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Diagnostic Utility of RT LAMP Compared to Real Time RT PCR in Dengue Virus Serotype Identification at a Tertiary Care Hospital of Chattogram District of Bangladesh

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Abstract

Background: Dengue virus (DENV) remains the most prevalent arbovirus globally, with rising incidence in tropical and subtropical regions. Its expanding geographical distribution and increasing disease severity highlight the need for early and accurate diagnosis. Rapid detection during the febrile phase is crucial to guide clinical management and prevent complications. While real-time reverse transcription polymerase chain reaction (RT-qPCR) is the gold standard for molecular detection, its limited availability in resource-constrained settings necessitates alternative methods. **Objective:** This study was evaluated the diagnostic performance of reverse transcription loop-mediated isothermal amplification (RT-LAMP) for DENV serotyping, compared with RT-qPCR. **Methodology:** This cross-sectional study was conducted in the Department of Microbiology at Chittagong Medical College, Bangladesh, from July 2023 to June 2024. Two hundred patients with clinically suspected dengue were recruited. Initial screening used an immunochromatographic test (ICT) for NS1 antigen; NS1-negative cases were further tested for anti-dengue IgM and IgG antibodies. Samples positive by NS1, IgM, or both were subjected to RT-qPCR and RT-LAMP for DENV serotyping. RT-qPCR served as the reference standard. **Results:** Of 200 suspected cases, 142 were ICT-positive. RT-qPCR confirmed DENV in 138 samples, while RT-LAMP detected 136 of these. Two RT-qPCR-positive samples were missed by RT-LAMP, and four were negative by both methods. RT-LAMP showed a sensitivity of 98.55%, specificity of 100%, positive predictive value of 100%, negative predictive value of 66.67%, and diagnostic accuracy of 98.59%. Receiver operating characteristic analysis showed an area under the curve (AUC) of 0.993 (95% CI: 0.980–1.000; p = 0.001), indicating excellent diagnostic capability. **Conclusion:** RT-LAMP offers a rapid, affordable, and reliable alternative to RT-qPCR for early DENV detection and serotyping. Its simplicity and minimal equipment requirements make it especially valuable in dengue-endemic and resource-limited settings, as well as during outbreaks.

Keywords: DENV, Dengue Serotyping, RT-LAMP, RT-qPCR

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Introduction

Dengue virus (DENV), a mosquito-borne RNA virus of the Flavivirus genus, is a growing global health concern. It comprises four well-established serotypes (DENV-1 to DENV-4), with a potential fifth (DENV-5) reported in Malaysia in 2013[1,2]. Each serotype contains multiple genotypes, contributing to complex regional transmission patterns1. Dengue incidence has risen sharply over the past two decades, now affecting over 100 countries across multiple continents[3]. Due to underreporting and mild or asymptomatic cases, the true disease burden remains

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underestimated[4]. Reported cases increased tenfold from 2000 to 2019, with 5.2 million cases in 2019 and continued surges in 2023, resulting in approximately 5,000 deaths across 80 countries[4].

Bangladesh remains highly endemic, with seasonal outbreaks intensified by rapid urbanization, climate change, and poor vector control[5-7]. Major cities such as Dhaka and Chattogram are particularly affected[8]. Since the first major outbreak in 2000, the country has faced recurring epidemics, with over 308,000 cases and nearly 1,600 deaths reported in 2023 alone3. DENV-3 dominated the 2000 outbreak, followed by shifts toward DENV-1 and DENV-2, with the latter re-emerging as the predominant strain in 2023, linked to increased disease severity[9,10]. While primary infection confers lifelong immunity to the same serotype, secondary infection with a different serotype may lead to severe disease due to antibody-dependent enhancement[11,12], highlighting the need for timely and accurate serotype-specific diagnostics.

Diagnostic approaches include NS1 antigen detection, serology (IgM/IgG), and molecular methods. Although RT-qPCR is the gold standard for early detection and serotyping13, its reliance on costly equipment and skilled personnel limits accessibility in low-resource settings. Virus isolation is impractical, and NS1/serology tests have limitations in sensitivity and timing14. Loop-mediated isothermal amplification (LAMP) offers a rapid, sensitive, and low-cost molecular alternative. RT-LAMP enables detection of viral RNA under isothermal conditions with minimal instrumentation, yielding results within an hour15. Its utility for point-of-care diagnosis has been demonstrated for various pathogens, including DENV[13].

