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A Cross-Sectional Study in Northeast India to Assess Malaria, Scrub Typhus, and Dengue in Patients with Acute Febrile Illness Using ELISA

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Research Article

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Abstract

Aim

Acute febrile illness (AFI) is a major reason for hospitalization in tropical and subtropical regions. Overlapping clinical features might also lead to confusion about the diagnosis of AFI. In India, dengue, scrub typhus, and malaria continue to be the most prevalent causes. Early laboratory validation using ELISA-based assays is essential for timely medication and a reduction in morbidity and death.

Methods

Using commercial ELISA kits to detect dengue NS1 antigen, dengue IgM antibodies, scrub typhus IgM antibodies, and malaria pLDH antigen in accordance with manufacturer instructions, a cross-sectional study of 200 patients presenting with AFI—95 urban and 105 rural residents—was carried out. Data on demographics, residential status, and length of fever were recorded, and statistical analysis was carried out using PAST software.

Result

The results showed that 51% of the patients were male and 49% were female. Patients from rural areas made up a higher proportion (53%) than those from metropolitan areas (47%). 16% of patients in cities were positive for dengue NS1, 7% for dengue IgM, 12% for scrub typhus, and 17% for malaria. Dengue (18%), scrub typhus (19%), and malaria (22%) were more prevalent among rural people. Longer fevers, particularly those lasting between 6 and 14 days, were related to greater positives. Malaria and scrub typhus were the leading causes of AFI.

Conclusion

ELISA-based diagnostics are useful for early detection of AFI etiology. Higher rural incidence highlights the importance of improved monitoring, early diagnostic access, and focused public health interventions.

Introduction

Dengue and scrub typhus have emerged as substantial contributors to non-malarial febrile diseases, especially in South and Southeast Asia [8]. Acute febrile illness (AFI) is a prominent cause of hospital attendance in tropical and subtropical countries. Four antigenically different dengue virus serotypes cause dengue, a mosquito-borne viral infection that continues to pose a serious threat to public health. Early clinical symptoms of dengue frequently overlap with those of other feverish illnesses, making

prompt identification and treatment more difficult [1,3]. Orientia tsutsugamushi is the source of scrub typhus, an underappreciated but potentially fatal rickettsial illness that often manifests as undifferentiated fever in its early stages and is increasingly being described as a co-endemic infection alongside dengue in India [8,9]. A prompt and accurate test diagnosis is crucial during the first 0–6 days of illness because improper or delayed treatment might result in serious consequences, longer hospital stays, and higher mortality [8,9]. Due to its applicability, scalability, and appropriateness for routine diagnostic labs in resource-constrained settings, serological and antigen-based assays—in particular, enzyme-linked immunosorbent assay (ELISA)—have become more popular [10,11]. It has been demonstrated that utilizing ELISA to identify the non-structural protein 1 (NS1) antigen for dengue diagnosis offers good sensitivity and specificity throughout the early viraemic phase, even before detectable antibodies emerge [1, 3].

Commercially available dengue NS1 ELISA tests are dependable instruments for early dengue diagnosis, providing consistent performance across various epidemiological scenarios, according to multi-country and clinical assessments [1,3]. The diagnostic and epidemiological relevance of serotyping procedures in acute dengue infections has increased due to advancements in NS1-based ELISA techniques [2]. The IgG-to-IgM ratio is a significant immunological marker in the early stages of the disease, and antibody-based ELISA techniques are essential for distinguishing between primary and secondary dengue infections in addition to antigen detection [5,6].

Several studies have found that secondary dengue infections are linked with greater IgG responses early in the disease, which may be efficiently measured using ELISA-based techniques [4–6]. Similarly, ELISA-based detection of IgM antibodies against Orientia tsutsugamushi has emerged as a cornerstone for scrub typhus diagnosis, with comparable sensitivity to molecular techniques in the early feverish phase [7, 9].

Comparative studies of molecular and serological assays show that, whereas nucleic acid amplification methods have great specificity, ELISA is still more practical for early screening and large-scale testing in endemic locations [9,11]. Despite ELISA's demonstrated effectiveness in individual disease diagnosis, few studies have focused on its combination application for simultaneous early detection of dengue and scrub typhus in patients presenting with AFI within the first week of symptom onset [8]. Given the comparable clinical presentations, common endemicity, and unique treatment implications of dengue and scrub typhus, there is an urgent need to test ELISA-based diagnostic techniques for early and reliable distinction [8, 9]. As a result, this study intends to investigate the relevance of ELISA-based antigen and antibody detection in the early identification of dengue. scrub typhus in patients with acute fever (0–6 days), offering data to support prompt clinical decision-making in endemic environments [1,11].

