Original Article

A Comparative Study of LAMP and PCR in Relation to Time and Cost

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Abstract

Background*: The Loop-mediated isothermal amplification (LAMP) represents a very sensitive, easy to use, and less time consuming diagnostic method.*

Aims: *The aim was to establish a simple, cost-effective, molecular technique.*

Materials and methods: *An analytical study was conducted using two hundred acute serum samples using two different molecular techniques; qPCR and LAMP to standardize a cost-effective and less time-consuming technique.*

Results: *The cost of in-house LAMP reagents was one-ninth of the cost of commercial qPCR. Consume cost was 23 times less than qPCR besides, lab setup cost was 92 times less than qPCR. More importantly, LAMP requires 5-6 times less time duration than qPCR.*

Conclusion: *Due to its simple short-time operation with low cost, it would be a prevalent molecular technique globally, particularly in Bangladesh.*

Key Words:
Loop-mediated isothermal amplification, qPCR, Low cost, Short-time operation.

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Introduction

A new molecular technique, the Loop-mediated isothermal amplification (LAMP) initially developed by Notomi *et al*.[1,2] that represents an extremely sensitive, easy to use, and less time consuming diagnostic method. LAMP can amplify up to 10^9 copies in less than 1 hour under isothermal conditions (65°C) using simple incubators such as water baths or heating blocks making this approach suitable for fieldwork.[3,4] Since LAMP does not require any significant equipment types, it represents an ideal diagnostic tool for use in areas with limited resources.[5,6] Extraction of the Nucleic acid is the first and foremost step in many molecular biology experiments such as, qPCR, RT-PCR for which, several commercial kits have been developed to extract nucleic acid from different types of specimens which is not required for LAMP. To decrease sample processing, time, and cost, direct pathogen detection without nucleic acid extraction using a simple heat-treatment of blood or serum can be used in LAMP.[7]

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Materials and methods

A prospective analytical study was conducted from January - December 2017 at the Department of Microbiology, Immunology and Virology, BSMMU, Bangladesh. Two hundred acute serum samples were tested using two different molecular techniques; qPCR and LAMP to standardize a cost-effective and less time-consuming technique. Ten µl of serum was diluted with 30 µl PCR grade water then heated in a heating block at 100°C for 5 minutes. The amplification reaction was performed in a water bath at 62°C for 45 minutes. HNB dye (Sigma, USA) was used to detect the positive reaction by its colour change [8-9]. PCR was performed following its standard technique and required 4-5 hours. All data of this study were analyzed by the Statistical Package for the Social Sciences (SPSS) version 20, USA. The study was approved by the BSMMU's Institutional Review Board (IRB). Written informed consent was obtained from each patient.

Results

Table I

Comparison of reagent cost of LAMP and qPCR

Cost categories	Cost items	Cost estimates (Taka)	
		qPCR	LAMP
A: Laboratory setup	qPCR machine (ABI-7500DX)	56,00,000	Not required
	Vortex (Lasogene)	47,000	Not required
	Centrifuge machine (Plate)	5,60,000	Not required
	Bio-Hazard safety cabinet-clar-2A2	8,50,000	Not required
	Heat block	2,10,000	Not required
	Water bath	Not required	1,15,000
	Expert human resource	Same	Same
Total (A) =		7,267,000	1,15,000
B: Consume	Gloves (5.2/pc)	42 (8 pc)	20.8 (4 pc)
	Hexisol (40 tk./bottle)	Same	Same
	Tips (300/ 500pc)	16 (10pc)	6.64
	Tips rack (600/ rack)	Same	Same
	Micropipettes (25000/ 1pc)	Same	Same
	Serum separation tube (8/pc)	8	Not required
	Serum separation tips (4/ tips)	4	Not required
	Test tube rack (200)	Same	Same
	Eppendorf tube (800/ 500pc)	6.25 (10 pc)	1.25 (2 pc)
	Eppendorf rack	Same	Same
	PCR tube (3200/ 100pc)	3.2	Not required
	PCR tube rack	600	Not required
	Tissue role (50)	Same	Same
	Liquid soap (56)	Same	Same
	Refrigerator	Same	Same
Total (B)		679	29
C: Reagents			
RNA extraction kit (Geneaid, Biotech, Ltd, UK) 100 RXN		700	Not required
Reagent cost		1800	165
Total (C)		2500	165

Table I illustrates that lab setup cost for qPCR requires 72,67,000 taka, whereas LAMP by water bath requires 1,15,000 taka. The qPCR consumption cost was 679 taka per test where LAMP requires 29 takas per test. Reagent cost for qPCR requires 2,500 taka; however, LAMP requires 265 takas. The reagent cost of LAMP was also less than qPCR. The cost of in-house LAMP reagents was one-ninth of the cost of commercial qPCR. By contrast, consumption cost was 23 times less than qPCR, and lab set up cost was 92 times less than qPCR (Table-I).

Table II

Comparative time analysis of LAMP and qPCR

qPCR		LAMP	
Steps name	Time	Steps name	Time
Sample processing and preparation	30 minutes	Heat preparation	5 minutes
Extraction procedure	120 minutes	Extraction procedure	Not required
Initial denaturation	120 hours	Isothermal amplification	45 minutes
Denaturation			
Annealing			
Elongation			
Total	270 minutes	50 minutes	

Table II shows that LAMP's sample processing and preparation time required only 5 minutes where qPCR required 30 minutes. The extraction procedure was not required for LAMP where qPCR required 120 minutes. LAMP's amplification time required 45 minutes, whereas qPCR required 270 minutes. The sample processing and preparation time of LAMP was one-sixth timeless than qPCR. The amplification time of LAMP was a one-sixth timeless than qPCR. Overall LAMP requires 5-6 times less time duration than qPCR (Table-II).

Discussion

There is an urgent requirement for prompt, easy, and accurate laboratory diagnosis of microorganisms to treat infection early and prevent its complications. Conventional methods, which include organism isolation, immunoassay, RT-PCR, and real-time PCR, ICT, and ELISA, have many drawbacks in diagnosis as they are time-consuming, costly, and require special equipment.

The relative sensitivity and specificity of LAMP assay with qPCR were 99% and 100%. The corresponding PPV and NPV of LAMP with qPCR were 100% and 99% respectively. These results were similar to the findings of[10-11-12] Comparing with qPCR, a good agreement was observed between these two tests, indicating that the LAMP assay can be an alternative to qPCR to detect organisms immediately from human blood.

The laboratory setup cost of LAMP requires a minimal amount of expense and can be performed using only a water bath. The cost of in-house LAMP reagents and consumption cost was less than qPCR. LAMP could be performed in a short period without any expert person.

In the study of Parida *et al. (*2005), the LAMP assay developed had allowed the rapid[13] and accurate identification of the organisms[14] due to its simple operation. It would be a valuable tool for the rapid detection in well-equipped laboratories, small-scale clinical laboratories, and field situations like peripheral health care settings in developing countries.

Conclusion

Due to its simple short-time operation without sophisticated equipment, it would be a valuable tool for the rapid detection of organisms in all types of laboratory settings in Bangladesh. It would be an immensely popular molecular technique for its low cost, accuracy, and rapid detection ability throughout the world.

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