

Original Article

Loop-Mediated Isothermal Amplification (LAMP) for the Rapid Diagnosis of Herpes Simplex Virus Type 1 (HSV-1)

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Abstract

Background and Aims: considering difficulties in usual laboratory methods in detection of viral infections, improved DNA-based diagnostic techniques are more reliable. Loop mediated isothermal amplification method (LAMP) is a nucleic acid amplification method that amplifies DNA using six primers which has been developed to diagnose viruses as a rapid and high efficiency test. In this study, the LAMP was used for detection of Herpes simplex virus.

Methods: The set of primers were designed, for detection of HSV-based on gG fragment. The genome of the virus was extracted from the culture supernatant of infected cells. LAMP technique was optimized in term of temperature, time and the ingredients used for test. For detection of HSV-1 infection, sensitivity and specificity of this technique were determined by various dilutions of virus and infected samples with HSV-2 that was taken from Day Hospital of Tehran.

Results: The sensitivity and specificity of HSV-1 specific LAMP method, reached about 500 copies/tube and 99.9% respectively. Furthermore, both the agarose gel electrophoresis containing Ethidium Bromide (EtBr) and the turbidity assay directly detected HSV-1 virus LAMP products in reactions.

Conclusion: In this study, the reliability of LAMP for detection of HSV- was approved; therefore this rapid, accurate, and cost-effective detection and quantification method may perhaps be an investigative tool which can be valid for detection of target viral genome in clinical specimens in the absence of the necessary facilities for performing PCR.

Keywords: Loop mediated isothermal amplification method (LAMP); Herpes simplex virus type 1 (HSV-1); Sensitivity; Specificity

Introduction

Nucleic acids amplification methods are the most valuable tests in all fields of science with brilliant applications in medical diagnosis. One of the best known and most popular methods for replicating nucleic

acids is polymerase chain reaction (PCR) method for amplifying definite target DNA/RNA sequences present within living sources Traditional PCR or newly developed Real-Time PCR require a very expensive device, use of toxic and carcinogen substances, the fluorescence detectors, and skilled personnel for performance (1).

According to the points mentioned above, a simple method is required that can overcome the limitations of previous methods and be carry out in normal and small medical

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laboratories especially applicable in a resource-limited situation.

Loop-Mediated Isothermal Amplification (LAMP) is an original method for replicating nucleic acids that introduced by Notomi *et al* (2000) (2). In this method, four primers (two inner primers and two outer primers) plus two loop primers that generally, recognize six to eight regions of the target DNA are used which makes the test highly sensitive (3). In addition, the thermal cycler is not necessary and reaction is performed in a water bath or heat block under isothermal conditions (4, 5). In the last decade, LAMP assay as a rapid, efficient and cost-effective method which has been set up to detect different kind of viral infection is considered to be use for the diagnosis of viral infection, and for prevention of viral outbreaks.

Methods

Cell culture and virus propagation

HeLa and MDCK cell lines were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 5% heat inactivated FCS, 100 IU/ml penicillin and 100 µg/ml streptomycin. All culture incubations were performed at 37°C, in an 5% CO₂ atmosphere in a humidified incubator. Cells productively infected with HSV-1 (the KOS strain) at 75–85% confluences.

Cells at the stage of 75-85% confluency were infected with HSV-1. The infected cells were scraped from tissue culture flask 72 hours post inoculation and centrifuged 5 min at 500 x g.

DNA extraction

The cell pellet was removed and resuspend in 10 ml 1X cold PBS, centrifuged for the second time and resuspend in 1 volume of digestion buffer (3 ml nuclei lysis buffer and 100 µl of 10 mg/ml Proteinase K and 400 µl of 10% SDS). The treated cells were incubated at 45°C with shaking for 12-18 hours in tightly capped tubes. Then, it was precipitated in 1 ml of 6 M NaCl and was centrifuged twice at 1700 x g. In the last step, 1/10 of the total volume 3 M sodium acetate (pH 5.2) and 2.5-3 times total volume cold 100% isopropanol were added and was shaken gently until the DNA was precipitated. The DNA was washed with 70% ethanol, dried and resuspend in distilled H₂O at 37°C overnight in Thermomixer. The DNA concentration was measured and 1-5 µl (approximately 200 ng) was loaded into agarose gel (1%) in 1X TAE buffer.

