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Original Research Article

Comparative Evaluation of Rapid Diagnostic Tests and ELISA for Early Detection of Dengue in Febrile Patients

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Conflict of interest: Nil

Abstract:

Background: Dengue is a mosquito-borne viral infection causing significant illness in tropical regions. Early and accurate diagnosis during the febrile phase is essential to guide treatment, prevent complications, and support outbreak control. Rapid Diagnostic Tests (RDTs) and ELISA are widely used, but their comparative accuracy in the early phase needs evaluation.

Objective: To compare the diagnostic accuracy of Rapid ICT assays with ELISA for detecting dengue NS1 antigen, IgM, and IgG in patients presenting within five days of fever.

Methodology: A cross-sectional study was conducted in the Department of Microbiology, Nalanda Medical College and Hospital, Patna, India. A total of 120 clinically suspected dengue cases were enrolled. Blood samples were collected, serum separated and tested using commercial Rapid ICT kits and ELISA for NS1, IgM, and IgG. Diagnostic performance was assessed using sensitivity, specificity, PPV, NPV, and Kappa agreement.

Results: Rapid ICT showed high sensitivity (83.3%) and specificity (86.7%) for NS1 detection. IgM demonstrated moderate sensitivity (70%) and specificity (76.7%), while IgG showed good accuracy (78.6% sensitivity, 80% specificity). Substantial agreement with ELISA was recorded for all markers (Kappa 0.68–0.72). Rapid ICT provided fast and cost-effective results suitable for field and resource-limited settings.

Conclusion: Rapid ICT assays offer reliable and timely detection of dengue during the early febrile phase. While NS1 results are highly accurate, IgM and IgG may require ELISA confirmation. Combining both methods strengthens diagnostic accuracy and supports effective clinical and public health decisions.

Keywords: Dengue; Early Febrile Phase; ELISA; IgG; IgM; NS1 Antigen; Rapid Diagnostic Test; Rapid ICT.

Introduction

Dengue fever is one of the most significant mosquito-borne viral infections affecting humans, particularly in tropical and subtropical regions [1]. Transmitted primarily by Aedes aegypti and Aedes albopictus, the disease has become a major global public health concern due to increasing urbanization, climate change, population mobility, and expanding vector habitats [2]. According to the World Health Organization, dengue incidence has risen dramatically in recent decades, with millions of infections reported annually and frequent outbreaks causing substantial morbidity, mortality, and economic burden. Early and accurate diagnosis is therefore critical to guide clinical management, prevent complications such as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS), and support timely public health interventions [3].

The early febrile phase, which typically spans the first 1–5 days of illness, presents a diagnostic challenge because clinical symptoms such as high fever, headache, myalgia, retro-orbital pain, and rash often

overlap with other acute febrile illnesses like chikungunya, malaria, influenza, and leptospirosis [4]. Laboratory confirmation is essential to differentiate dengue from these conditions. During this early window, viral replication is at its peak, and specific biomarkers can be detected before the onset of antibodies. Hence, choosing an appropriate diagnostic test during this phase is crucial for accurate and rapid identification of dengue infection [5].

Two major categories of laboratory tests are commonly used for dengue diagnosis: Rapid Diagnostic Tests (RDTs) and Enzyme-Linked Immunosorbent Assays (ELISA). Rapid tests, also known as point-of-care immunochromatographic assays, are widely used due to their simplicity, low cost, quick turnaround time, and minimal equipment requirement. Most RDTs detect dengue NS1 antigen and IgM/IgG antibodies [6]. The NS1 antigen is a nonstructural glycoprotein secreted by the dengue virus during the early phase of infection, making it a valuable marker for early detection. Because RDTs can be performed in peripheral or resource-limited settings without specialized laboratory infrastructure, they play an important role in screening large populations, especially during outbreaks [7].

Given these factors, there is a critical need to evaluate and compare the performance of RDTs and ELISA specifically during the early febrile phase, when rapid diagnosis can significantly influence patient outcomes [8]. Understanding their respective advantages, limitations, and diagnostic accuracy will help guide clinicians and public health professionals in selecting the most appropriate diagnostic approach. This comparison also provides insights into improving diagnostic algorithms, enhancing outbreak response, and reducing the risk of severe disease progression [9].

Therefore, the present study aims to compare the effectiveness, sensitivity, and specificity of rapid tests versus ELISA in detecting dengue infection during the early febrile phase. By assessing their diagnostic reliability within this crucial time window, the study seeks to support evidence-based decision-making to improve dengue case detection and patient management.

