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Diagnostic Performance of a Rapid Immunochromatographic Test Compared with IgM ELISA for Scrub Typhus in Clinically Suspected Cases: A Prospective hospital based cross-sectional study

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ABSTRACT

Background: Scrub typhus is an important cause of acute undifferentiated febrile illness in India. Because its clinical manifestations overlap with other tropical febrile illnesses, laboratory confirmation is important. IgM ELISA is widely used for serological diagnosis, whereas rapid immunochromatographic tests (ICTs) offer rapid point-of-care results.

Methods: A prospective hospital-based cross-sectional study was conducted among 90 clinically suspected scrub typhus cases at JJM Medical College, Davangere, Karnataka, from June 2019 to May 2020. Serum samples were tested by Scrub Typhus Detect IgM ELISA and SD Bioline Tsutsugamushi rapid ICT method. Diagnostic performance of ICT was assessed using IgM ELISA as the reference serological method.

Results: Of 90 clinically suspected cases, 35 (38.9%) were IgM ELISA positive. Among these, 31 were ICT positive and 4 were ICT negative. Of 55 ELISA-negative samples, 52 were ICT negative and 3 were ICT positive. The ICT demonstrated a sensitivity of 88.6%, specificity of 94.5%, positive predictive value of 91.2%, negative predictive value of 92.9%, and overall diagnostic accuracy of 92.2%.

Conclusion: The rapid ICT demonstrated good sensitivity, high specificity and acceptable diagnostic accuracy. It may be useful as an initial screening/point-of-care test, particularly where conventional laboratory facilities are limited. However, the false-negative results observed in the present study support confirmation by IgM ELISA when feasible, especially when clinical suspicion remains high.

Keywords: *Scrub typhus, Orientia tsutsugamushi, IgM ELISA, Rapid Immunochromatographic test, sensitivity, specificity.*

INTRODUCTION

Scrub typhus is an acute febrile zoonotic disease caused by the obligate intracellular bacterium Orientia tsutsugamushi and transmitted to humans through the bite of infected larval trombiculid mites, commonly referred to as chiggers [1-5]. It is an important cause of acute undifferentiated febrile illness in the Asia-Pacific region, including India, and continues to be an under-recognized infection because of its variable clinical presentation. The geographical distribution of the disease and the presence of diverse strains of O. tsutsugamushi contribute to considerable epidemiological and diagnostic heterogeneity across endemic regions.

In India, scrub typhus has been reported from several geographical regions and is increasingly recognized as an important cause of acute febrile illness. Outbreaks and sporadic cases have been reported from southern, northern, northeastern and central India, emphasizing that the disease is not restricted to a single geographical area [5-7].

The clinical manifestations of scrub typhus are diverse and may range from a self-limiting febrile illness to severe and potentially fatal disease. Patients may present with fever, headache, myalgia, rash, lymphadenopathy, hepatosplenomegaly

and an eschar. Complications may include pulmonary involvement, hepatitis, renal dysfunction, meningoencephalitis and cardiovascular manifestations. Importantly, the classical eschar may be absent, and therefore its absence cannot be used to exclude scrub typhus.

The nonspecific nature of the clinical presentation creates considerable diagnostic overlap with other tropical infections, particularly dengue, malaria, leptospirosis and enteric fever. Consequently, clinical diagnosis alone may be difficult and laboratory confirmation plays an important role in establishing the diagnosis.

Several laboratory methods are available for the diagnosis of scrub typhus. Indirect immunofluorescence assay (IFA) is widely regarded as an important reference serological method; however, its routine application may be limited by the requirement for specialized equipment, trained personnel, technical expertise and relatively greater cost [2,6]. IgM ELISA provides a more practical alternative for routine diagnostic laboratories and is widely used in Indian studies

Rapid immunochromatographic tests (ICTs) have emerged as useful point-of-care diagnostic tools because they can provide results within minutes and require minimal laboratory infrastructure. Such characteristics are particularly valuable in peripheral healthcare facilities, emergency departments and resource-limited settings where conventional serological facilities may not be readily accessible. A rapid test that provides adequate diagnostic performance could facilitate earlier recognition of scrub typhus and support timely clinical decision-making.

Therefore, the present study was undertaken to evaluate the diagnostic performance of a rapid immunochromatographic test in comparison with IgM ELISA . The study specifically assessed the sensitivity, specificity, positive predictive value, negative predictive value and overall diagnostic accuracy of the rapid ICT using IgM ELISA as the reference serological method. The findings were intended to determine the potential role of rapid ICT as an initial screening or point-of-care diagnostic test in the local clinical setting.

