



## Original Article

# Detection of Dengue Serotypes by Two Molecular Techniques, qPCR and LAMP

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### Abstract

**Background:** Dengue virus is a mosquito-borne flavivirus and the most widely prevalent arbovirus in tropical and subtropical regions. Detection of this virus by a new molecular technique named Loop-mediated isothermal amplification (LAMP) is a very sensitive, easy, and less time-consuming diagnostic method. It can amplify up to  $10^9$  copies in less than 1 hour under isothermal conditions (65°C).

**Aims:** To establish a dengue LAMP as simple, less time-consuming, more specific, and sensitive than the qPCR to detect dengue serotypes.

**Materials and Methods:** This prospective analytical study was conducted from January-December 2017 at the Department of Virology, BSMMU, Dhaka, Bangladesh. A total of 290 serum samples were used, confirmed by ICT (immune chromatographic test). These samples were used to perform qPCR for the detection of dengue serotypes. After that, dengue LAMP was performed using the same samples to establish the LAMP technique as a suitable, time-saving molecular technique that is more sensitive than qPCR.

**Results:** A total of 290 dengue confirmed samples by ICT were used for qPCR to detect dengue serotypes. Among which 137 dengue I and 113 dengue II RNA positive serum samples were confirmed by qPCR, and those were also positive by LAMP assay. Besides, 20 both dengue NS1 and dengue IgM negative by ICT were tested by qPCR, and 01 positive dengue II serotype was detected by qPCR, whereas 02 dengue II serotypes were detected by dengue LAMP technique from the same samples.

**Conclusion:** RT-LAMP assay had been developed in this study which allowed the rapid and accurate identification of dengue virus serotypes.

**Keywords:** Dengue LAMP, qPCR, ICT.

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## Introduction

According to a World Health Organization report, an estimated 500,000 people with severe dengue require hospitalization each year, and about 2.5%

of those affected die.<sup>1</sup> Dengue virus is a mosquito-borne flavivirus and the most widely prevalent arbovirus in tropical and subtropical regions of Asia, Africa, and Central and South America.

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Currently available diagnostic tests used for dengue diagnosis in most dengue-endemic countries include commercially available IgM- and IgG-based EIAs, Immunochromatographic assay (ICT). Dengue NS1 antigen test is a rapid diagnostic test for dengue virus infection. NS1 is usually detectable early in the acute phase of dengue infection, but it gives the possibility of false-positive reaction in detecting clinical patients.<sup>5</sup> NS1 antigen cannot identify serotypes.<sup>6</sup> Due to the ability of molecular techniques to provide rapid diagnostic information, reverse transcription (RT)-PCR, nested PCR, and real-time PCR is used to diagnose dengue virus serotypes.<sup>7</sup> Another molecular technique, the Loop-mediated isothermal amplification (LAMP), initially developed by Notomi *et al.* (2000), represents a very sensitive<sup>8</sup>, easy, and less time-consuming diagnostic method. LAMP can amplify up to  $10^9$  copies in less than 1 hour under isothermal conditions (65°C).<sup>9</sup>

### Materials and Methods

This prospective analytical study was conducted from January - December 2017 for twelve months at the Department of Virology, BSMMU, Bangladesh. A total of 290 serum samples were used, from the patients who had 1-5 days fever, for the study after performing ICT. Among these, 250 were positive for either dengue IgM or dengue IgG, 20 were negative for both dengue IgM and IgG, and 20 were healthy control. These samples

### Results

**Table 1: Detection of acute dengue serum samples (n= 250) and healthy controls (n= 20) and negative samples (20) by ICT**

Type of serum samples	Number of detected samples
Dengue NS1 positive but dengue IgM negative	150
Dengue IgM positive but dengue NS1 negative	100
Both dengue NS1 and dengue IgM negative	20
Healthy controls	20

were used to performed q PCR for the detection of dengue serotypes. After that, dengue LAMP was performed using the same samples to establish the LAMP technique as a suitable, short-time consuming molecular technique that is more sensitive than qPCR in detecting dengue serotypes. The study was approved by the Institutional Review Board (IRB) of BSMMU (No. BSMMU/2017/2142) on the 27<sup>th</sup> of February 2017). Written informed consent was obtained from each patient.