Given Bangladesh's recurrent dengue outbreaks and limited molecular diagnostic capacity, this study aimed to evaluate the performance of RT-LAMP in comparison to RT-qPCR for DENV serotyping during the 2023 outbreak in Chattogram.

Methodology

Study Settings and Population: This was a cross-sectional study done among clinically suspected dengue patients in the Department of Microbiology at Chittagong Medical College (CMC), Chattogram, Bangladesh from July 2023 to June 2024 for a period of one year. Suspected dengue patients attending the outpatient department and those admitted to the in-patient departments (Medicine, Paediatrics, and Dengue wards) of Chittagong Medical College Hospital (CMCH). A purposive sampling technique was used to recruit study participants and A total of 200 clinically suspected dengue patients were enrolled in the study. Patients with clinically suspected dengue, as per WHO 2009 dengue case classification, were included and diagnosed cases of febrile illness other than dengue were excluded. As per WHO 2009 modified dengue case classification16 individuals with a recent travel/residency in dengue-endemic areas, fever of 2–7 days, and at least two of the following: nausea, vomiting, rash, aches, positive tourniquet test, leucopenia, or any warning signs.

Study Procedure: After proper counseling and informed written consent, 3 mL of venous blood was collected aseptically using a red-top vacutainer tube. Intravenous blood was taken following standard venipuncture protocol17. Each sample was anonymized with a unique ID. Blood samples were allowed to clot, then centrifuged at 3,000–5,000 rpm for 10–15 minutes to separate serum. Serum was aliquoted into two RNase-free 1.5 mL Micro-centrifuge tubes. One tube of the processed serum was used for NS1 and IgM/IgG testing, another tube was used for RT-qPCR and RT-LAMP assays. Following NS1 and IgM/IgG testing, serum samples were stored at -80°C until RNA extraction, RT-qPCR, and RT-LAMP procedures were performed.

Laboratory Procedure: Dengue NS1 antigen was detected using an immunochromatographic test (ICT) with a commercially available BiolineTM Dengue NS1 Ag kit (Korea), following manufacturer's instructions. Detection of dengue-specific IgM and IgG antibodies was performed using Bioline Dengue Duo (Korea) ICT kits.

RNA Extraction: Viral RNA was extracted from 142 serum samples positive for either NS1 or IgM/IgG, using the PureLink™ Viral RNA/DNA Mini Kit (Invitrogen, Thermo Fisher Scientific) as per manufacturer protocol. Serum samples were thawed and centrifuged at 4,000 rpm for 10 minutes to remove debris.

RT-qPCR for Dengue Virus Detection: Dengue viral RNA was amplified using a commercial Bosphore dengue virus real Time RT-PCR Detection Kit (Anatolia geneworks, Turky). Amplification was performed in a QuantStudio 5 Real-Time PCR system. Each reaction included extracted RNA template, master mix, and specific primers/probes. Thermal cycling conditions followed manufacturer guidelines.

RT-LAMP Assay for Dengue Virus Detection: RT-LAMP was performed on RNA extracted from the