REVIEW OF LITERATURE:

Early reports from India established scrub typhus as an important cause of acute febrile illness in southern India. Chrispal et al. described scrub typhus as an important cause of acute undifferentiated febrile illness in Vellore and documented severe complications and mortality in affected adults [7].

Varghese et al. subsequently described the clinical profile and improving mortality trend of scrub typhus in South India, reinforcing the importance of early recognition and treatment [8].

Gurung et al. described an outbreak in Sikkim, demonstrating disease across adult and paediatric age groups. Sinha et al. reported an outbreak in north-western India, supporting the expanding geographical recognition of scrub typhus in India [9,10].

The diagnostic literature demonstrates that no single serological assay performs identically across all settings. Zhang et al. evaluated a rapid diagnostic test against micro-immunofluorescence assay in China and supported the potential utility of rapid testing . Kingston et al. showed that performance of a recombinant 56-kDa antigen-based rapid assay varied according to the IFA antibody titre used as the positivity threshold, illustrating the effect of reference standard and cut-off selection [11,12].

Prakash et al. evaluated IgM ELISA in India and supported its usefulness for serological diagnosis . Anitharaj et al. evaluated the InBios rapid IgM test against IgM ELISA in southern India and reported sensitivity of 99.25% and specificity of approximately 94.9%. Elangovan et al. evaluated ICT and ELISA using a serum panel and reported sensitivity of 98.7% and specificity of 96.3% for ICT, with excellent concordance between the two assays [12-15].

Notably, not all studies have demonstrated such high sensitivity. Pote et al. evaluated ICT, IgM ELISA and IFA in 600 patients with acute undifferentiated fever in central India. Their ICT sensitivity was only 38%, although specificity was 100%, while IgM ELISA performed substantially better. This strongly discordant result demonstrates the variability of rapid-test performance and supports local validation of commercial assays [16].

Narayanappa et al. evaluated a rapid antibody detection test in children using IFA as the reference standard and reported sensitivity of 88.3% and specificity of 89.3%. Saha et al. reported sensitivity of 100% and specificity of 86.87% for a rapid immunochromatographic test when IgM ELISA was used as the reference standard. More recent Indian evidence also supports continued evaluation of rapid assays. Ramalingam et al. reported sensitivity of 93.55% and specificity of 99.38% for a rapid card test, compared with 96.30% sensitivity and 100% specificity for IgM ELISA [17,18].

Systematic reviews have emphasized the considerable burden of scrub typhus and the dominant role of IgM ELISA in published Indian studies. The variability in rapid-test performance may reflect differences in antigen composition, circulating O. tsutsugamushi strains, stage of illness, antibody titer, patient selection and reference standards [19-23].

MATERIALS AND METHODS:

Study Design and Setting

A prospective hospital based cross-sectional study was conducted in the Department of Microbiology, JJM Medical College, Davangere, Karnataka, in collaboration with the Departments of General Medicine and Pediatrics. Patients attending the inpatient and outpatient departments of Bapuji Hospital and Chigateri General Hospital were included. The study period was June 2019 to May 2020. Ethical approval was obtained from the Institutional Ethics Committee and informed consent was obtained from participants or their guardians where applicable.

Study Population

Ninety clinically suspected, non-repetitive cases of scrub typhus of all age groups and sexes were included. Patients with acute febrile illness and compatible clinical features such as headache, chills, rash, eschar, myalgia/polyarthralgia, lymphadenopathy, hepatosplenomegaly, edema, meningoencephalitic manifestations, respiratory symptoms or relevant mite exposure were eligible. Patients with laboratory-confirmed alternative diagnoses such as malaria, dengue, enteric fever or measles were excluded.

Laboratory Methods

Serum samples were tested using Scrub Typhus Detect IgM ELISA (InBios International Inc., Seattle, USA) and SD Bioline Tsutsugamushi rapid ICT (Standard Diagnostics Inc.). The ELISA used in the study detects IgM antibodies against recombinant O. tsutsugamushi antigens. Rapid ICT used in this study qualitatively detects IgM, IgG,IgA antibodies against the 56 kDa recombinant protein O.tsutsugamushi antigens. For the present diagnostic analysis, IgM ELISA was used as the reference serological method

Statistical Analysis

Data were entered into Microsoft Excel and analyzed using IBM SPSS version 23.0. Categorical variables were expressed as frequencies and percentages. Sensitivity, specificity, positive predictive value, negative predictive value and overall diagnostic accuracy of ICT were calculated using IgM ELISA as the reference method. Tools like ROC curve was utilized to depict the diagnostic accuracy of RDT. p value < 0.05 was taken as statistically significant.