### Sample processing:

Fifteen  $\mu$ l of serum was diluted with 150 $\mu$ l PCR grade water. The mixture was centrifuged at 14000 rpm for 5 minutes. The viral RNA was extracted from 140  $\mu$ l of serum samples by using a commercially available kit (Geneaid, Biotech Ltd, UK) according to the manufacturer's instructions, for techniques, qPCR, and LAMP. The real-time PCR assay was performed by Step one PCR machine (Applied Biosystem, USA) with a commercially available kit (Genesig primer design<sup>TM</sup> Ltd, UK) according to the manufacturer's instructions. RNA concentration was measured in ng/ $\mu$ l by spectrophotometer (Nano-drop 2000/2000C) of 260/280 (Thermo Scientific, USA). Primer and target sequences and a total of 25  $\mu$ l master mix for RT-LAMP Assay were used according to the procedure described by Parida *et al.*, 2005.<sup>10</sup>

Table 1 illustrates a total of 290 ICT confirmed serum samples were enrolled in the study. About 150 dengue NS1 positive but dengue IgM negative samples were selected by ICT along with dengue IgM positive but dengue NS1 negative 100 samples were taken after confirmed by the same technique. Besides, 20 patients who had a fever but found negative for both dengue NS1 and dengue IgM by ICT also were enrolled in this study. At the same time, 20 healthy controls who had no fever or any other problems were confirmed by ICT were also included.

**Table 2: Detection of dengue serotypes by different molecular detection methods, LAMP assay, and PCR (n=290)**

ICT confirmed serum samples type	Study samples used for serotype standardization	Number of samples detected by qPCR	Number of samples detected by LAMP
Dengue NS1 positive but dengue IgM negative (150), and dengue IgM positive but dengue NS1 negative(100) (n=250)	Dengue I	137	137
	Dengue II	113	113
Both dengue NS1 and dengue IgM negative (n=20)	Dengue I	00	00
	Dengue II	01	02
Healthy controls (20)		00	00

Table 2 shows a total of 290 dengue confirmed samples by ICT were used for qPCR to detect dengue serotypes. Real-time LAMP was used as a detection method for the dengue LAMP products in the study. Firstly, 150 dengue NS1 positive but dengue IgM negative and 100 dengue IgM positive but dengue NS1 negative serum samples detected by ICT were used for qPCR. Among which 137 dengue I and 113 dengue II RNA positive serum samples were confirmed by qPCR, and those were then tested by LAMP assay, and the same 137 and 113 samples were detected for dengue I and dengue II serotypes, respectively. Secondly, 20 both dengue NS1 and dengue IgM negative by ICT were tested by qPCR, and no dengue I serotype was detected by qPCR or LAMP. However, among those samples, one positive dengue II serotype was detected by qPCR, and two dengue II serotypes were detected by dengue LAMP technique. Twenty healthy controls were used in both qPCR and LAMP techniques and found negative in both molecular methods (Table 2).

## Discussion

There is an urgent requirement for prompt, easy, and accurate laboratory diagnosis of dengue serotypes to treat dengue infection early and prevent its complications.

The dengue ICT strip test had high specificity<sup>11</sup>, but false-positive results may occur in individuals with active infection due to other flaviviruses, including West Nile virus and yellow fever virus. Negative results may occur if the specimen was

collected more than seven days following symptom onset.

In the present study, at first, acute serum samples were tested by ICT; after that dengue virus RNA was detected by LAMP assay and compared with qPCR by considering the qPCR as a gold standard. All of the 250 dengue qPCRs confirmed serum samples were also positive by dengue LAMP assay. Different serotypes were included to compare the serotype specificity, detection ability of dengue LAMP assay with qPCR.

Among the ICT negative serum samples (n=20), one serum sample were detected by qPCR reaction, and two serum samples were amplified and detected by dengue LAMP reaction. For confirmation of the positivity of these two samples, LAMP amplification was performed three times for that very samples and found the same positive result. This additional positively may be due to more sensitivity and specificity of dengue LAMP assay. Hu *et al.* (2015) recommended that dengue LAMP reaction is sensitive enough to detect 10-copies of RNA template, unlike PCR, which has detection limits of up to 100 copies.<sup>12</sup> Dengue serotype can be determined by LAMP assay within 45-60 minutes; however, it takes 270 minutes by qPCR to complete the whole run.

### Conclusion

RT-LAMP assay had been developed in this study which allowed the rapid and accurate identification of dengue virus serotypes. Due to its simple and rapid operation, it could be a valuable tool for the detection and differentiation of dengue virus serotypes in laboratories all over the globe, particularly in Bangladesh.

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