Table 1: Sequences of RT-LAMP Primers Used in this Study

Virus serotype	Target (genome position)	Primer	Sequence (5'→ 3')
DEN-1	10469–	F3	GAGGCTGCAAACCATGGAA
	10667	B3	CAGCAGGATCTCTGGTCTCT
	(199 bp)	FIP	GCTGCGTTGTGTCTTGGGAGGTTTTCTGTACGCATGGGGTAGC
		BIP	CCCAACACCAGGGGAAGCTGTTTTTTTGTTGTTGTGCGGGGG
		FLP	CTCCTCTAACCACTAGTC
		BLP	GGTGGTAAGGACTAGAGG
DEN-2	10449–	F3	TGGAAGCTGTACGCATGG
	10659	B3	GTGCCTGGAATGATGCTG
	(211 bp)	FIP	TTGGGCCCCCATTGTTGCTGTTTTAGTGGACTAGCGGTTAGAGG
		BIP	GGTTAGAGGAGACCCCCCCAATTTTGGAGACAGCAGGATCTCTGG
		FLP	GATCTGTAAGGGAGGGG
		BLP	GCATATTGACGCTGGGA
DEN-3	10289–	F3	GCCACCTTAAGCCACAGTA
	10506	B3	GTTGTGTCATGGGAGGG
	(218 bp)	FIP	TGGCTTTTGGGCCTGACTTCTTTTTTGAAGAAGCTGTGCAGCCTG
		BIP	CTGTAGCTCCGTCGTGGGGATTTTCTAGTCTGCTACACCGTGC
		FLP	CCTTGGACGGGGCT
		BLP	GGAGGCTGCAAACCGTG
DEN-4	10289–	F3	CTATTGAAGTCAGGCCAC
	10517	B3	ACCTCTAGTCCTTCCACC
	(229 bp)	FIP	TGGGAATTATAACGCCTCCCGTTTTTTCCACGGCTTGAGCAAACC
		BIP	GGTTAGAGGAGACCCCTCCCTTTTAGCTTCCTCCTGGCTTCG
		FLP	GGCGGAGCTACAGGCAG
		BLP	TCACCAACAAAACGCAG

same 142 samples using serotype-specific primers(Macrogen, Korea) designed based on 3′ untranslated region (UTR)13 of the DENV genome. targeting the DENV genome. 25µl reaction mix was prepared by adding 12.5µL of master mix (WarmStart Colorimetric RT-LAMP 2X Master Mix with UDG, New England Biolab), 1 µL of extracted RNA, 2.5 µL of RT-LAMP primer mix (Macrogen, Korea), 9 µL of nuclease free water. Reactions were incubated at 63°C for 60 minutes and visualized by color change or turbidity.

Statistical Analysis: Data were analyzed using SPSS version 25. Descriptive statistics (mean, standard deviation, frequency, and percentage) were calculated. Chi square test and ROC analysis was done to detect the Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of RT-LAMP by using RT-qPCR as the gold standard.

Ethical Consideration: Ethical approval was obtained from the Ethical Review Committee of Chittagong Medical College (Memo no: 59.27.0000.013.19. PG.009.2023/1005, dated 02.10.2023). Written informed consent was obtained from all participants or guardians of minors, following the Declaration of Helsinki, Good Clinical Practice guidelines, and national regulations. Study aims and procedures were explained in Bengali or the local language.

Results

Among 200 suspected dengue patients, 106(53.0%) were positive for dengue NS1. Of 94 patients tested further, 32(34.0%) were IgM positive, and 4(4.2%) were positive for both IgM and IgG. Additionally, 23 (24.0%) showed IgG positivity alone. A total of 165 patients had at least one positive test. Based on CDC guidelines, 142 cases were identified as recent dengue infections (positive for NS1 and/or IgM). (Table 2)

Table 2: ICT Test Result for Dengue NS1 and Dengue IgM, IgG (N=200)

Number of patients, n	Dengue NS1	Dengue IgM	Dengue IgG
106	Positive	Not done	Not done
4	Negative	Positive	Positive
32	Negative	Positive	Negative
23	Negative	Negative	Positive
35	Negative	Negative	Negative
200	**Total**		

Of the 142 confirmed dengue cases, RT-qPCR detected 138 (97.2%) while RT-LAMP detected 136 (95.8%). Two samples were positive by RT-qPCR but negative by RT-LAMP. Four samples were negative by both assays. The difference was statistically significant ($p < 0.0001$) (Table 3).

