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Diagnostic Accuracy of a NAAT-LAMP Assay with ELISA Reader-Based Optical Density Detection for Early Dengue Diagnosis: A Prospective Study

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Abstract: Early diagnosis of dengue is critical for effective clinical management and outbreak control. Although RT-PCR is the reference standard during the acute phase, its routine use is limited by cost and infrastructure requirements. Serology-based rapid diagnostic tests (RDTs) demonstrate reduced sensitivity in early infection. This study evaluated the diagnostic accuracy of a NAAT-LAMP assay with ELISA reader-based optical density (OD) detection for early dengue diagnosis. In this prospective diagnostic accuracy study, 598 patients presenting with acute febrile illness of ≤6 days were enrolled. RT-PCR served as the reference standard. Viral RNA was amplified using RT-LAMP targeting the conserved 3′ untranslated region of the dengue virus genome, with Hydroxynaphthol Blue dye for colorimetric detection. Optical density values were measured using an ELISA microplate reader employed solely as a spectrophotometric device. Diagnostic performance indices, ROC analysis, and agreement statistics were calculated and compared with NS1, IgM, and IgG RDTs. RT-PCR confirmed dengue infection in 299 (50.0%) cases. NAAT-LAMP demonstrated a sensitivity of 90.3%, specificity of 97.0%, and overall diagnostic accuracy of 93.6%, with near-perfect agreement with RT-PCR (κ = 0.873). Mean OD values were significantly higher in RT-PCR-positive samples ($p < 0.001$). ROC analysis showed excellent discrimination (AUC = 0.939; optimal OD cut-off 0.60). RDTs showed lower sensitivity, while combined RDT algorithms reduced specificity. NAAT-LAMP with ELISA reader-based OD detection is a reliable, objective, and scalable alternative to RT-PCR for early dengue diagnosis, particularly suited to resource-limited settings

Keywords: Dengue; NAAT-LAMP; RT-PCR; ELISA reader; diagnostic accuracy.

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INTRODUCTION

Dengue virus infection is one of the most important mosquito-borne viral diseases worldwide, with an estimated 390 million infections occurring annually, of which approximately one-quarter are clinically apparent [1,2]. India bears a substantial proportion of this burden due to hyperendemic transmission, circulation of all four dengue virus serotypes (DENV-1 to DENV-4), rapid urbanization, and favorable climatic conditions for vector proliferation [3-5]. Recurrent outbreaks impose significant strain on healthcare systems and emphasize the need for early and reliable laboratory diagnosis to support timely clinical management and public health interventions [6].

Early diagnosis of dengue during the acute febrile phase (≤6 days) is particularly challenging because clinical manifestations are nonspecific and overlap with other endemic febrile illnesses such as malaria, chikungunya, leptospirosis, enteric fever, and viral fevers [7-9]. Laboratory confirmation during this phase is critical for appropriate patient monitoring, prevention of progression to severe dengue, and rational utilization of healthcare resources [10]. However, commonly used serological assays have well-recognized limitations in early infection. NS1 antigen detection, although useful in primary dengue, demonstrates reduced sensitivity in secondary infections, during later days of illness, and in infections caused by certain serotypes, particularly DENV-4, due to immune complex formation and declining antigenemia [11-14]. Similarly, IgM and IgG antibody assays are limited by delayed seroconversion and significant cross-reactivity with other flaviviruses, leading to false-negative or false-positive results in endemic regions [15-17].

Reverse transcription polymerase chain reaction (RT-PCR) is regarded as the reference standard for early dengue diagnosis owing to its high sensitivity and specificity during the viremic phase [18-20]. Despite its diagnostic accuracy, widespread implementation of RT-PCR in routine clinical settings remains limited, particularly in low- and middle-income countries, because of high costs, dependence on thermocyclers, infrastructure requirements, and the need for trained personnel [21,22]. These constraints have created a diagnostic gap between high-performance molecular assays and widely available but less reliable serological tests [23].

Nucleic acid amplification tests (NAATs) based on loop-mediated isothermal amplification (LAMP) have emerged as promising alternatives to conventional PCR for infectious disease diagnostics [24]. LAMP enables rapid nucleic acid amplification under isothermal conditions, eliminating the need for thermocyclers while maintaining high analytical sensitivity and specificity [25]. Several studies have demonstrated the utility of RT-LAMP for dengue virus detection, particularly when primers target conserved genomic regions such as the 3′ untranslated region (3′UTR), allowing detection across all dengue serotypes [26]. However, interpretation of LAMP results based solely on visual color change may be subjective and prone to inter-observer variability.