Methodology

Study Design: This study adopted a hospital-based, cross-sectional comparative design to evaluate the diagnostic performance of Rapid Diagnostic Tests (RDTs) against Enzyme-Linked Immunosorbent Assay (ELISA) for early detection of dengue infection during the acute febrile phase (Day 1–5 of illness).

Study Setting and Area: This study was conducted in the Department of Microbiology, Nalanda Medical College and Hospital (NMCH), Patna, Bihar, India for 7 months from March 2025 to Sept 2025.

Study Population: The study population consisted of clinically suspected dengue patients presenting with acute fever (≤5 days) to the hospital's outpatient and inpatient departments. Patients of both genders and all age groups were assessed.

Selection Criteria

Inclusion Criteria

- Patients presenting with acute febrile illness ≤5 days duration.
- Clinical suspicion of dengue based on WHO criteria (fever with ≥2 symptoms such as headache, retro-orbital pain, myalgia, rash, nausea/vomiting).
- Individuals are willing to provide written informed consent.
- Both male and female patients of all age groups.

Exclusion Criteria

- Fever lasts more than 5 days, as antibody levels significantly vary after Day 5.
- Confirmed diagnosis of malaria, chikungunya, typhoid, or any other co-infection.
- Patients who had received blood transfusion within the last three months.
- Hemolyzed, insufficient, or improperly labeled blood samples.
- Patients unwilling to provide consent.

Sample Size: A total of 120 clinically suspected dengue cases were included in the study using a consecutive sampling approach. The sample size was estimated considering the expected difference in sensitivity between Rapid Diagnostic Tests (RDTs) and ELISA, with a 95% confidence level and adequate precision to compare diagnostic performance.

Sample Collection: Under aseptic precautions, 3–5 mL of venous blood was collected from each participant using a sterile disposable syringe or vacutainer. The blood was transferred into plain tubes and allowed to clot at room temperature for 20–30 minutes, followed by centrifugation at 3000 rpm for 10 minutes to separate the serum. The serum was aliquoted into two portions: one was used immediately for the Rapid Test (NS1/IgM/IgG), and the other was stored at 2–8°C until processing for ELISA. All samples were labeled with a unique identification code and handled according to standard biosafety guidelines.

Diagnostic Tests Performed

- **Rapid Diagnostic Test (RDT)**

A commercially available immunochromatographic (ICT) rapid test kit was used to detect:

- **NS1 antigen**
- **IgM antibody**
- **IgG antibody**

- **Procedure:** Two to three drops of serum were added to the sample well of the test strip, which was then allowed to react for 15–20 minutes at room temperature. Results were interpreted as positive, negative, or invalid according to the manufacturer's guidelines, and the validity of the internal control line was confirmed for each kit.

- **ELISA Testing**

NS1 antigen ELISA and IgM capture ELISA were performed using validated commercial kits.

- **Procedure:** Serum samples, along with controls and standards, were pipetted into microplate wells coated with anti-NS1 or anti-IgM antibodies and incubated at 37°C for 60 minutes. The wells were washed 4–5 times using an automated washer, followed by the addition of enzyme conjugate and a second incubation. After washing, TMB substrate was added and color development was allowed for 10–15 minutes, after which the reaction was stopped using the stop solution. Absorbance was measured at 450 nm using an ELISA plate reader, and cut-off values were calculated according to the manufacturer's instructions.

Data Recording: A structured data collection form was used to record patient demographics, clinical presentation, duration of fever, Rapid Diagnostic Test (NS1/IgM/IgG) results, ELISA (NS1/IgM) results, and the final interpretation for each participant.

Statical Analysis: Data was analyzed using SPSS, R, or MS Excel to evaluate the diagnostic performance of the tests. Key statistical parameters, including sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and overall accuracy, were calculated to assess the reliability of the Rapid Diagnostic Test compared with ELISA. Agreement between the two methods was determined using the Kappa statistics. Descriptive statistics were used to summarize patient demographics and clinical characteristics. A P-value <0.05 was considered statistically significant, indicating a meaningful difference or association between the variables under study.

Result

The present study was conducted on 120 dengue-suspected patients presenting in the early febrile phase at the Department of Microbiology, Nalanda Medical College and Hospital, Patna. The aim was to evaluate the diagnostic performance of Rapid ICT assays compared to standard ELISA for NS1 antigen, IgM, and IgG detection. Patients were assessed for demographic characteristics, clinical symptoms, and laboratory findings, including rapid and ELISA-based serological tests. The study population comprised both males and females across different age groups, with a predominance of young adults, and most patients reported fever of short duration.

