Review Article

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Comparative Advantage of the Novel Loop-Mediated Isothermal Amplification Technique over the Conventional Polymerase Chain Reaction

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Abstract

The role of gene amplification technology in the area of diagnostic medicine and other aspects of molecular biology has been very laudable. Molecular diagnostic has demystified the gray areas in disease diagnosis, making the process of disease management much easier and efficient. Several advancements in molecular diagnostics have been largely due to the introduction of the polymerase chain reaction (PCR). Hence, there is no doubt about the rank of the PCR, as one of the greatest molecular biological tools that existed in the last few decades. However, in this article we highlight the comparative advantages of a novel gene amplification technique called loop-mediated isothermal amplification (LAMP) over the conventional PCR. This innovative gene amplification technique has provided a solutions to the odds and limitations of the PCR through the unique features of the technique. The most interesting aspect of the LAMP, which is in contrast to the PCR is the affordability of the tool, hence, gives hope to the poor and resource-limited settings of the society. Other unique features of this novel technique are highlighted in this article.

Key words: Amplification, Diagnosis, DNA polymerase, Gene, Molecular diagnostic

INTRODUCTION

Accurate diagnosis is key in effectively treating, preventing, and achieving excellent prognosis. Over the last few decades, nucleic acid-based diagnostic techniques have tremendously improved disease diagnosis, limiting some of the challenges of disease diagnosis that previously existed. Despite the tremendous success recorded by the introduction of molecular diagnostic, widely credited to the use of polymerase chain reaction (PCR) there are still loop holes left to be filled. Development of a novel gene amplification technique called loop-mediated isothermal amplification (LAMP), has since gone to address those gray areas in molecular diagnostics. The LAMP is a unique nucleic acid amplification technique developed 14 years ago.[1] It has since been applied in numerous nucleic acid researches, and in clinical application as a screening tool.[2] It is a single tube technique that amplifies with high precision, few copies of DNA into billion copies within an hour.[1,3] It is a very rapid, sensitive, and efficient gene amplification technique.[1]

LAMP is increasingly gaining attention among researchers due to outstanding results obtained from numerous research work carried out with the technique (Table 1). It is an ideal tool for diseases diagnosis, with unique qualities which eliminate the odds of the PCR and other pre-existing molecular methods.[4] Techniques such as the nucleic acid sequence-based amplification, self-sustained sequence replication, strand displacement amplification, rolling circle amplification, and most prominently PCR are among the pre-existing molecular techniques.[5-9] These listed techniques have some common limiting factor which is well-addressed by LAMP. Most apparent is the relatively high cost of application, this has limits there use mostly to highly resourced facilities, like teaching hospitals, and

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Table 1: Pathogens of human and veterinary importance detected by LAMP

Target host	Pathogens (genome)	Classification of pathogen	References
Human	West nile virus	Virus(RNA)	[17]
Human	Japanese encephalitis virus	Virus (RNA)	[20]
Human	Norovirus	Virus (RNA)	[21]
Human	Dengue virus (1, 2, 3, and 4)	Virus (RNA)	[18]
Human	Avian influenza virus (H5N1)	Virus (RNA)	[22]
Human	HIV	Virus (RNA)	[23]
Human	BK virus	Virus (DNA)	[24]
Human	Rubella virus	Virus (DNA)	[25]
Human	Human papilloma virus (6, 11, 16, and 18)	Virus (DNA)	[26]
Human	Vericella-zoster virus	Virus (DNA)	[27]
Human	Adeno virus keratoconjunctivitis	Virus (DNA)	[28]
Human	Human herpes virus 6	Virus (DNA)	[29]
Avian	Newcastle disease virus	Virus (DNA)	[30]
Avian	Infectious bursal disease virus	Virus (RNA)	[31]
Avian	Mareks disease virus	Virus (DNA)	[32]
Avian	Infectious bronchitis virus	Virus (RNA)	[33]
Avian	Chicken infectious anemia virus	Virus (DNA)	[34]
Avian	Goose circo virus	Virus (DNA)	[35]
Canine	Canine parvo virus	Virus (DNA)	[36]
Equine	Equine Rota virus	Virus (DNA)	[37]
Caprine	Capripox virus	Virus (DNA)	[38]
Caprine	Capripox arthritis encephalitis virus	Virus (DNA)	[39]
Porcine	Porcine cico virus	Virus (DNA)	[40]
Porcine	Porcine cytomegalovirus	Virus (DNA)	[41]
Porcine	Classical swine fever virus	Virus (DNA)	[42]
Camel	Camel pox virus	Virus (DNA)	[43]
Human	*Vibrio cholarae*	Bacteria	[44]
Human	*Camplylobacter* spp.	Bacteria	[45]
Human	*Mycobacterium ulceran*	Bacteria	[46]
Human/animal	*Bacillus antracis*	Bacteria	[47]
Human/animal	*Listeria monocytogene*	Bacteria	[48]
Human/animal	*Escherichia coli*	Bacteria	[49]
Human/animal	*Staphylococcus aureus*	Bacteria	[50]
Human/animal	*Brucella* spp.	Bacteria	[51]
Porcine	*Yesrsiniaenterolitica*	Bacteria	[52]
Fish	*Vibrio alginolyticus*	Bacteria	[53]
Dog	*Anaplasmaphagocytophilum*	Rickettsia	[54]
Human	*Pneumocystis pneumonaea*	Fungi	[55]
Human	*Candida* spp	Fungi	[56]
Human	*Plasmodium falciparum*	Protozoan	[57,58]
Human	*Gardiaduodenalis*	Protozoan	[59]
Human	*Schistosmamansoni*	Protozoan	[60]
Human	*Leishmania* spp.	Protozoan	[61]
Human/animal	*Trypanosoma* spp.	Protozoan	[62]
Human	*Taenia* spp.	Tape worm	[63]
Ovine	*Theileria* spp.	Protzoan	[64]
Canine	*Babesiacanis*	Protozoan	[65]
Canine	*Echinococcusgranulosus*	Tape worm	[66]