Objective quantification of LAMP-associated colorimetric changes using widely available laboratory instruments, such as ELISA microplate readers functioning as spectrophotometric devices, offers a practical solution to this limitation [27]. Such an approach preserves the simplicity and rapidity of NAAT-LAMP while enabling reproducible and quantitative result interpretation without employing ELISA-based enzymatic amplification.

In this context, the present study was undertaken to evaluate the diagnostic accuracy of a NAAT-LAMP assay with ELISA reader-based optical density detection for early dengue diagnosis, using RT-PCR as the reference standard, and to assess its potential utility as a scalable diagnostic strategy in resource-limited, dengue-endemic settings.

MATERIALS AND METHOD

Study Design and Population

A prospective diagnostic accuracy study was conducted on 598 patients presenting with acute febrile illness (≤6 days duration). Patients of all age groups and both sexes were included after informed consent. Ethical approval was obtained from the Institutional Ethics Committee.

Reference Standard

RT-PCR for dengue virus detection was performed on extracted RNA and served as the gold standard.

NAAT-LAMP Assay

Viral RNA was amplified using RT-LAMP targeting the conserved 3′UTR region of the dengue virus genome. Amplification was carried out using Hi-PCR® REDy Master Mix (HiMedia; Cat. No.

MBT089A-50R) at 63°C for 35-40 minutes. Hydroxynaphthol Blue dye was added prior to amplification for closed-tube colorimetric detection.

OD Measurement

Following amplification, reaction mixtures were transferred to 96-well microplates. Optical density was measured at ~650 nm using an ELISA microplate reader, which was used solely as a spectrophotometric device.

Rapid Diagnostic Tests

NS1 antigen, IgM, and IgG RDTs were performed according to manufacturer instructions.

Statistical Analysis

Sensitivity, specificity, PPV, NPV, accuracy, and Cohen's kappa were calculated. ROC curve analysis was performed to determine optimal OD cut-off values. Statistical significance was assessed using Mann-Whitney U and chi-square tests where appropriate

RESULTS

A total of 598 patients presenting with acute febrile illness were included in the study. The baseline demographic and clinical characteristics are summarized in Table 1. The mean age of participants was 33.4 ± 16.1 years, with the majority belonging to the 25-44-year age group. Males constituted 56.2% of the study population. Most patients presented early, with a mean fever duration of 3.3 ± 1.2 days, and over half of the cases reporting fever of ≤3 days, indicating enrollment predominantly during the acute viremic phase.

The distribution of diagnostic test results is shown in Table 2. RT-PCR confirmed dengue infection in 299 (50.0%) patients. The NAAT-LAMP assay detected dengue virus RNA in 279 (46.7%) cases, whereas rapid diagnostic tests showed lower positivity rates, with NS1, IgM, and IgG RDTs detecting 34.1%, 31.9%, and 24.6% of cases, respectively.

Comparison of NAAT-LAMP with RT-PCR demonstrated strong concordance (Table 3). NAAT-LAMP identified 270 true positives and 290 true negatives, with 29 false negatives and 9 false positives. The assay achieved a sensitivity of 90.3%, specificity of 97.0%, and an overall diagnostic accuracy of 93.6%. Agreement analysis showed a Cohen's kappa value of 0.873, indicating near-perfect agreement with RT-PCR. Optical density analysis revealed significantly higher OD values in RT-PCR-positive samples compared with RT-PCR-negative samples ($p < 0.001$). ROC curve analysis demonstrated excellent discriminatory ability, with an AUC of 0.939 and an optimal OD cut-off of 0.60.

The diagnostic performance of rapid diagnostic tests compared with RT-PCR is summarized in Table 4. Individual RDTs showed lower sensitivities, with NS1, IgM, and IgG demonstrating sensitivities of 65.9%, 56.2%, and 43.8%, respectively, although specificity remained high. A combined RDT algorithm improved sensitivity to 94.0% but resulted in reduced specificity (85.6%) and lower overall accuracy compared with NAAT-LAMP.