Materials and Methodology

Study design and setting

Over the course of a predefined research period, this cross-sectional research study was carried out in a tertiary care teaching hospital. Patients from both the outpatient and inpatient departments were recruited if they had an acute febrile illness lasting 0–6 days. The Institutional Ethics Committee (4311/2022) authorized the study, and each participant or their legal guardian provided signed informed permission.

Sample Size and sampling technique

The study adopted a consecutive sampling strategy. All eligible patients presenting with acute febrile illness who satisfied the established inclusion criteria were included sequentially until the needed sample size was reached. This non-probability sampling strategy guaranteed thorough coverage of the instances encountered throughout the research period while minimizing selection bias. The sample size was 200 people with acute febrile sickness. A standardized proforma was used to collect demographic information about each participant, such as age, gender, and location of residence (rural or urban)[4,12].

Sample Collection and Processing

Approximately 3–5 mL of venous blood was obtained aseptically from each subject using universal biosafety measures. Blood samples were allowed to coagulate before centrifugation at 3000 rpm for 10 minutes to separate serum. Serum samples were held at 2–8°C and examined within 24 hours, or kept at -20°C until analysis[10,11,13].

Procedure

The immunoassay techniques for the laboratory diagnosis of acute febrile diseases, such as dengue, scrub typhus, and malaria, are compiled in Figue 01. Dengue IgM ELISA was employed for patients who presented after six days of fever, while dengue NS1 antigen detection was carried out during the early stages of disease (0–6 days). An indirect ELISA based on recombinant Orientia tsutsugamushi antigens was used to detect scrub typhus IgM, while a sandwich ELISA was used to detect Plasmodium pLDH antigen to identify malaria infection. The assay type, underlying concept, important methodological stages, and the corresponding cut-off values or formulae utilized for result interpretation are all described in the Fig. 01.

Figure 01

: **Procedure of ELISA test of selected parameter**

Quality Control and Test Validity

Each ELISA run comprised positive and negative controls included in the test kits. The assay's validity was confirmed by ensuring that the control optical density readings were within the permissible ranges stated by the manufacturer. Assays that did not fulfil validity standards were rerun[10,11].

Statistical analysis

PAST (PAleontological STatistics) software was used to statistically analyse the gathered data. To produce the study findings, tables, and figures needed for interpretation and presentation, all data were input, processed, and analysed within the program.

Result

The study involved 200 individuals with acute febrile sickness. Of them, 103 (51%) were men and 97 (49%) were women, indicating a virtually equal gender distribution. According to their place of residence, 95 (47%) patients lived in cities, while 105 (53%) lived in villages. Among urban participants, 43 had fever during 0–5 days, whereas 140 reported fever for 6–14 days. Rural participants, on the other hand, had a larger burden, with 81 cases in the 0–5 day group and 236 cases in the 6–14 day fever group, showing that rural residents sought healthcare later.

Serological Characteristics of the Urban Population (n = 95)

In the urban group, Dengue NS1 antigen positivity was observed in 15 patients (16%), while 80 patients (84%) tested negative. Dengue IgM MAC ELISA was positive in 7 cases (7%) and negative in 88 cases (93%). Scrub typhus IgM antibodies were detected in 11 patients (12%), whereas 84 patients (88%) were negative. Malaria antigen positivity was found in 16 patients (17%), with 79 patients (83%) testing negative.

Serological Characteristics of the Rural Population (n = 105)

Among rural individuals, Dengue serology (NS1/IgG/IgM) was positive in 19 (18%) and negative in 86 (82%). Dengue IgM MAC ELISA testing after 14 days of fever revealed positive in 14 patients (13%), whereas 91 patients (87%) were negative. Scrub typhus IgM positive was detected in 20 individuals (19%), while 85 patients (81%) tested negative. Malaria antigen positive was greatest in rural cases, with 23 (22%), whereas 82 (78%) were negative (Table 01).