The performance of Rapid ICT assays was analyzed in terms of sensitivity, specificity, positive predictive value, negative predictive value, and agreement with ELISA using Kappa statistics. Findings from this study are summarized in Tables 1–5 and Figures 1–5, providing a comprehensive overview of the demographic profile, symptom prevalence, diagnostic accuracy, and agreement between rapid and standard serological methods in early dengue detection.

Table 1: Demographics

Characteristic	Value
Male	72
Female	48
Age <15	20
Age 15–30	50
Age 31–45	30
Age >45	20
Mean age (years)	29.5
Fever duration (days)	3.3

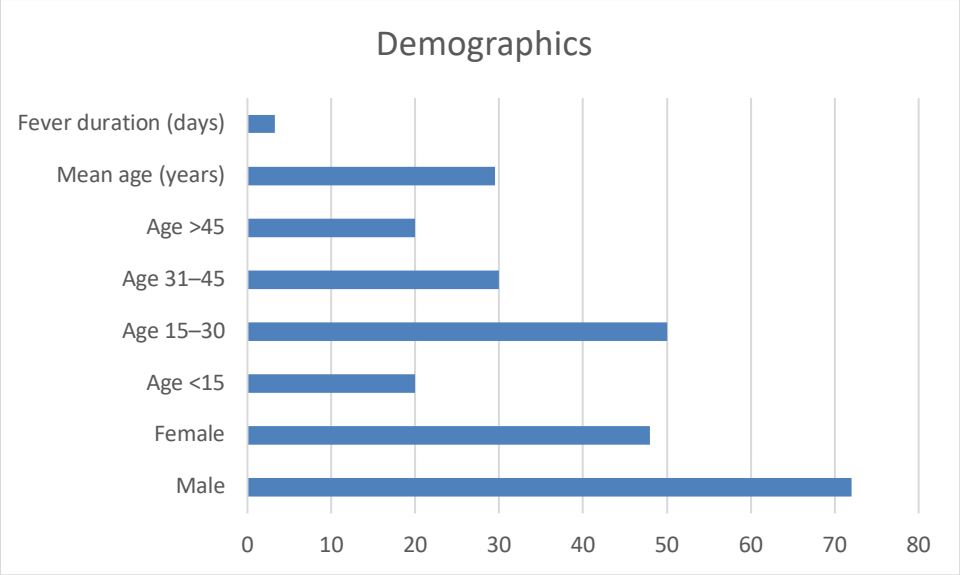

Figure 1: Gender and age distribution of dengue-suspected patients

According to Table 1, the study included 120 dengue-suspected patients in the early febrile phase. Males were slightly more affected (72; 60%) than females (48; 40%). Most patients were young adults aged 15–30 years (41.7%), followed by 31–45 years (25%), <15 years (16.7%), and >45 years (16.7%). The mean age was 29.5 years, and the mean fever duration was 3.3 days, confirming early-phase illness. This demographic profile provides context for interpreting diagnostic test performance across age and sex groups.

Table 2: Rapid ICT vs ELISA for Dengue NS1 Antigen

ELISA NS1	Rapid ICT Positive	Rapid ICT Negative	Total
Positive	50	10	60
Negative	8	52	60
Total	58	62	120