Analysis of RT-PCR Ct values and fever duration in relation to NAAT-LAMP results is presented in Table 5. No statistically significant difference in mean Ct values was observed between NAAT-LAMP-positive and NAAT-LAMP-negative samples ($p = 0.48$). Similarly, NAAT-LAMP positivity did not vary significantly across fever duration categories ($p = 0.51$), indicating stable performance of the assay across the early phase of illness.

Overall, NAAT-LAMP demonstrated the most favorable balance of sensitivity, specificity, and diagnostic accuracy among all evaluated diagnostic strategies, approaching the performance of RT-PCR while outperforming serology-based rapid tests.

Table 1. Baseline demographic and clinical characteristics of the study population (n = 598)

Variable	Category	n (%) / Mean ± SD
Age (years)	Mean ± SD	33.4 ± 16.1
	<5	30 (5.0)
	5-14	46 (7.7)
	15-24	94 (15.7)
	25-44	285 (47.7)
	45-64	128 (21.4)
	≥65	15 (2.5)
Sex	Male	336 (56.2)
	Female	262 (43.8)
Fever duration (days)	Mean ± SD	3.3 ± 1.2
	≤3 days	343 (57.4)
	4-5 days	229 (38.3)
	≥6 days	26 (4.3)

Table 2. Distribution of diagnostic test results among suspected dengue cases (n = 598)

Diagnostic test	Positive n (%)	Negative n (%)
RT-PCR for dengue	299 (50.0)	299 (50.0)
NAAT-LAMP assay	279 (46.7)	319 (53.3)
NS1 RDT	204 (34.1)	394 (65.9)
IgM RDT	191 (31.9)	407 (68.1)
IgG RDT	147 (24.6)	451 (75.4)

Table 3. Diagnostic performance of NAAT-LAMP compared with RT-PCR (n = 598)

A. Cross-tabulation

NAAT-LAMP result	RT-PCR positive	RT-PCR negative	Total
Positive	270 (TP)	9 (FP)	279
Negative	29 (FN)	290 (TN)	319
Total	299	299	598

B. Diagnostic indices and OD analysis

Parameter	Estimate
Sensitivity (%)	90.3
Specificity (%)	97.0
Positive predictive value (%)	96.8
Negative predictive value (%)	90.9
Overall diagnostic accuracy (%)	93.6
Cohen's kappa (κ)	0.873
Mean OD (RT-PCR positive)	1.66 ± 0.71
Mean OD (RT-PCR negative)	0.29 ± 0.31
p-value (OD comparison)	<0.001
ROC AUC	0.939
Optimal OD cut-off	0.60

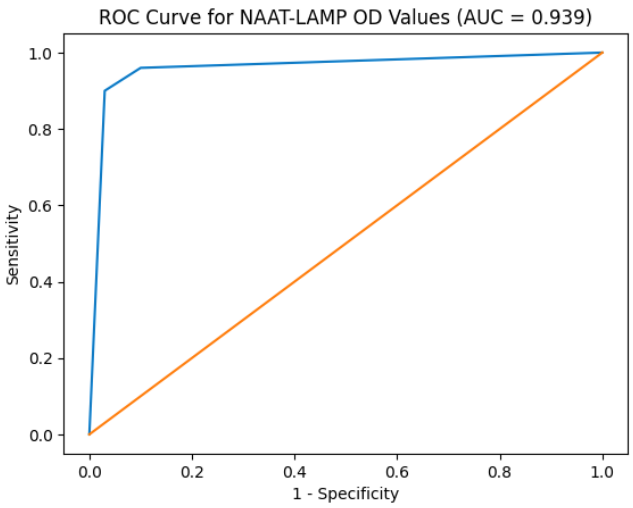

Figure 1. Receiver operating characteristic (ROC) curve of NAAT-LAMP optical density values for prediction of RT-PCR positivity. The area under the curve (AUC) was 0.939, indicating excellent diagnostic performance.

