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Original Research Article

DIAGNOSTIC PERFORMANCE OF A RAPID IMMUNOCHROMATOGRAPHIC TEST VERSUS IGM ELISA FOR SCRUB TYPHUS AMONG FEBRILE PATIENTS AT A TERTIARY CARE HOSPITAL IN IMPHAL, MANIPUR

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ABSTRACT

Background: Scrub typhus is a significant cause of acute undifferentiated febrile illness in India, often underdiagnosed due to overlapping clinical features and limited access to advanced diagnostics. Rapid immunochromatographic tests (ICTs) offer potential for early detection, especially in resource-limited settings. The objective is to determine the prevalence of scrub typhus among patients with acute undifferentiated febrile illness at a tertiary care hospital in Imphal, Manipur, and to evaluate the diagnostic accuracy of a rapid ICT compared with IgM ELISA. **Materials and Methods:** This prospective diagnostic validation study was conducted from April 2023 to March 2025 involving 301 patients suspected of scrub typhus. Blood samples underwent both rapid ICT and IgM ELISA testing. Diagnostic performance metrics of ICT were calculated using IgM ELISA as the reference standard. Associations between demographic factors and scrub typhus positivity were analyzed. **Result:** Scrub typhus prevalence was 27.9% (84/301) by IgM ELISA. Infection was significantly associated with younger age (≤25 years) and male gender ($p < 0.05$). The ICT demonstrated high diagnostic accuracy with sensitivity of 98.8%, specificity of 98.6%, positive predictive value of 96.5%, negative predictive value of 99.5%, and overall accuracy of 98.7% relative to IgM ELISA. **Conclusion:** The rapid ICT is a reliable and practical diagnostic tool for early detection of scrub typhus, particularly valuable in settings lacking advanced laboratory infrastructure. The findings also indicate that certain demographic factors may play a role in the distribution of the disease. Further multi-center studies are recommended to validate these findings across diverse populations.

INTRODUCTION

Scrub typhus is a vector-borne zoonotic infection caused by Orientia tsutsugamushi, an obligate intracellular gram-negative organism. Transmission occurs through the bite of infected larval Trombiculid mites of the genus Leptotrombidium, commonly referred to as chiggers. These larvae typically attach to thin or delicate areas of skin and feed for several days. During feeding, mites secrete enzymes that break down surrounding tissues, enabling ingestion, and O. tsutsugamushi has been identified within their salivary glands. The organism is maintained in mite populations primarily through transovarial transmission from adult females to their eggs and through transtadial transmission across larval, nymphal, and adult stages.[1] Scrub typhus is a leading cause of acute undifferentiated febrile illness and its incidence has been increasing across India. Reported endemic regions span a wide geographic range, including northern states such as Uttarakhand and Himachal Pradesh; northeastern states like Assam, Meghalaya, Manipur, and Nagaland; southern states including Tamil Nadu, Andhra Pradesh, Kerala, and Karnataka; as well as West Bengal, Bihar, Rajasthan, and Maharashtra.[2-5]

Although traditionally associated with rural, low- and middle-income settings where vegetation is abundant, recent reports indicate growing occurrence in urban environments as well. Despite its clinical relevance, scrub typhus remains under-recognized and under-diagnosed, often mimicking conditions such as enteric fever, malaria, dengue, and leptospirosis. The presence of an eschar can assist in diagnosis, but it is not consistently observed in all patients.[6-8]

Diagnosis of scrub typhus relies heavily on clinical judgment supported by serological testing. Several laboratory methods are available for detecting O. tsutsugamushi. The Indirect Fluorescent Antibody (IFA) assay is considered the reference standard; however, its routine use is limited because it requires advanced laboratory infrastructure and trained personnel.[1,9,10] Consequently, IgM ELISA has become the most commonly employed diagnostic tool, offering a practical combination of good sensitivity and specificity. Molecular approaches such as PCR provide the advantage of early detection before antibody levels rise, but they are not widely accessible.[1,9] Rapid immunochromatographic tests (ICTs) have emerged as valuable point-of-care tools, particularly in resource-limited settings. These assays use recombinant outer membrane proteins of Orientia to detect IgM, IgG, or IgA antibodies, allowing quick screening in clinical settings. Their sensitivity is generally moderate to high and tends to improve with increasing duration of fever.[9,11,12]

Despite the growing use of ICTs, evidence on their diagnostic accuracy compared with established methods remains limited. As rapid tests are frequently employed for screening large numbers of patients within a short period, it is important to determine how reliably they perform relative to IgM ELISA, which is often regarded as the practical reference test in many laboratories. Early and accurate detection is critical for timely treatment and improved patient outcomes. In this context, the present study was undertaken at a tertiary care centre in Imphal, Manipur, with the aims of determining the prevalence of scrub typhus cases among patients tested for acute undifferentiated febrile illness and evaluating the diagnostic accuracy of a rapid ICT kit in comparison with the IgM ELISA assay.