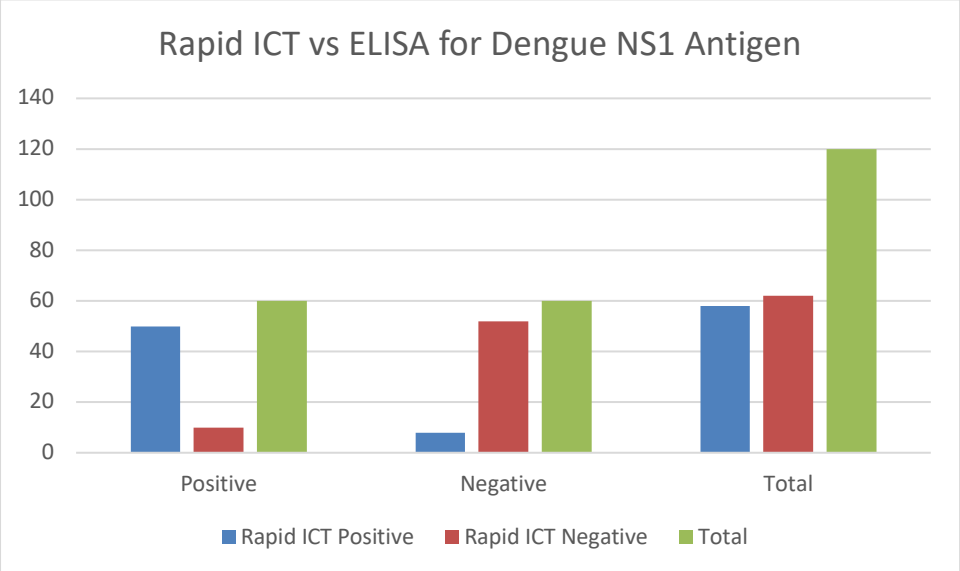

Figure 2: Comparison of Rapid ICT and ELISA for Dengue NS1 antigen detection showing true positives, true negatives, false positives, and false negatives

According to Table 2, the Rapid ICT assay detected 50 out of 60 ELISA-positive NS1 samples, showing a sensitivity of 83.3%. Among ELISA-negative samples, 52 were correctly identified as negative by ICT, yielding a specificity of 86.7%. The positive predictive value (PPV) was 86.2%, and the negative predictive value (NPV) was 83.9%. These results indicate that the Rapid ICT assay provides high

accuracy for early detection of dengue NS1 antigen, making it suitable for rapid screening in the early febrile phase.

Table 3: Rapid ICT vs ELISA for Dengue IgG			
ELISA IgM	**Rapid ICT Positive**	**Rapid ICT Negative**	**Total**
Positive	42	18	60
Negative	14	46	60
Total	56	64	120

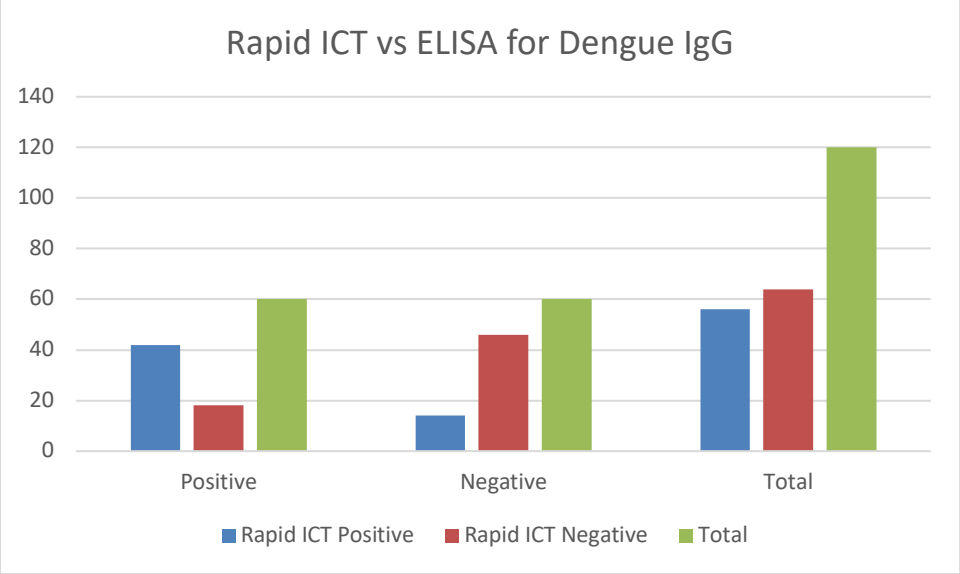

Figure 3: Comparison of Rapid ICT and ELISA for Dengue IgM antibody detection showing true positives, true negatives, false positives, and false negatives.

According to Table 3, the Rapid ICT assay correctly identified 42 out of 60 ELISA-positive IgM samples, resulting in a sensitivity of 70.0%. Among ELISA-negative samples, 46 were correctly identified as negative by ICT, giving a specificity of 76.7%. The positive predictive value (PPV) was 75.0% and the negative predictive value (NPV) was 71.9%. These results indicate that the Rapid ICT assay provides moderate accuracy for IgM detection during the early febrile phase of dengue infection.