RESULTS:

A total of 90 clinically suspected cases were enrolled. Thirty-five (38.9%) were positive by IgM ELISA and 55 (61.1%) were negative. Rapid ICT was positive in 34 cases and negative in 56 cases. Thirty-one of the 35 ELISA-positive cases were also ICT positive, while four were ICT negative. Among the 55 ELISA-negative cases, three were ICT positive and 52 were ICT negative.

Table 1. Demographic characteristics of IgM ELISA-positive cases (n=35)

Variable	Number	Percentage (%)
Age 0–10 years	7	20.0
Age 11–20 years	6	17.1
Age 21–30 years	4	11.4
Age 31–40 years	13	37.1
Age 41–50 years	4	11.4
>50 years	1	2.9
Male	17	48.6
Female	18	51.4
Rural residence	28	80.0
Urban residence	7	20.0

The highest proportion of IgM ELISA-positive cases was observed in the 31–40 yr age group (37.1%), followed by children aged 0–10 yr (20.0%) and those aged 11–20 yr (17.1%). A slight female predominance was observed (51.4%), while the majority of cases were from rural areas (80.0%).

Table 2. District-wise distribution of IgM ELISA-positive cases (n=35)

District	Number	Percentage (%)
Davangere	21	60.0
Chitradurga	8	22.9
Bellary	2	5.7
Haveri	4	11.4
Total	35	100

Among the IgM ELISA-positive cases, the majority were from Davangere district (60.0%), followed by Chitradurga (22.9%), Haveri (11.4%) and Bellary (5.7%). This distribution largely reflects the geographical catchment of the study hospitals.

Table 3. Selected clinical characteristics of IgM ELISA-positive cases (n=35)

Clinical feature	Number	Percentage (%)
Fever	35	100.0
Headache	14	40.0
Maculopapular rash	2	5.7
Eschar	1	2.9
History of mite bite	3	8.6

Fever was present in all IgM ELISA-positive cases (100%), while headache was the next most frequent clinical feature (40.0%). Maculopapular rash (5.7%), history of mite bite (8.6%) and eschar (2.9%) were relatively uncommon.

Table 4. Results of IgM ELISA

IgM ELISA	No. of cases	Total
Positive	35	38.9
Negative	55	61.1
Total	90	100

Of the 90 clinically suspected cases evaluated, 35 (38.9%) were positive for IgM antibodies by ELISA, while 55 (61.1%) were negative.

Table 5. Results of Rapid ICT

Rapid ICT	No. of cases	Total
Positive	34	37.8
Negative	56	62.2
Total	90	100

Rapid ICT was positive in 34 (37.8%) of the 90 clinically suspected cases and negative in 56 (62.2%) cases.

Table 6. Comparison of rapid ICT with IgM ELISA

Rapid ICT	IgM ELISA positive	IgM ELISA negative	Total

Positive	31	3	34
Negative	4	52	56
Total	35	55	90

When rapid ICT results were compared with IgM ELISA, 31 of the 35 ELISA-positive cases were also positive by ICT, whereas four were ICT negative. Among the 55 ELISA-negative cases, 52 were also negative by ICT, while three showed positive ICT results. Thus, 83 of the 90 samples showed concordant results between the two assays.

Table 7. Diagnostic performance of rapid ICT using IgM ELISA as reference method.

Parameter	Value (%)
Sensitivity	88.6
Specificity	94.5
Positive predictive value	91.2
Negative predictive value	92.9
Overall diagnostic accuracy	92.2

Using IgM ELISA as the reference serological method, the rapid ICT demonstrated a sensitivity of 88.6% and specificity of 94.5%. The positive predictive value was 91.2%, negative predictive value was 92.9%, and overall diagnostic accuracy was 92.2%.