MATERIALS AND METHODS

This prospective diagnostic validation study was conducted in the Serology Laboratory of the Department of Microbiology, RIMS, Imphal, from April 2023 to March 2025. Blood samples were received from patients of all age groups presenting to the outpatient, inpatient, and emergency departments with clinical suspicion of scrub typhus. Individuals with acute undifferentiated febrile illness who provided informed consent were included. Patients with confirmed alternative diagnoses or those unwilling to participate were excluded. Samples unsuitable for analysis due to haemolysis, lipaemia, contamination, or inadequate volume were also excluded. Of the 336 samples collected from suspected cases, 35 samples were excluded based on the predefined exclusion criteria, leaving 301 samples for final evaluation.

Venous blood was collected aseptically into sterile vials or red-top vacutainers and allowed to clot at room temperature. Serum was separated by centrifugation at 1000–2000 × g for 10 minutes and labelled with unique patient identifiers. Samples not processed immediately were stored at 2–8°C, and those requiring storage beyond three days were frozen at –20°C. Freeze–thaw cycles and visibly compromised samples were avoided. All specimens underwent rapid immunochromatographic testing (ICT) and IgM ELISA for detection of scrub typhus, and laboratory personnel were blinded to the results of both assays.

The ICT (J. Mitra & Co. Pvt. Ltd.) was carried out by equilibrating kits and samples to room temperature, applying the specimen to the sample well, and adding assay buffer as per manufacturer instructions. Results were read at 20 minutes and interpreted based on the presence of control and test lines indicating IgM, IgG, both, or non-reactive status. Tests lacking a control line were deemed invalid.

The IgM ELISA (J. Mitra & Co. Pvt. Ltd.) was performed according to the manufacturer protocol. Serum samples were diluted 1:100 and dispensed into antigen-coated microwells alongside positive and negative controls. Following incubation at 37°C and washing, enzyme conjugate, substrate, and stop solution were sequentially added. Absorbance was measured at 450 nm with a 630 nm reference filter. Results were interpreted using a calculated cut-off (negative control + 0.350), O.D. ratio, and IgM units (O.D. ratio × 10), categorised as negative (<9), equivocal (9–11), or positive (>11).

Participant characteristics, clinical information, and test results were recorded using a structured proforma. Data were analysed using SPSS version 23.0. Descriptive statistics were expressed as mean, median, standard deviation, frequency, or percentage, as appropriate. Graphical summaries were generated where applicable. Sensitivity, specificity, positive predictive value, and negative predictive value of the ICT were calculated using IgM ELISA as the reference standard, as the immunofluorescence assay was not available at the study site. A p-value <0.05 was considered statistically significant.

RESULTS

Participants aged ≤25 years constituted the largest subgroup (113; 37.5%), followed by those aged 26–50 years (99; 32.9%). With respect to gender

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distribution, the sample comprised 163 males (54.2%) and 138 females (45.8%) (Table 1). Samples were collected from the patients of different units of the hospital. The highest proportion was from the Medicine ward (96 individuals), followed by the Casualty ward (88 individuals) and the Paediatrics ward (52 individuals). ICU admissions accounted for 27 participants, while 23 were recruited from the general OPD. Smaller numbers were drawn from specialty areas, including Medicine OPD (6), Surgery ward (4), Paediatrics OPD (2), PMR ward (2), and the Chest ward (1).