Table 4: Overall Diagnostic Accuracy (NS1 + IgM + IgG)					
Marker	**Sensitivity (%)**	**Specificity (%)**	**PPV (%)**	**NPV (%)**	**Accuracy (%)**
NS1	83.3	86.7	86.2	83.9	85
IgM	70	76.7	75	71.9	73.3
IgG	78.6	80	84.6	72.7	79.2

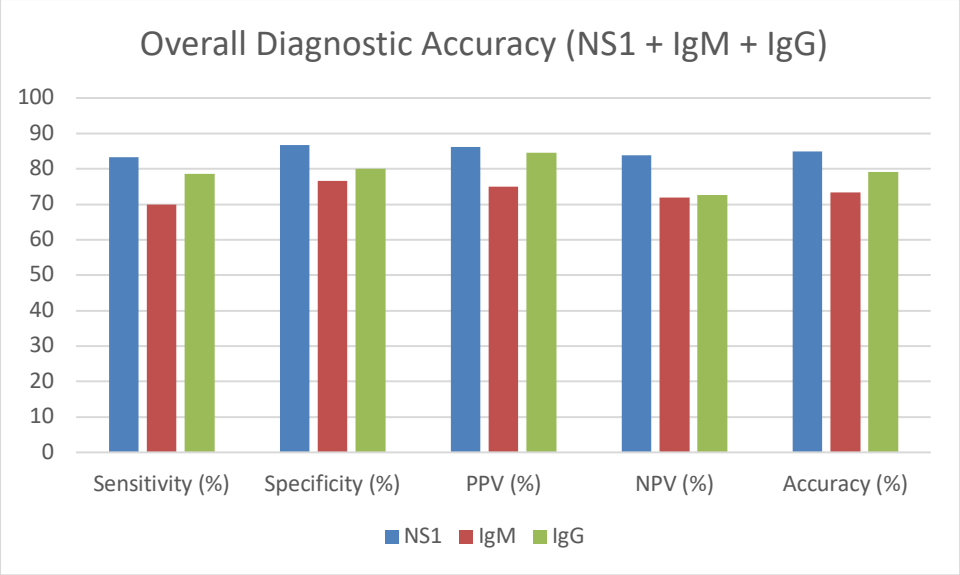

Figure 4: Comparison of Rapid ICT and ELISA for Dengue IgG antibody detection showing true positives, true negatives, false positives, and false negatives.

Table 5: Kappa Agreement Between RDT and ELISA		
Marker	**Kappa Value**	**Agreement Level**
NS1	0.7	Substantial
IgM	0.68	Substantial
IgG	0.72	Substantial

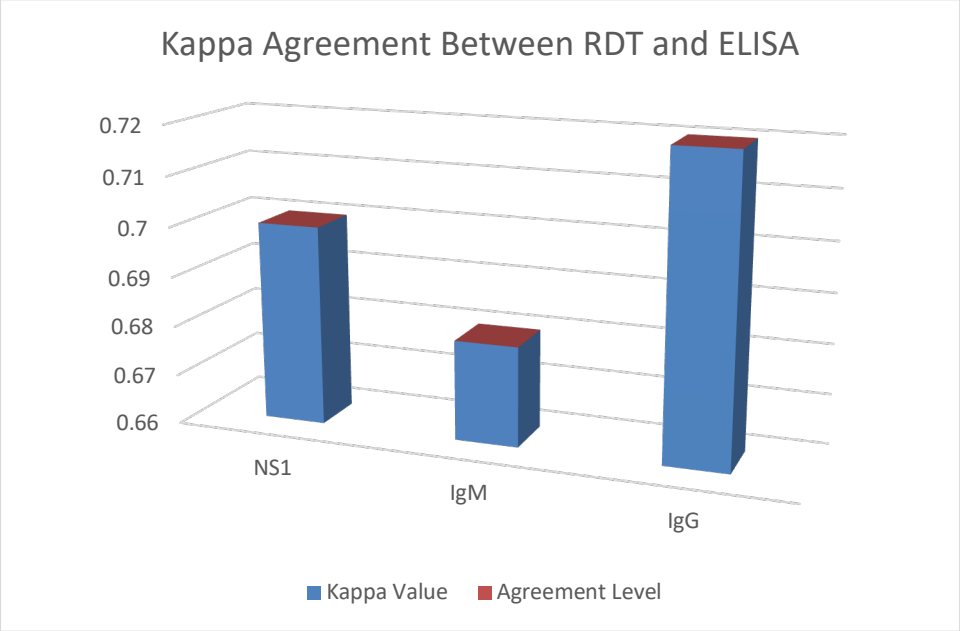

Figure 5: Kappa agreement values between Rapid ICT and ELISA for NS1, IgM, and IgG markers indicating substantial agreement

According to Table 5, the agreement between Rapid ICT assays and ELISA was substantial for all markers. The Kappa values were 0.70 for NS1, 0.68 for IgM, and 0.72 for IgG, indicating good concordance between the rapid and standard diagnostic methods. These results support the reliability of Rapid ICT assays as a diagnostic tool for dengue detection in the early febrile phase.