Table 8. Diagnostic accuracy of rapid tests in selected published studies

Study	Year	Setting	Reference standard	Test	Sensitivity (%)	Specificity (%)
Anitharaj et al. [14]	2016	Southern India	IgM ELISA	InBios rapid IgM	99.25	94.87
Elangovan et al. [15]	2019	India	Serum reference panel	IgM ICT	98.7	96.3
Pote et al. [16]	2018	Central India	Latent-class/serological comparison	SD Bioline ICT	38.0	100
Narayanappa et al. [17]	2023	India; children	IFA	Rapid antibody test	88.3	89.3
Saha et al. [18]	2023	India	IgM ELISA	Rapid ICT	100	86.87
Ramalingam et al. [22]	2025	Tamil Nadu, India	RT-PCR/combined comparison	Rapid card	93.55	99.38
Present study	2020	Davangere Karnataka	IgM ELISA	SD Bioline ICT	88.6	94.5

The diagnostic performance of rapid assays varied considerably across the published studies included in Table 8 [13,16,19-21]. Sensitivity ranged from 38.0 to 100%, while specificity ranged from 86.87 to 100%. The sensitivity and specificity observed in the present study were within the range reported in many previous studies. [14-18,22].

Table 9. Studies with results discordant with the present study

Study	Finding	Comparison with present study	Interpretation
Anitharaj et al. [14], 2016	Sensitivity 99.25%; specificity 94.87%	Much higher sensitivity; similar specificity	May reflect kit, population, timing and reference-method differences
Elangovan et al. [15], 2019	Sensitivity 98.7%; specificity 96.3%	Higher sensitivity and slightly higher specificity	Serum-panel design and assay characteristics may influence performance
Pote et al. [16] , 2018	Sensitivity 38%; specificity 100%	Markedly lower sensitivity; higher specificity	Demonstrates substantial inter-study and kit variability
Narayanappa et al. [17], 2023	Sensitivity 88.3%; specificity 89.3%	Very similar sensitivity; lower specificity	Different age group and IFA reference standard
Saha et al. [18], 2023	Sensitivity 100%; specificity 86.87%	Higher sensitivity; lower specificity	Different population and assay performance
Ramalingam et al. [22], 2025	Sensitivity 93.55%; specificity 99.38%	Higher sensitivity and specificity	Different study design and reference framework

The comparison of studies with results differing from the present study highlights substantial inter-study variability in rapid-test performance. Differences in reference standards, patient populations, age groups, assay platforms and clinical settings may contribute to the observed variation [13-18,22].

DISCUSSION:

The present study evaluated a rapid immunochromatographic assay against IgM ELISA among 90 clinically suspected scrub typhus cases. Thirty-five cases (38.9%) were positive for IgM ELISA. This proportion is very similar to the 39% seropositivity reported by Ramyasree et al. in a tertiary care hospital in Andhra Pradesh, supporting the observation that scrub typhus contributes substantially to the burden of acute febrile illness in southern India [19].

In the present study, scrub typhus was observed across a broad age range, with the highest proportion of IgM ELISA-positive cases occurring in the 31–40 yr age group. A slight female predominance was observed. The marked predominance of cases from rural areas may reflect greater exposure to mite-infested vegetation and agricultural or outdoor environments; however, this finding should be interpreted cautiously because the study population was hospital-based and the distribution of the underlying population at risk was not assessed. The occurrence of cases across different age groups emphasizes that scrub typhus should be considered in the differential diagnosis of acute febrile illness irrespective of age.

The clinical profile of the present study was predominantly nonspecific. Fever was present in all ELISA-positive patients, while headache was the next common manifestation. Rash and eschar were uncommon. This finding is clinically important because absence of eschar should not exclude scrub typhus. Chrispal et al. and other Indian studies have similarly demonstrated that scrub typhus can present without the classical eschar and may be difficult to distinguish clinically from other causes of acute febrile illness [7,8].

The predominance of cases from Davangere district is likely related, at least in part, to the location of the study centre and its referral catchment, and therefore should not be interpreted as evidence of higher population-level prevalence in the district.

The rapid ICT demonstrated 88.6% sensitivity and 94.5% specificity, with PPV of 91.2%, NPV of 92.9% and overall diagnostic accuracy of 92.2%. The sensitivity was lower than that reported in several studies. Anitharaj et al. reported

99.25% sensitivity and 94.87% specificity [14,15,13] for the InBios rapid IgM assay, while Elangovan et al. reported 98.7% sensitivity and 96.3% specificity for ICT. Prakash et al. also reported a high performance of a rapid IgM assay. Therefore these studies represent results that are more favourable than those observed in the present study.