Sensitivity and Specificity of the LAMP reaction

The sensitivity of the LAMP assay was determined using 10-fold serial dilutions of standard plasmid (encoding HSV-1 gG) as template. Additionally, the specificity of this method examined with CSF infected with HSV-2 that was taken from Day Hospital of Tehran.

Specific primer sets for HSV-1 gG fragment

The LAMP assays were carried out for detection of HSV-1 using primers introduced by Enomoto *et al* in 2004 (Table 1).

Table 1. Names and sequence of primers for HSV-1 LAMP assay.

Primer	Sequence
HS1F3	5'-GCCGTTGTTCCCATATCCC-3'
HS1B3	5'-TACTTGGCATGGGGGGTG-3'
HS1F1P	5'-GTTGGGTGGTGGAGGAGACGTCCTTTTGGTTCTTGTCGGT-3'
HS1B1P	5'-GGTCGTCCTCGCATGAAGCGGCGTGGTAAGGCTGATG-3'
HS1LPF	5'-TTGGTGGGAACCCCGATAC-3'
HS1LPB	5'-AACATGACCCAGACCGGCAC-3'

This primers set is specific for HSV-1 gG fragment enable to detect specifically human herpes virus 1 gG frange

The LAMP reaction

The LAMP reaction was carried out in a 25 μ l reaction mixture containing the following reagents 4mMMgSO₄, 0.8 M betaine, 1.0 mM dNTP, 0.2 μ M each of F3 and B3 primers, 1.6 μ M each of FIP and BIP, 8UBstDNA polymerase (New England Biolabs, Beverly, MA), 1X *Bst* polymerase reaction buffer and appropriate amount of template genomic DNA. The reaction was carried out at 63 °C for 1 h and inactivated at 80 °C for 10 min (2). The amplified products were analyzed by 1.5% agarose gel electrophoresis and stained with Gel Red (iNtron Biotechnology, Inc.).

Results

Gel agarose electrophoresis of LAMP products

Figure 1 shows electrophoretic ladder-like pattern of LAMP-amplified HSV-1 gG products that is considered as a positive result for HSV-1 LAMP test. Furthermore, both the agarose gel electrophoresis and the turbidity assay directly detected HSV-1 LAMP products in positive samples.

The sensitivity of the LAMP assay was tested using different concentrations of HSV-1 DNA and plasmid containing HSV-1 gG (Fig. 1). The specificity of the assay was tested using DNA extracted from KOS-HSV-1-infected HeLa cells and HSV-2 that was approved by Real Time PCR (Fig. 2).

Discussion

Laboratory diagnosis of HSV-1 is an important public health tool, given the proven ability of this viral infection to cause enormous morbidity and financial burden particularly in immune compromised individuals (7, 8). HSV-1 causes devastating infections of newborns, including fatal encephalitis or inflammation of the brain (8, 9). Therefore, the rapid and easy diagnostic tests should be available. There are a number of molecular tests developed for rapid detection of infectious viruses (1). In the current study, we used a novel nucleic acid amplification method, LAMP, for the detection of HSV-1 infection. Using this method, virus