Table 01
The result of screening as per ethnicity

Result	Urban (n-95)			
	Dengue NS1 Ag ELISA	**Dengue Mac ELISA**	**Scrub typhus ELISA**	**Malaria parasite ELISA**
Positive	15 (16%)	7 (4%)	10 (5%)	14 (7%)
Negative	62 (34%)	133 (73%)	133 (73%)	133 (73%)
Rural (n-105)				
Positive	19 (6%)	14 (4%)	18 (6%)	23 (7%)
Negative	56 (18%)	220 (70%)	216 (68%)	215 (68%)

Statistics: PAST software was used to conduct statistical analyses. Categorical variables were reported using frequencies and percentages. The Chi-square test was used to assess the relationship between urban and rural distribution, gender, fever duration, and diagnostic positive for dengue, scrub typhus, and malaria. All analyses were judged statistically significant with a p-value of < 0.05.

Discussion

AFI remains a major diagnostic issue in tropical and subtropical countries because to the clinical overlap of dengue, scrub typhus, and malaria. Early etiological diagnosis is crucial for lowering morbidity, avoiding complications, and guiding suitable treatment measures. The current study examined the role of ELISA-based diagnostic tests in the early identification of dengue virus infection, scrub typhus, and malaria in both urban and rural populations.

In this study, dengue NS1 antigen positive was greater during the early febrile phase, demonstrating the efficacy of NS1 antigen detection within the first week of illness. Several investigations have shown that the NS1 antigen may be identified on day one of fever and is detectable until day nine, making it an important early diagnostic marker before antibody seroconversion occurs [1,3,12]. NS1 ELISA assays have been evaluated in many countries and shown to be highly sensitive and specific, particularly in initial dengue infections [1]. The current study's findings are consistent with earlier publications, highlighting the value of NS1 ELISA as an early diagnostic tool.

Patients who presented after six days of fever were more likely to have dengue IgM MAC-ELISA positive, which is consistent with the predicted pattern of immune response. IgM antibodies usually start to show up on the third or fifth day of the illness and reach their peak in the second week [1,5,6]. When paired with IgG measurements, prior research has highlighted the significance of IgM-based tests for detecting dengue in the later stages of illness and for distinguishing between main and secondary dengue [5,6]. The found IgM positivity supports current diagnostic criteria advised by WHO, even though IgG serotyping was not the main goal of this investigation [12].

A considerable percentage of AFI cases were caused by scrub typhus, especially in rural individuals. This finding is consistent with previous research from India that found scrub typhus to be a significant but underdiagnosed source of feverish illnesses other than malaria [8,15]. According to research showing that ELISA performs better than conventional Weil-Felix testing, the use of IgM ELISA for scrub typhus diagnosis showed dependable identification throughout the acute phase [7,9]. Agricultural methods, delayed access to treatment, and more exposure to vector habitats might all contribute to the higher positivity in rural areas [4,5].

Rural people had greater positive rates for malaria antigen detection using Malaria Ag Microlisa (pLDH-based sandwich ELISA), which is consistent with endemic transmission patterns in settings with inadequate resources. When compared to microscopy, ELISA-based malaria tests offer higher sensitivity, especially in instances with low parasitaemia [16]. The results demonstrate the ongoing need of malaria screening in AFI, especially in areas where scrub typhus and dengue are becoming more well known [8,10,11].

Dengue, scrub typhus, and malaria positive rates were consistently higher in rural groups, according to the comparative urban–rural research. Previous research has demonstrated similar epidemiological patterns, highlighting the impact of socioeconomic, environmental, and healthcare accessible determinants on disease burden [8]. The need for improved early diagnostic services in peripheral healthcare settings is further highlighted by the longer fever duration and delayed presentation seen in rural patients [5,8].

Overall, the current study shows that ELISA-based diagnostic tests are practical, dependable, and efficient instruments for the early etiological identification of AFI. Their use decreases unnecessary empirical therapy and facilitates prompt clinical decision-making. The results corroborate WHO guidelines that promote laboratory-based confirmation of feverish diseases in endemic areas [12].

Conclusion

The study emphasizes the substantial burden of acute febrile diseases in both urban and rural populations, with malaria, dengue fever, and scrub typhus recognized as primary causes. Higher positive rates were seen in rural areas, indicating the spatial diversity in illness distribution. Early ELISA-based diagnosis has shown useful for prompt identification, proper clinical care, and effective public health surveillance.

Abbreviations

AFI
Acute Febrile Illness
ELISA
Enzyme–Linked Immunosorbent Assay

NS1
Non–Structural Protein 1
IgM
Immunoglobulin M
IgG
Immunoglobulin G
pLDH
Plasmodium Lactate Dehydrogenase
OD
Optical Density
WHO
World Health Organization

Declarations

Author contributions: Jayanta Deb helped with the study design, sample collection, laboratory investigations, data compilation, and paper preparation. Saurabh Gupta contributed to the study design, laboratory technique supervision, data analysis, and manuscript critical review. Tapan Majumdar gave methodological direction, result interpretation, and text critical evaluation. All authors reviewed and approved the final article.