Table 3: Type Specific RT-qPCR and RT-LAMP Assay for Dengue Serotyping (n=142)

Test name	RT-qPCR positive	RT-qPCR negative	Total	P value
RT-LAMP positive	136 (95.77%)	0 (0.0%)	136 (95.77%)	
RT-LAMP negative	2 (1.40%)	4 (2.81%)	6 (4.23%)	<0.0001
Total	**138 (97.18%)**	**4 (2.81%)**	**142 (100.0%)**	

Note: 142 serum samples, that were classified as recent dengue virus infection, were tested by both RT-qPCR, and RT-LAMP assay; Positive = detected; Negative =undetected; p-value <0.0001which is statistically significant [Chi-square test done (level of significance ≤ 0.05)

ROC curve analysis revealed that RT-LAMP had a sensitivity of 98.5%, specificity of 100%, PPV of 100.0%, and NPV of 66.7%, with an overall accuracy of 98.59%. The AUC was 0.993 (95% CI: 0.980–1.000; p = 0.001), indicating excellent diagnostic performance compared to RT-qPCR (Figure I, Table 4).

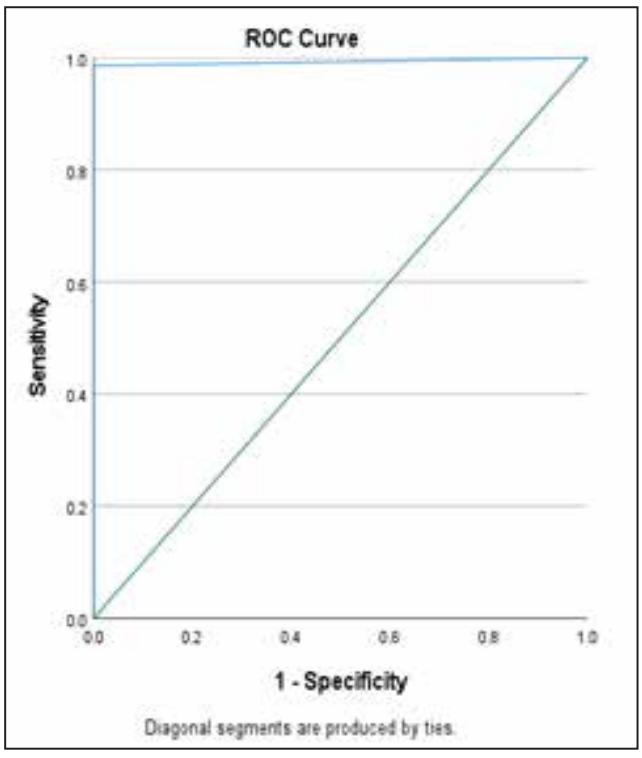

Figure I: ROC Curve Analysis to Evaluate the RT-LAMP Assay in Comparison to RT-qPCR for Serotyping of Dengue

Table 4: Results of ROC curves of RT-LAMP

Parameters	Percentage	AUC	P value	Cut off value
Sensitivity	98.55	0.993		
Specificity	100.00	(95% CI:		
Positive predictive value	100.00	0.980–1.000)	0.001	≥ 0.5
Negative predictive value	66.67			
Accuracy	98.59			

Note: p-value ≤ 0.05 was considered significant.

RT-qPCR identified DENV-1 in 11 (8%), DENV-2 in 99 (70%), and DENV-3 in 17 (12%) cases. RT-LAMP showed similar distribution: DENV-1 (8%), DENV-2 (69%), and DENV-3 (12%). No DENV-4 was detected. Both assays identified 3 cases (2%) of triple serotype infection (DENV-1,2,3), and mixed DENV-2,3 infection in 8 (RT-qPCR) and 7 (RT-LAMP) cases. Four (3%) samples were undetected by RT-qPCR, and six (4%) by RT-LAMP (Table 5).

Table 5: Distribution of Dengue virus serotypes detected by RT-qPCR and RT-LAMP

Serotypes	Category	RT-qPCR	RT-LAMP
Undetected		4(3.0%)	6(4.0%)
Detected	DEN-1	11(8.0%)	11(8.0%)
	DEN-2	99(70.0%)	98(69.0%)
	DEN-3	17(12.0%)	17(12.0%)
	DEN-4	0	0
	DEN-1,2,3	3(2.0%)	3 (2.0%)
	DEN-2,3	8(5.0%)	7 (5.0%)
Total		**138**	**136**

Note: Dengue ICT detected positive patients= 142; Dengue serotype detected patients=138; 4 (3%) were undetected by RT-qPCR and 6(4%) were undetected by RT-LAMP

Among NS1-positive patients (n=106), both RT-qPCR and RT-LAMP detected all cases. In IgM-positive cases (n=32), 28 (87.5%) were detected by both methods. For patients positive for both IgM and IgG (n=4), RT-qPCR detected all, while RT-LAMP detected 2 (50%). The mean CT values (RT-qPCR) for detected cases were: NS1-positive (24.61), IgM-positive (25.70), and IgM plus IgG-positive (27.13) (Table 6).