Table 4. Diagnostic performance of rapid diagnostic tests compared with RT-PCR (n = 598)

Test / Algorithm	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
NS1 RDT	65.9	97.7	96.6	74.1	81.8
IgM RDT	56.2	92.3	88.0	67.8	74.2
IgG RDT	43.8	94.6	89.1	62.7	69.2
Combined RDT (any NS1/IgM/IgG)	**94.0**	**85.6**	**86.7**	**93.4**	**89.8**

Table 5. Association of NAAT-LAMP results with RT-PCR Ct values and fever duration

A. Ct values among RT-PCR-positive samples (n = 299)

NAAT-LAMP result	n	Mean Ct ± SD	Median (IQR)	p-value*
Positive	270	27.36 ± 4.06	27.55 (24.42-30.28)	
Negative	29	28.00 ± 4.18	27.50 (25.30-30.80)	0.48

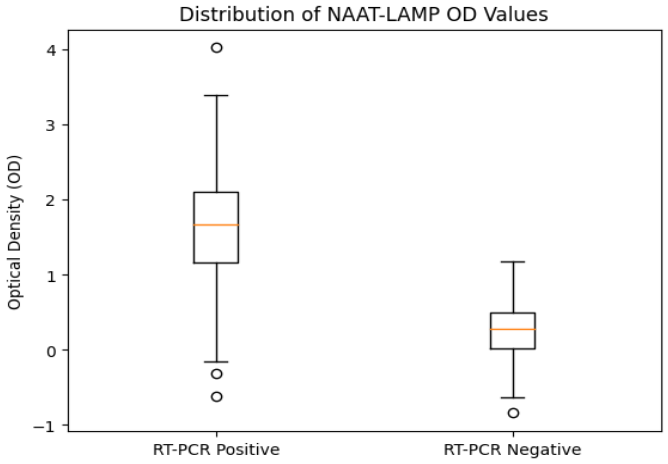

Figure 2. Distribution of NAAT-LAMP optical density values among RT-PCR-positive and RT-PCR-negative samples. RT-PCR-positive samples showed significantly higher OD values ($p < 0.001$).

B. Fever duration and NAAT-LAMP positivity (n = 598)

Fever duration	NAAT-LAMP negative n (%)	NAAT-LAMP positive n (%)	p-value†
≤3 days	184 (53.6)	159 (46.4)	
4-5 days	124 (54.1)	105 (45.9)	
≥6 days	11 (42.3)	15 (57.7)	0.51

* Mann-Whitney U test

† Chi-square test

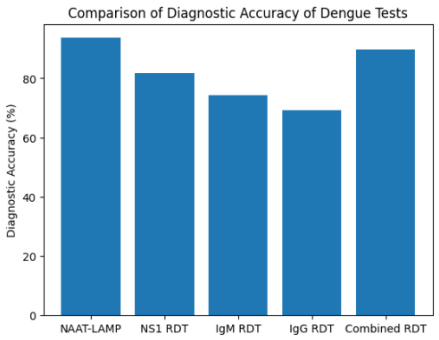

Figure 3. Comparative diagnostic accuracy of NAAT-LAMP, individual rapid diagnostic tests, and a combined RDT algorithm using RT-PCR as the reference standard

DISCUSSION

Early and accurate diagnosis of dengue remains a cornerstone of effective clinical management and outbreak control, particularly in endemic regions where multiple febrile illnesses coexist. In the present study, the NAAT-LAMP assay quantified using ELISA reader-based optical density (OD) measurement demonstrated high diagnostic accuracy when compared with RT-PCR, supporting its potential role as a reliable alternative molecular diagnostic approach for early dengue detection.

The sensitivity (90.3%) and specificity (97.0%) observed for NAAT-LAMP in this study are comparable to molecular assays reported in earlier evaluations. Similar performance characteristics have been described by Parida et al., 2005 and Teoh et al., 2013, who demonstrated that RT-LAMP assays targeting conserved regions of the dengue genome provide high sensitivity across all serotypes while avoiding the infrastructural constraints of conventional PCR [24-26]. The near-perfect agreement with RT-PCR ($\kappa = 0.873$) observed in our study further reinforces the robustness of NAAT-LAMP as a molecular diagnostic tool, consistent with findings reported by Johnson et al., 2005 and Lanciotti et al., 1992 for nucleic acid-based dengue detection [18,19].