Conflicts of Interest: The authors declare that they have no conflicts of interest relevant to this study.

Ethical approval: The Institutional Ethics Committee of Agartala Government Medical College (AGMC) accepted this study, which was carried out in compliance with institutional rules (Approval No.: 4311/2022).

Consent to participate: Every individual participant in the study gave their informed consent.

Consent to publication: Not applicable

Availability of data and materials: Any qualified researcher will have unrestricted access to the raw data used to support the conclusions in this manuscript.

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References

1. Guzman MG, Jaenisch T, Gaczkowski R, Ty Hang VT, Sekaran SD, Kroeger A, Vazquez S, Ruiz D, Martinez E, Mercado JC, Balmaseda A. Multi-country evaluation of the sensitivity and specificity of two commercially-available NS1 ELISA assays for dengue diagnosis. PLoS Negl Trop Dis. 2010;4(8):e811.

2. Prommool T, et al. High performance dengue virus antigen-based serotyping-NS1-ELISA (plus): A simple alternative approach to identify dengue virus serotypes in acute dengue specimens. PLoS Negl Trop Dis. 2021;15(2):e0009065.
3. Pal S, Dauner AL, Mitra I, Forshey BM, Garcia P, Morrison AC, Halsey ES, Kochel TJ, Wu SJ. Evaluation of dengue NS1 antigen rapid tests and ELISA kits using clinical samples. PLoS ONE. 2014;9(11):e113411.
4. Nascimento EJ, George JK, Velasco M, Bonaparte MI, Zheng L, DiazGranados CA, Marques ET, Huleatt JW. Development of an anti-dengue NS1 IgG ELISA to evaluate exposure to dengue virus. J Virol Methods. 2018;257:48–57.
5. Cucunawangsih LNP, Kurniawan A, Immunoglobulin. G (IgG) to IgM ratio in secondary adult dengue infection using samples from early days of symptoms onset. BMC Infect Dis. 2015;15(1):276.
6. Changal KH, Raina AH, Raina A, Raina M, Bashir R, Latief M, Mir T, Changal QH. Differentiating secondary from primary dengue using IgG to IgM ratio in early dengue: an observational hospital based clinico-serological study from North India. BMC Infect Dis. 2016;16(1):715.
7. Jayagandan S, Ratchagadasse VR, Philomenadin FS, Dhodapkar R. Evaluation of diagnostic accuracy of the novel Scrub Typhus IgM ELISA Kit. Diagnostic Microbiology and Infectious Disease. 2025 Jul 24:117025.
8. Rao PN, Van Eijk AM, Choubey S, Ali SZ, Dash A, Barla P, Oraon RR, Patel G, Nandini P, Acharya S, Mohanty S. Dengue, chikungunya, and scrub typhus are important etiologies of non-malarial febrile illness in Rourkela, Odisha, India. BMC Infect Dis. 2019;19(1):572.
9. Kannan K, John R, Kundu D, Dayanand D, Abhilash KP, Mathuram AJ, Zachariah A, Sathyendra S, Hansdak SG, Abraham OC, Gunasekaran K. Performance of molecular and serologic tests for the diagnosis of scrub typhus. PLoS Negl Trop Dis. 2020;14(11):e0008747.
10. Shah K, Maghsoudlou P. Enzyme-linked immunosorbent assay (ELISA): the basics. Br J Hosp Med. 2016;77(7):C98–101.
11. Hayrapetyan H, Tran T, Tellez-Corrales E, Madiraju C. Enzyme-linked immunosorbent assay: types and applications. ELISA: methods and protocols. 2023 Feb 17:1–7.
12. World Health Organization review on 23 April. 2024;Dengue and severe dengue website: - https://www.who.int/news-room/fact-sheets/detail/dengue-and-severe-dengue
13. The official website J, Mitra. & Co. Pvt. Ltd; Dengue IgM Test kit (ELISA) accessed on 23.12.2025 at 10.30am. Website: - https://jmitra.co.in/product-details/dengue-igm-elisa-test-kit/
14. The official website J, Mitra. & Co. Pvt. Ltd;Scrub Typhus ELISA Test Kit accessed on 23.12.2025 at 10.30am.Website:- https://jmitra.co.in/product-details/scrub-typhus-igm-microlisa/
15. Singh OB, Panda PK. (2022). Scrub Typhus. Stat Pearls Publishing. access on 23.12.2025 at 10.10 am.
16. The official website J, Mitra. & Co. Pvt. Ltd; Malaria Ag ELISA Test Kit accessed on 23.12.2025 at 10.30am.Website:-https://jmitra.co.in/wpcontent/uploads/2021/10/manual-Malaria-Ag-Microlisa.pdf