Discussion

This study assessed the diagnostic performance of Rapid ICT assays compared to standard ELISA for NS1 antigen, IgM, and IgG detection in 120 dengue-suspected patients during the early febrile phase. According to the demographic data, most patients were young adults aged 15–30 years, with a slightly higher prevalence among males. This age and

gender distribution is consistent with patterns observed in regions with high dengue transmission, where younger, active populations are more frequently exposed to the vector. The mean fever duration of 3.3 days confirms that the majority of patients were in the early phase of dengue infection, making the evaluation of early diagnostic markers particularly relevant.

Rapid ICT assays demonstrated high sensitivity (83.3%) and specificity (86.7%) for NS1 antigen detection. Haider et al., (2022) confirms their reliability for early-phase diagnosis, as NS1 antigen is typically detectable within the first 5 days of illness [10]. The high performance of NS1 detection by ICT Kabir et al., (2011) suggested that these assays can serve as effective point-of-care tools for early identification of dengue, allowing timely clinical management and limiting progression to severe disease [11].

For IgM detection, the Rapid ICT assay showed moderate sensitivity (70%) and specificity (76.7%). The moderate sensitivity can be attributed to the fact that IgM antibodies generally appear later in the course of infection, and early sampling may result in false-negative results. Blacksell et al., (2011) highlighted the importance of combining NS1 antigen detection with IgM testing in order to improve diagnostic accuracy, particularly in cases presenting within the first few days of fever [12].

IgG detection by Rapid ICT exhibited slightly higher accuracy than IgM, indicating its utility in identifying secondary infections. Early recognition of secondary dengue infections is clinically significant, as they carry a higher risk of complications, including plasma leakage and hemorrhagic manifestations. Substantial agreement between Rapid ICT and ELISA for all markers, as indicated by Kappa values (0.68–0.72), demonstrates the reliability of rapid tests in comparison with standard laboratory assays.

Zhang et al., (2022) and Hoermann et al., (2022) highlighted the practical advantages of Rapid ICT assays [13,14]. They are simple to perform, require minimal equipment, and provide results within 20–25 minutes, making them highly suitable for resource-limited settings where access to advanced laboratory facilities is limited. The lower cost of Rapid ICT assays further supports their utility in large-scale screening and outbreak response scenarios.

Despite these advantages, some limitations were observed. Medialdea et al., (2021) showed that in Moderate sensitivity for IgM suggests that false-negative results may occur, particularly in secondary infections or very early febrile cases [15]. Therefore Andre et al., (2022) revealed that, confirmatory testing using ELISA remains important for accurate diagnosis, particularly in epidemiological studies or clinical trials [16]. Combining NS1, IgM, and IgG detection in a single rapid test could enhance overall sensitivity and specificity, providing a more robust diagnostic tool for early dengue detection.

Overall, the study supports the integration of Rapid ICT assays into routine dengue diagnostic protocols, particularly for early-phase detection, outbreak response, and primary care settings. Their performance, in combination with conventional laboratory methods, can improve patient management, enable timely interventions, and contribute to more effective surveillance and control of dengue transmission.

Conclusion

Rapid ICT assays provide a reliable, rapid, and cost-effective tool for the detection of dengue NS1 antigen, IgM, and IgG in the early febrile phase. NS1 detection shows high sensitivity and specificity, while IgM detection has moderate accuracy, highlighting the potential need for confirmatory testing. Substantial agreement with ELISA confirms that Rapid ICT can be effectively employed in resource-limited settings for timely diagnosis, aiding clinical decision-making and outbreak management. Integration of rapid tests with conventional laboratory methods can enhance dengue detection and patient care.

The use of Rapid ICT assays can significantly reduce the turnaround time for diagnosis, enabling early initiation of supportive treatment and monitoring. Their ease of use and minimal equipment requirements make them particularly suitable for field settings and peripheral healthcare centers. Moreover, combining NS1, IgM, and IgG detection in a single assay can improve overall diagnostic accuracy, making Rapid ICT a valuable tool for both clinical management and public health surveillance. Adoption of these rapid diagnostic strategies can contribute to better outbreak control and reduced disease burden.

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