Among 142 recent dengue cases, RT-LAMP detected all NS1-positive samples (106/106; 100%). Among IgM-positive cases, 28/32 (87.5%) were detectable. In patients positive for both IgM and IgG, 2/4 (50%) were detected (Table 7).

Table 6: Frequency of Dengue Detection among Different Positive Serological Tests by RT-qPCR (n=142)

Serology	RT-qPCR with mean CT-value		
	Positive	Negative	(mean CT-value)
NS1 positive (n=106)	106(100.0%)	0	24.61
IgM positive (n=32)	28(87.5%)	4(12.5%)	25.70
IgM, IgG positive (n=4)	4(100.0%)	0	27.13
Total	**138(97.2%)**	**4 (2.8%)**	

Note: Dengue ICT detected positive patients= 142; Dengue serotype detected patients=138; 4 (3%) were undetected by RT-qPCR and 6(4%) were undetected by RT-LAMP

Table 7: Frequency of Dengue Detection among Different Positive Serological Tests by RT-LAMP (n=142)

Serology	RT-LAMP		Total
	Positive	Negative	
NS1 Positive	106(100.0%)	0(0.0%)	106(100.0%)
IgM Positive	28(87.5%)	4(12.5%)	32(100.0%)
IgM, IgG both Positive	2(50.0%)	2(50.0%)	4(100.0%)
Total	**136 (95.8%)**	**6(4.2%)**	

Discussion

Dengue remains a significant public health challenge in endemic regions like Bangladesh, where limited diagnostic infrastructure, financial constraints, and inadequate public awareness hinder early and accurate diagnosis. The circulation of multiple dengue virus (DENV) serotypes, including cases of co-infection, can exacerbate disease severity depending on host immune status[18]. Early detection and serotype identification are therefore essential for both clinical management and outbreak control.

Among 200 clinically suspected dengue cases in this study, 142(71.0%) were confirmed as recent infections. Of these, 106 (53%) were NS1-positive, aligning with findings, reported 85.1% NS1 positivity in the acute phase[19], underscoring the utility of NS1 antigen detection in early diagnosis. To assess RT-LAMP's diagnostic performance, these 142 confirmed cases were tested using both RT-qPCR and RT-LAMP assays. RT-qPCR identified 138 (97.18%) positive cases, while RT-LAMP detected 136 (95.77%). Four IgM-positive samples with symptom onset beyond six days were negative in both molecular assays, likely due to declining viral load or cross-reactivity with other flaviviruses, consistent with WHO guidelines[20]. The negative results may also be attributed to RNA degradation, primer-probe mismatches, or low viral copy numbers below the RT-qPCR detection threshold. Notably, RNA viruses like DENV have high mutation rates due to the lack of proofreading activity in their RNA-dependent RNA polymerase, which can compromise primer binding[16].

Two samples positive by RT-qPCR were missed by RT-LAMP. These cases also tested positive for both IgM and IgG, indicating a later stage of infection with likely reduced viremia. RT-qPCR, known for its high sensitivity, can detect low viral loads, whereas RT-LAMP may fail to amplify RNA near its detection limit. Teoh et al. observed a similar trend, where low viral loads resulted in false negatives in RT-LAMP despite RT-qPCR positivity[21]. The diagnostic accuracy of RT-LAMP was evaluated using ROC analysis, which yielded an AUC of 0.993, with sensitivity and specificity of 98.55% and 100%, respectively, when compared to RT-qPCR. These findings are consistent with earlier reports where RT-LAMP closely matched RT-qPCR performance[21]. Although RT-LAMP did not surpass RT-qPCR in sensitivity, it offered a significant time advantage, requiring only 30 minutes versus 150 minutes for RT-qPCR. Similar time and performance benefits have been reported in several studies[21,22]. Interestingly, a study found RT-LAMP to be even more sensitive than RT-qPCR23, achieving 100% sensitivity and specificity compared to RT-qPCR's 85.2% sensitivity.