Figures

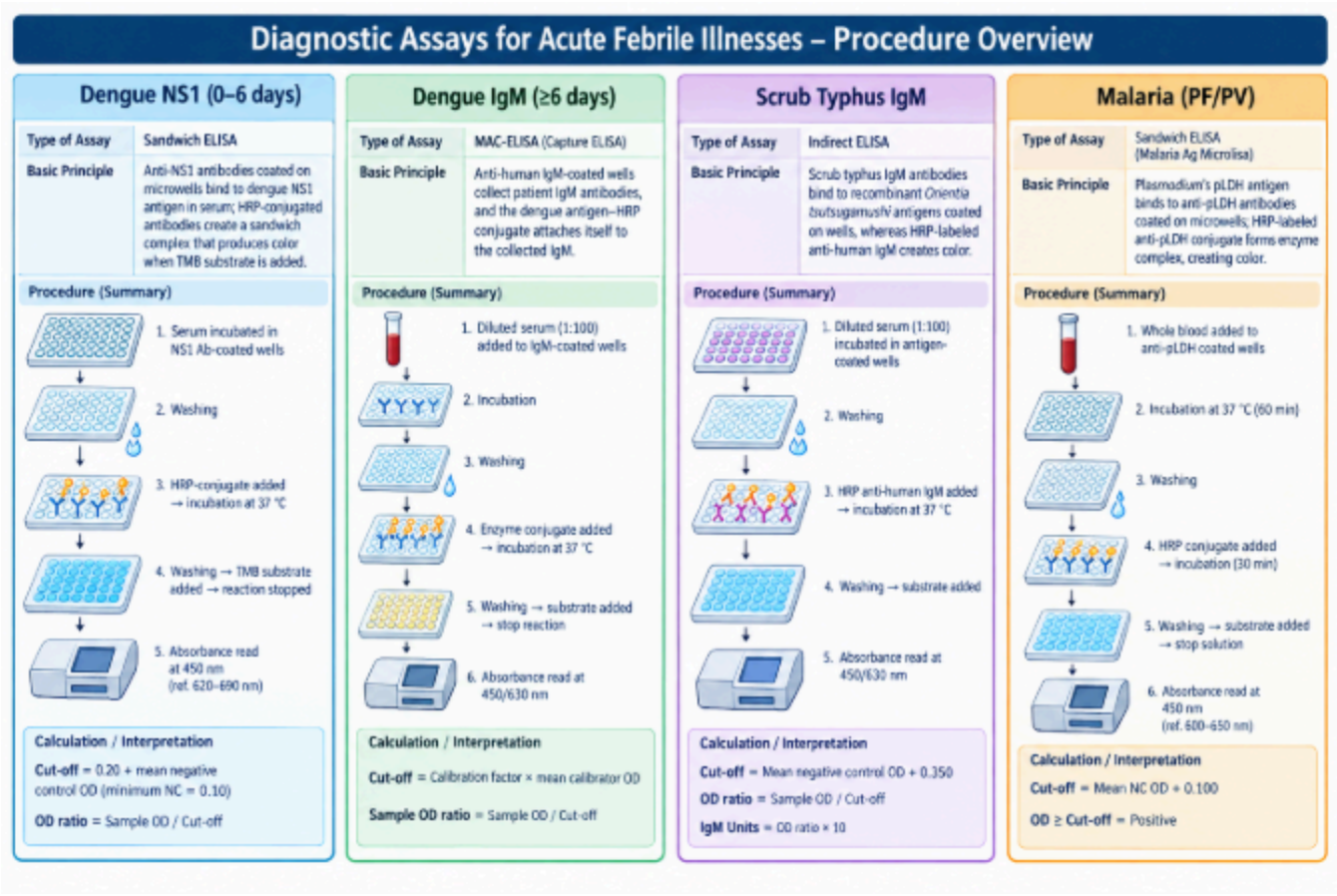


Figure 1

Procedure of ELISA test of selected parameter