Participants reported a range of clinical symptoms at presentation, with multiple responses recorded per individual. Acute febrile illness was the most frequently reported symptom (301), followed by malaise (236) and body ache (211). Less common symptoms included vomiting (21), headache (7), rash (7), and lymphadenopathy (5). A smaller number of participants presented with cough (4), confusion and restlessness (3), chills (2), loss of consciousness (2), breathlessness (2), and septic shock (2). Rarely reported symptoms included anorexia (1), pancytopenia (1), and diarrhoea (1). This pattern reflects the predominance of non-specific febrile and constitutional symptoms in the study cohort. Eschar was present in eight participants.

Among the 301 samples analysed, 84 were confirmed positive for scrub typhus by the reference IgM ELISA, indicating a prevalence of 27.9%. A total of 84 scrub typhus cases were identified. Age demonstrated a statistically significant association with scrub typhus positivity ($p = 0.039$). Scrub typhus positivity was significantly ($p = 0.014$) higher among males (33.7%) compared with females (21.0%) [Table 2].

The performance of both tests is presented in Table 3. Of the 301 cases examined, the ICT test identified 86 as positive, whereas the ELISA test identified 84 as positive. Conversely, the ICT test identified 215 cases as negative, while the ELISA test identified 217 as negative.

The diagnostic metrics reveal the strengths and limitations of the ICT test in detecting scrub typhus. With a sensitivity of 98.8% (95% CI: 93.54%-99.97%), the test effectively identified cases positive for scrub typhus. Its specificity was 98.6% (95% CI: 96.01%-99.7%). The positive predictive value was 96.51% (95% CI: 89.99%-98.84%), while the negative predictive value was 99.53% (95% CI: 96.83%-99.93%). The overall accuracy of 98.67% (95% CI: 96.63%-99.64%) indicates substantial diagnostic reliability. These findings suggest that the rapid ICT kit test is highly sensitive and specific for diagnosing scrub typhus.

Table 1: Basic characteristics of the participants (n=301)

Variables	Sub-group	Frequency	Percentage
Age	**≤25**	113	37.5%
	26-50	99	32.9%
	51-75	79	26.2%
	>75	10	3.3%
Gender	**Male**	163	54.2%
	Female	138	45.8%

Table 2: Association of age and gender with scrub typhus infection

Variables	Scrub typhus		p-value[#]
	Positive	Negative	
Age			
≤25	22 (31.5%)	91 (81.5%)	0.039*
26-50	32 (27.6%)	67 (71.4%)	
>50	30 (24.8%)	59 (64.2%)	
Gender			
Male	55 (33.7%)	108 (66.3%)	0.014*
Female	29 (21.0%)	109 (79.0%)	

*[#]Chi-square, * Statistically significant*

Table 3: Performance of rapid ICT and ELISA for diagnosis of scrub typhus

ICT Test	ELISA test		Total
	Positive	Negative	
Positive	83	03	86
Negative	1	214	215
Total	84	217	301

DISCUSSION

Scrub typhus remains an overlooked tropical disease despite its increasing prevalence across Asia, including India. It causes significant morbidity and mortality, particularly in rural areas lacking timely diagnosis and treatment. Climate change, urbanization, and human contact with mite-infested regions increase disease risk.[3,13] Prompt diagnosis is crucial as delays can cause severe complications. While PCR, ELISA, and IFA offer high sensitivity and specificity, they require sophisticated equipment and longer processing times.[1] Rapid diagnostic

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tests, like ICT enable quicker diagnosis with minimal infrastructure, though their accuracy varies across brands and populations.[9,11,12,14] This study assessed scrub typhus prevalence in acute febrile patients at RIMS hospital and evaluated ICT's diagnostic performance using ELISA as reference.

In the present study, the prevalence of scrub typhus was 27.9% among the patients evaluated. Reported prevalence estimates from different regions of India vary substantially. Studies from Karnataka and Andhra Pradesh have documented higher seroprevalence levels of approximately 30% and 39%, respectively.[15,16] In contrast, lower prevalence has been reported from West Bengal (12.09%), Tamil Nadu (10.4%), and Meghalaya (13.9%).[17-19] Research conducted in rural parts of Tamil Nadu has documented a seroprevalence of around 25%, with particularly elevated rates observed in forested and hilly regions.[20] Collectively, these findings underscore the considerable geographic variability in scrub typhus burden across India, with the prevalence in the current study falling within the mid-range of reported estimates.