Importantly, however, the present sensitivity was not an outlier. Narayanappa et al. reported a sensitivity of 88.3% and specificity of 89.3% for a rapid antibody detection test using IFA as the reference standard in children, closely approximating the sensitivity of 88.6% observed in the present study. In contrast, Saha et al. reported 100% sensitivity but lower specificity of 86.87%. Thus, both higher sensitivity and lower specificity have been reported, indicating that rapid-test performance is context dependent [17,18].

A particularly discordant study was reported by Pote et al. in central India. In 600 patients with acute undifferentiated fever, the sensitivity of SD Bioline ICT was only 38%, although specificity was 100%. This contrasts markedly with the 88.6% sensitivity and 94.5% specificity observed in the present study. The difference illustrates why performance estimates from one setting should not be assumed to apply universally to another commercial platform may perform differently depending on the study population, disease spectrum, timing of specimen collection, antibody titer, antigenic variation and the method used to establish the reference diagnosis [16].

The four ICT-negative/ELISA-positive cases in this study are clinically important. False-negative antibody tests may occur when samples are collected early in illness before sufficient IgM antibody has developed, or when antibody concentration is near the detection threshold.

Differences between assay antigens and circulating O. tsutsugamushi strains may also contribute. Kingston et al. demonstrated that rapid-test performance can change according to the IFA antibody titer used as the positivity threshold, highlighting the influence of reference criteria [12].

The three ICT-positive/ELISA-negative results represent the converse discordance. These may reflect assay-specific reactivity, cross-reactivity, differences in analytical thresholds, or the imperfection of IgM ELISA as a reference method. Because no diagnostic test is a perfect gold standard, sensitivity and specificity in the present study should be interpreted as performance estimates relative to IgM ELISA rather than absolute measures of diagnostic accuracy.

The specificity of 94.5% in the present study was comparable to that reported by Anitharaj et al. and Prakash et al., although lower than the 96.3% reported by Elangovan et al. and the [14,13] 100% reported by Pote et al. High specificity is useful in clinical settings because a positive rapid test can provide supportive evidence for early treatment decisions. Nevertheless, predictive values are prevalence-dependent and should not be extrapolated directly to populations with different disease prevalence.

The findings therefore support the use of rapid ICT as an initial screening or point-of-care test rather than as a complete replacement for serological confirmation. In settings where ELISA or IFA is unavailable, a rapid test can provide useful information rapidly and may facilitate earlier clinical management. However, a negative rapid test should not by itself exclude scrub typhus when clinical suspicion remains high. In such cases, confirmatory or repeat serological testing should be considered.

This study has several strengths, including its prospective hospital-based design, simultaneous testing of the same serum samples by ICT and IgM ELISA, and inclusion of both clinical and demographic information. The principal limitations are the relatively small sample size, single-center design, use of IgM ELISA rather than IFA as the reference method, absence of PCR testing, and lack of paired acute and convalescent sera. These limitations should be considered when interpreting the diagnostic accuracy estimates.

CONCLUSION:

In the present study, 38.9% of clinically suspected scrub typhus cases were positive by IgM ELISA. The rapid immunochromatographic test demonstrated 88.6% sensitivity, 94.5% specificity and 92.2% overall diagnostic accuracy. The results indicate that rapid ICT is a useful screening and point-of-care tool, particularly in resource-limited settings; however its sensitivity is variable and false-negative results may occur. Therefore IgM ELISA remains preferable for serological confirmation where available. Larger multicentric studies incorporating IFA and molecular methods are required to further define the performance of individual rapid assays across different Indian epidemiological settings.

Strengths of the Present Study:

The strengths of the present study include its prospective design, inclusion of clinically suspected patients from a tertiary care teaching hospital, and simultaneous evaluation of two commonly used serological diagnostic methods. The use of IgM ELISA as the reference

serological method enable assessment of the diagnostic performance of the rapid immunochromatographic assay under routine clinical conditions. In addition, the study evaluated both demographic and clinical characteristics of laboratory-confirmed scrub typhus cases, thereby providing a comprehensive overview of the disease profile in the study population.

Limitations:
The present study had certain limitations. First, IgM ELISA rather than indirect immunofluorescence assay (IFA) was used as the reference standard because IFA was not available at the study centre. Second, molecular techniques such as polymerase chain reaction (PCR), which are capable of detecting early infection before antibody production, were not performed. Finally, paired acute and convalescent serum samples were not available to assess seroconversion.

Conflict of Interest : There is no conflict of interest

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