub typhus in Southern India: Evaluation of InBios Scrub typhus Detect IgM Rapid Test and comparison with other serological tests. *J Clin Diagn Res* 2016; 10: DC07-DC10.
13. Singhsilarak T, Leowattana W, Looareesuwan S, Wongchotigul V, Jiang J, Richards AL, et al. Short report: Detection of *Orientia tsutsugamushi* in clinical samples by quantitative real-time polymerase chain reaction. *Am J Trop Med Hyg* 2005; 72: 640-641.
14. Bakshi D, Singhal P, Mahajan SK, Subramaniam P, Tuteja U, Batra HV. Development of a real-time PCR assay for the diagnosis of Scrub typhus cases in India and evidence of the prevalence of new genotype of *O. tsutsugamushi*. *Acta Trop* 2007; 104: 63-71.
15. Saisongkorh W, Chenchittikul M, Silpapojakul K. Evaluation of nested PCR for the diagnosis of Scrub typhus among patients with acute pyrexia of unknown origin. *Trans R Soc Trop Med Hyg* 2004; 98: 360-366.