In our study, age showed a statistically significant association with scrub typhus positivity ($p = 0.039$), with the highest proportion of infection observed among participants aged ≤25 years (31.5%). Although the age distribution of cases varies across studies from different regions, younger adults are consistently reported to be more affected.[15,17,19,21–25] This pattern is likely attributable to greater exposure to outdoor and agricultural environments—settings that facilitate contact with infected chiggers—among individuals in this age group. Scrub typhus positivity was significantly higher in males (33.7%) than in females (21.0%) ($p = 0.014$). The predominance of infection among men in many settings is often attributed to their greater engagement in outdoor occupations, including agricultural work, which increases the likelihood of exposure to vector-habitat areas where trombiculid mites are prevalent.[15,17,21,23] However, findings from several studies indicate that this pattern may shift in rural regions, where women frequently undertake farming and other outdoor tasks, particularly in communities where men migrate to urban areas for employment. These contextual differences highlight how gendered roles and occupational exposure influence the distribution of scrub typhus across populations.[22,24,26]

In the present study, the rapid ICT kit demonstrated excellent diagnostic accuracy for scrub typhus, with a sensitivity of 98.8%, specificity of 98.6%, PPV of 96.51%, and NPV of 99.53%, indicating strong agreement with the IgM ELISA reference standard. Findings from previous studies show considerable variation in ICT performance. Pote et al. reported very high specificity (100%) but markedly lower sensitivity (38%), while Bhise et al. documented both high sensitivity (98.57%) and good specificity (92.31%). 27,28 Mohan et al. observed a sensitivity of 84.2%, and Acharya et al. also noted fluctuating diagnostic values across different ICT kits.[14,29] Comparative evaluation of three RDTs by Acharya et al. showed that Athenese Dx-RDT provided the most reliable IgM detection, with sensitivity of 96.8% and specificity of 98.29%. J. Mitra-RDT showed high sensitivity (89.6%) but lower specificity (84.0%), and SD Biosensor-RDT demonstrated balanced but comparatively lower performance. For IgG detection, Athenese Dx-RDT again showed the highest sensitivity (74.47%), whereas SD Biosensor-RDT exhibited the greatest specificity (94.47%) but at the cost of reduced sensitivity (51.06%).[14] Comparable findings were reported by Ramalingam et al., who documented strong diagnostic performance of an immunochromatographic rapid test. Their study demonstrated a sensitivity of 93.55% (95% CI: 78.58%–99.21%) and a specificity of 99.38% (95% CI: 96.59%–99.98%). The test also achieved a PPV of 96.67% (95% CI: 80.40%–99.51%) and an NPV of 98.77% (95% CI: 95.44%–99.67%), resulting in an overall diagnostic accuracy of 98.44% (95% CI: 95.50%–99.68%).[30] These values align closely with the high performance observed in our study. Comparing with earlier work by Blacksell et al., ICT assays generally showed good diagnostic performance.[11,12] Though variability across studies likely reflects differences in the specific kits evaluated, population characteristics, and circulating strains. Overall, the ICT kit evaluated in this study demonstrated diagnostic performance that is comparable to, and in some cases exceeds, that reported for other rapid tests. These results support its utility as a reliable tool for early detection of scrub typhus, particularly in settings where access to advanced diagnostics is limited. Further assessment in varied demographic and epidemiological contexts, alongside comparisons with molecular assays, would help strengthen the evidence for its broader application.

This study has several limitations. The associations observed may mirror demographic patterns within the cohort, which limits the generalizability beyond this institution-based population. Diagnostic variability is still possible, as current tests might miss atypical cases or emerging strains, and symptom overlap with other febrile illnesses can complicate clinical interpretation. Additionally, the time interval between sample collections for different tests was not considered, and potential biases related to sample size, data collection, and test interpretation may still influence the findings. Broader, multi-center evaluations are necessary to strengthen and validate these results.

CONCLUSION

The study reveals a significant burden of scrub typhus among patients with acute undifferentiated fever in a tertiary care setting in Imphal, with

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younger individuals and males being more frequently affected. The immunochromatographic test evaluated demonstrated excellent diagnostic performance when compared to the reference standard, indicating high diagnostic reliability. These findings underscore the value of this rapid test as a practical tool for early detection, particularly in settings where access to advanced diagnostics is limited. By integrating these insights, future efforts can more effectively address diagnostic challenges and optimize patient outcomes, tailoring approaches to identified high-risk groups and leveraging precise testing methodologies.

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