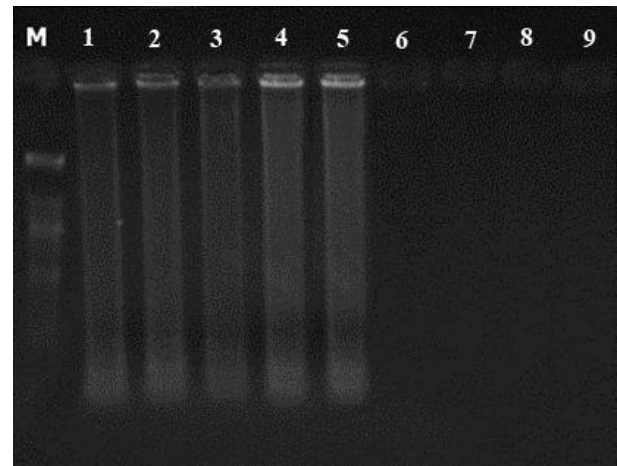


Fig. 1. Detection of Analytical Sensitivity (detection limit) of LAMP by 1.5% agarose gel electrophoresis. M: 1kb DNA Ladder; lane 1-4: 10^6 - 10^3 copies/tube; lane 5: 500 copies/tube; lane 6: 250 copies/tube; lane 7: 100 copies/tube; lane 8: 10 copies/tube and lane 9: Negative control.

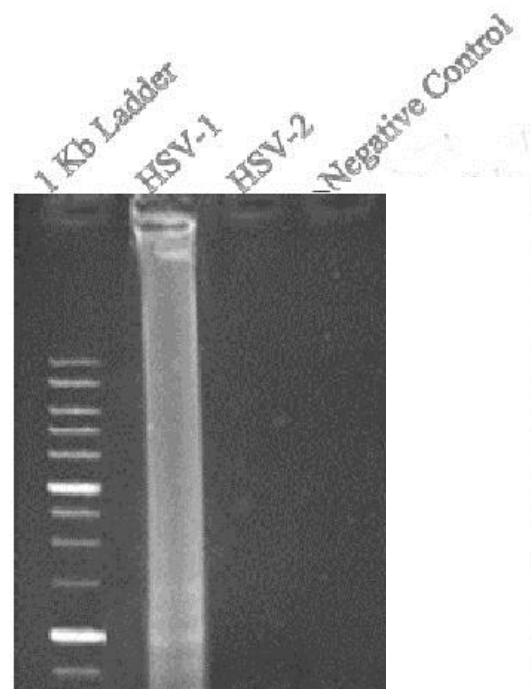


Fig. 2. Determination of Analytical Specificity. In this study, as a result of the electrophoresis, LAMP reaction only was designed HSV-1 virus.

could be identified within 45 min of DNA extraction (10). Since there is high homology among the nucleotide sequences of HSV-1, we chose sequence specific for HSV-1 in the gG genes fragment. Consequently, we used published primers to target these regions.

Recent studies consider the high sensitivity of virus detection by PCR in comparison with viral antigen detection or cell culture; although a qualitative study with Real-Time PCR is most reliable and efficient method that has been developed for detection of infectious agents yet. In generally, PCR-based techniques require special devices that may be unavailable in every laboratory (11). Notomi *et al* (2000) first described an autocycling strand displacement DNA synthesis using the *Bst* DNA polymerase, the LAMP method is carried out widely in diagnostic clinical laboratories for detection of bacteria and viruses (12). The LAMP assay as a rapid and cost-effective method does not require thermocycler and gel electrophoresis in some cases (using turbidity) and the inhibitory effects of substances in samples are largely eliminated (4, 13). A reliable LAMP mostly depends on the specificity of the primer sets (4).

Adequate Mg^{2+} concentration is essential to form Magnesium pyrophosphate salt in LAMP reaction, the insoluble salt causes in growing turbidity as the reaction proceeds. Therefore, different concentration of $MgSO_4$ (4-8mM) were experienced in LAMP mixture to optimize its effect on turbidity but after the reactions were completed no visual changes were observed.

In conclusion, this method employs a DNA polymerase and a set of specific primers that recognize a total of six distinct sequences on the target DNA/cDNA. Expensive apparatus are not necessary to obtain a high level of accuracy, and there is fewer preparation steps compared to conventional PCR-based and real-time PCR assays. Current study briefly summarized the applications of LAMP method in imperative pathogenic microorganisms. In addition, LAMP assays were applied to the rapid detection of most significant human pathogens in various clinical specimens.

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