Serotype analysis revealed DENV-2 as the predominant circulating strain (70.0%), followed by DENV-3 (12%), DENV-1 (8.0%), mixed DENV-2 and 3(5.0%), and triple serotype infections (2.0%). These findings are consistent with studies from Chattogram and nationwide surveillance data, where DENV-2 accounted for the majority of cases[24-27]. The absence of DENV-4 in this study may reflect its lower replication efficiency in Aedes mosquitoes or underdiagnosis due to mild or asymptomatic presentations[5,28].

All NS1-positive samples were successfully serotyped by both RT-qPCR and RT-LAMP, while among IgM-positive samples, 87.5% were detected by RT-qPCR and RT-LAMP. However, among samples positive for both IgM and IgG, only 50.0% were detected by RT-LAMP, compared to 100.0% by RT-qPCR. These results suggest that NS1-positive samples, typically collected during early infection, are optimal for molecular detection due to high viral load. Some studies reported similar patterns, where NS1-positive samples showed significantly higher RT-qPCR detection rates[29,30]. A study also found maximal sensitivity of NS1 and RT-qPCR assays within the first three days of illness[31]. In contrast, antibody-positive samples often yield lower molecular detection due to reduced viremia as immune responses

clear circulating virus[32].

Although RT-qPCR remains the reference standard for DENV detection and serotyping, this study demonstrates that RT-LAMP offers comparable diagnostic performance. Its ease of use, rapid turnaround, and lower resource requirements make it a valuable tool for dengue surveillance and clinical application, especially in resource-limited settings.

This study was limited by its single-center design and relatively small sample size, which may not reflect the broader dengue patient population in Chittagong. The cross-sectional nature and time constraints prevented follow-up sampling, particularly for IgG-positive cases, limiting confirmation of secondary infections. Due to budget limitations and the high cost of reagents, serotyping was restricted to ICT-positive samples only. Consequently, the study could not assess RT-LAMP's performance in ICT-negative suspected cases, which may have affected the overall evaluation of the assay's diagnostic utility.

Conclusion

This study demonstrates that the RT-LAMP assay offers diagnostic performance comparable to RT-qPCR for dengue virus (DENV) serotyping, with high sensitivity and specificity. Its ability to amplify nucleic acids under isothermal conditions using only a simple heat block eliminates the need for complex instrumentation, making it a practical tool for resource-limited settings. The assay is rapid, user-friendly, and requires minimal technical expertise, making it suitable for both centralized laboratories and decentralized rural healthcare facilities. Given its performance and operational advantages, RT-LAMP represents a promising alternative for molecular dengue diagnostics, particularly in endemic regions such as Bangladesh. The RT-LAMP assay is recommended for use in point-of-care settings during dengue outbreaks to facilitate rapid detection and serotype differentiation. Its adoption could significantly strengthen epidemiological surveillance and outbreak response efforts. Training programs should be implemented for healthcare and laboratory personnel to ensure proper technique, quality control, and accurate interpretation of results.

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Conflict of Interest

The authors declare that there is no conflict of interest

Financial Disclosure

No external funding was received.

Authors' contributions

Marium Sukanya: conceptualized and designed the study, analyzed the data, interpreted the results, and wrote up the draft manuscript. Lovely Barai, Mili Rani Saha, and Tanjila Rahman contributed to the data analysis, interpretation of the results, and critical review of the manuscript. Minhaj Rashidur Rahman and Md. Rokibul Hasan contributed to the analysis of the data. All authors read and approved the final manuscript.

Data Availability

Any inquiries regarding supporting data availability of this study should be directed to the corresponding author and are available from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate

Ethical approval for the study was obtained from the Institutional Review Board. As this was a prospective study the written informed consent was obtained from all study participants. All methods were performed in accordance with the relevant guidelines and regulations.

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