A key strength of the present study lies in the objective quantification of LAMP amplification using an ELISA microplate reader. While many LAMP assays rely on subjective visual interpretation of color change, which may introduce observer bias, OD-based measurement provides reproducibility and analytical rigor. Similar approaches using spectrophotometric or reader-based detection to enhance LAMP result interpretation have been proposed by Mori and Notomi, 2009 and Peeling et al., 2010, emphasizing the importance of objective readouts for field-deployable molecular diagnostics [25,27]. Importantly, in this study, the ELISA reader was used solely as a spectrophotometric device, without any ELISA-based enzymatic amplification, preserving methodological simplicity while improving result reliability.

The optical density analysis and ROC curve findings further support the diagnostic utility of NAAT-LAMP. The excellent AUC value (0.939) observed indicates strong discriminatory capacity between dengue-positive and dengue-negative cases. Comparable ROC performance has been reported in previous molecular diagnostic studies, underscoring that quantitative interpretation enhances the clinical applicability of NAAT-based assays [22,24]. The identification of an optimal OD cut-off also facilitates standardization, which is critical for large-scale implementation.

In contrast, serology-based rapid diagnostic tests showed lower sensitivity in the present study, particularly when used individually. The reduced sensitivity of NS1 antigen tests observed here aligns with reports by Blacksell et al., 2008 and Tricou et al., 2010, who documented declining NS1 detection in secondary dengue infections and later stages of illness [11,12]. Similarly, the suboptimal performance of IgM and IgG assays during early infection is consistent with delayed antibody kinetics and cross-reactivity described by Guzman and Harris, 2015 and Simmons et al., 2012 [5,6]. Although the combined RDT algorithm improved sensitivity, it did so at the expense of specificity, increasing the risk of false-positive diagnoses—an issue previously highlighted in endemic settings [15-17].

The lack of significant association between NAAT-LAMP positivity and RT-PCR Ct values suggests that assay performance was not strongly influenced by viral load within the range observed. This finding is consistent with earlier reports indicating that LAMP assays can efficiently amplify low-copy viral RNA due to their high amplification efficiency [24,25]. Additionally, the stable performance of NAAT-LAMP across different fever duration categories supports its suitability throughout the acute phase, which is clinically the most relevant diagnostic window.

From a translational perspective, the findings of this study have important implications. RT-PCR, while diagnostically superior, remains inaccessible in many routine laboratories due to cost and infrastructure requirements [21-23]. NAAT-LAMP, combined with ELISA reader-based OD detection, offers a pragmatic solution that bridges the gap between advanced molecular diagnostics and commonly available laboratory infrastructure. This approach aligns with global recommendations emphasizing the need for scalable, affordable diagnostic tools in dengue-endemic regions [6,27].

Overall, the present study adds to the growing body of evidence supporting NAAT-LAMP as a viable molecular diagnostic alternative for dengue. By demonstrating high accuracy, objective quantification, and operational feasibility, this work supports the integration of NAAT-LAMP into early dengue diagnostic algorithms, particularly in resource-limited settings.

CONCLUSION

NAAT-LAMP with ELISA reader-based OD detection is a reliable, scalable, and resource-appropriate diagnostic tool for early dengue detection. Its implementation can significantly enhance diagnostic capacity in dengue-endemic regions.

COMPLIANCE WITH ETHICAL STANDARDS

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Conflict of Interest

The authors declare that they have no conflict of interest regarding the publication of this paper.

Ethical Approval

This study was conducted in accordance with the ethical standards of the institutional research committee and with the Declaration of Helsinki. Ethical approval was obtained from the Institutional Ethics Committee of Jawaharlal Nehru Medical College and Acharya Vinoba Bhave Rural Hospital, Datta Meghe Institute of Higher Education and Research (Deemed to be University) Sawangi (Meghe), Wardha,
Approval number: DMIHER(DU)/IEC/2025/599

Informed Consent

Informed consent was obtained from all individual participants included in the study (or from legally authorized representatives where applicable).

Authors Contribution

Ms. Deepali conceived and designed the study, performed laboratory work, analyzed the data, and prepared the original manuscript draft. Dr. Pratibha supervised the study and critically reviewed the manuscript. Mr. Asim and Mr. Abhijeet assisted in laboratory investigations and data collection. Dr. Nandkishore provided academic guidance and departmental support during the study. Dr. Sarita provided mentorship and contributed to critical revision of the manuscript. All authors read and approved the final manuscript.

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