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

Rawnuck T1, Reza MS2, Khan MJR3, Khanam RA4, Munshi SU5

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.

[J Shaheed Suhrawardy Med Coll 2020; 12(2): 72-75]
DOI: https://doi.org/10.3329/jssmc.v12i2.56885

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

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

**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

| Cost categories       | Cost items                                      | Cost estimates (Taka) |
|-----------------------|-------------------------------------------------|-----------------------|
| **A: Laboratory setup** | qPCR machine (ABI-7500DX)                       | 56,00,000             |
|                       | Vortex (Lasogene)                              | 47,000                |
|                       | Centrifuge machine (Plate)                     | 5,60,000              |
|                       | Bio-Hazard safety cabinet-clar-2A2             | 8,50,000              |
|                       | Heat block                                     | 2,10,000              |
|                       | Water bath                                     | 115,000               |
|                       | Expert human resource                          | Same                  |
|                       |                                                 | Same                  |
| Total (A)             |                                                 | 7,267,000             |
|                       |                                                 | 1,15,000              |
| **B: Consume**        | Gloves (5.2/pc)                                | 42 (8 pc)             |
|                       | Hexisol (40 tk./bottle)                        | Same                  |
|                       | Tips (300/ 500pc)                              | 16 (10 pc)            |
|                       | Tips rack (600/ rack)                          | Same                  |
|                       | Micropipettes (25000/ 1pc)                     | Same                  |
|                       | Serum separation tube (8/pc)                   | 8                     |
|                       | Serum separation tips (4/ tips)                | 4                     |
|                       | Test tube rack (200)                           | Same                  |
|                       | Eppendorf tube (800/ 500pc)                    | 6.25 (10 pc)          |
|                       | Eppendorf rack                                 | Same                  |
|                       | PCR tube (3200/ 100pc)                         | 3.2                   |
|                       | PCR tube rack                                  | 600                   |
|                       | Tissue role (50)                               | Same                  |
|                       | Liquid soap (56)                               | Same                  |
|                       | Refrigerator                                   | Same                  |
| Total (B)             |                                                 | 679                   |
|                       |                                                 | 29                    |
| **C: Reagents**       | RNA extraction kit (Geneaid, Biotech, Ltd, UK) | 700                   |
|                       | Reagent cost                                   | 1800                  |
|                       |                                                 | 2,500                 |
| 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**

| 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 | Total                       | 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\textsuperscript{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\textsuperscript{13} and accurate identification of the organisms\textsuperscript{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.

**References**

1. Notomi T, Okayama H, Masubuchi H, Yonekawa T, Watanabe K, Amino N (2000). Loop-mediated isothermal amplification of DNA. Nucleic Acids Res; 28(12): E63.
2. Shu P, Chang S, Kuo Y, Yueh Y, Chien L, Sue C (2003). Development of Group- and Serotype-Specific One-Step SYBR Green I-Based Real-Time Reverse Transcription-PCR Assay for Dengue Virus Development of Group- and Serotype-Specific One-Step SYBR Green I-Based Real-Time Reverse Transcription-PCR Assay for Dengue. J Clin Microbiol; 41(6): 2408–16.
3. Lau YL, Lai MY, Teoh BT, Abd-Jamil J, Johari J, Sam SS (2015). Colorimetric detection of dengue by single-tube reverse-transcription-loop-mediated isothermal amplification. PLoS One; 10(9): 1–9.
4. Klungthong C, Gibbons R V., Thaisomboonsuk B, Nisalak A, Kalayanarooj S, Thirawuth V (2007). Dengue virus detection using whole blood for reverse transcriptase PCR and virus isolation. J Clin Microbiol; 45(8): 2480–5.
5. Markos T (2016). Loop-Mediated Isothermal Amplification Promising Molecular Diagnostic Tool for Application in Developing Countries. Adv Life Sci Technol; 49:34–43.
6. TBG (2016). Benefits of Molecular Diagnostics Research. http://www.tbgbio.com/en/ research_development/ front_benefits_of_molecular_diagnostics.
7. Njiru ZK (2012). Loop-mediated isothermal amplification technology: Towards point of care diagnostics. PLoS Negl Trop Dis; 6(6): 1–4.
8. Yung CF, Lee KS, Thein TL, Tan LK, Gan VC, Wong JGX, Lye DCL, Ng LC (2015). Dengue serotype-specific Differences in Clinical Manifestation, Laboratory Parameters, and Risk of Severe Disease in Adults, Singapore. Am J Trop Med Hyg; 92(5): 999–1005.
9. Nie K, Qi S Xiang, Zhang Y, Luo L, Xie Y, Yang M Ji (2012). Evaluation of a Direct Reverse Transcription Loop-Mediated Isothermal Amplification Method without RNA Extraction for the Detection of Human Enterovirus 71 Subgenotype C4 in Nasopharyngeal Swab Specimens. PLoS One; 7(12): e52486.
10. Sahni AK, Grover N, Sharma A, Khan ID, Kishore J (2013). Reverse transcription loop-mediated isothermal amplification (RT-LAMP) for the diagnosis of dengue. Med J Armed Forces India; 69(3): 246–53.
11. Notomi T, Okayama H, Masubuchi H, Yonekawa T, Watanabe K, Amino N (2000). Loop-mediated isothermal amplification of DNA. Nucleic Acids Res; 28(12): E63.
12. Ocwieja KE, Sherrill-Mix S, Liu CJ, Song HB, and Bushman F (2015). A reverse transcription loop-mediated isothermal amplification assay optimized to detect multiple HIV subtypes. J Virol Methods; 25(4): 1-14.
13. Parida M, Horioke K, Ishida H, Dash K, Saxena P, Jana AM (2005). Rapid Detection and Differentiation of Dengue Virus Serotypes by a Real-Time Reverse Transcription-Loop-Mediated Isothermal Amplification Assay Rapid Detection and Differentiation of Dengue Virus Serotypes by a Real-Time Reverse Transcription–Loop-Media. J Clin Microbiol; 43(6): 2895–2903.
14. Parida M, Sannarangaiah S, Dash PK PR (2008). Human papillomavirus molecular biology and disease association. Rev Med Virol; 18(1): 407–21.