LAMP: Loop-mediated isothermal amplification

in diagnostic centers found in countries with a robust economy.

STANDOUT PROPERTIES OF LAMP

LAMP employs four to six specially designed primers to hybridize six to eight distinct regions of a target gene. Hence gene amplification is done with high efficiency and precision.[1,3] In a LAMP reaction, all primers must have specific sequences to that of the target gene for amplification to commence, this strict principle of LAMP ensures high efficiency and reliability of the technique. LAMP uses a special polymerase enzyme called *Bst* DNA polymerase; this enzyme exhibits strand displacement activity as it extends annealed primer sequence.[10] This strand displacement activity is uniquely elicited by the LAMP polymerase enzyme without exonuclease activity at the 5'-3' as in the case of other strand displacement and isothermal nucleic acid amplification techniques.[1,11] Single-stranded DNA molecule can be obtained from this novel technique; this is achievable true the unique property of the *Bst* DNA polymerase.[11,12] Other standout properties of LAMP are enumerated below.

SIMPLICITY AND COST EFFECTIVENESS

The simplicity and cheapness of LAMP give room for its application both in resourced and resource-limited settings, the assay only requires a simple water bath or heat block, unlike the conventional PCR that requires a sophisticated instrument, such as thermo cycler, and the electrophoresis setup.[1,13] Although LAMP is a technically sound technique; however, it is simple to perform due to it straightforward principles, a semiskilled personnel can effectively perform the assay, and the protocols are highly comprehensive with no sophisticated instruments required. The reaction is rapidly and efficiently conducted in a single step. As illustrated in (Figure 1), LAMP result can be detected through visualization of turbidity resulting from accumulation of pyrophosphate ion released as byproduct in positive LAMP reaction.[11]

Real-time monitoring of LAMP can be accomplished, through spectrophotometric analysis using an inexpensive Real-time turbidimeter.[14] LAMP amplicon are quantified through Real-time monitoring of the amplification. The generation of a standard curve derived from plotting known concentration of gene copy number against time of positivity is followed to analyze the LAMP product.[2,14] Therefore, detection and quantification of LAMP product does not employ electrophoresis, which requires more time and extra working resources (Figures 1 and 2). Use of ethidium bromide in electrophoresis, a potential carcinogen is avoided. Mores on LAMP properties are listed in Table 2.

UNIQUE AND RAPID AMPLIFICATION

The ability of LAMP to amplify a target gene in a semi or unprocessed sample such as blood has taken the robustness of this technique to a greater level. The *Bst* DNA polymerase enzyme commonly used in LAMP has been demonstrated to be resistant to the presence of anticoagulants, hemin, N-acetylcysteine, Nacl, and other PCR-inhibiting substances.[15,16] Application of this property of LAMP will be beneficial in health care centers, where accurate and rapid diagnosis is most desired. The process will be much more rapid since the template extraction step has been omitted owing to this amazing property of LAMP.

EFFICIENCY

LAMP is a highly sensitive technique that amplifies few copies of template DNA in a reaction to a detectable level.[1] Forensic samples can be amplified with LAMP, as low as femtogram levels of DNA can be detected in a sample with few and degraded DNA copies.[4] It is considerably more sensitive than the PCR, the sensitivity of LAMP over PCR is evident in these studies, which

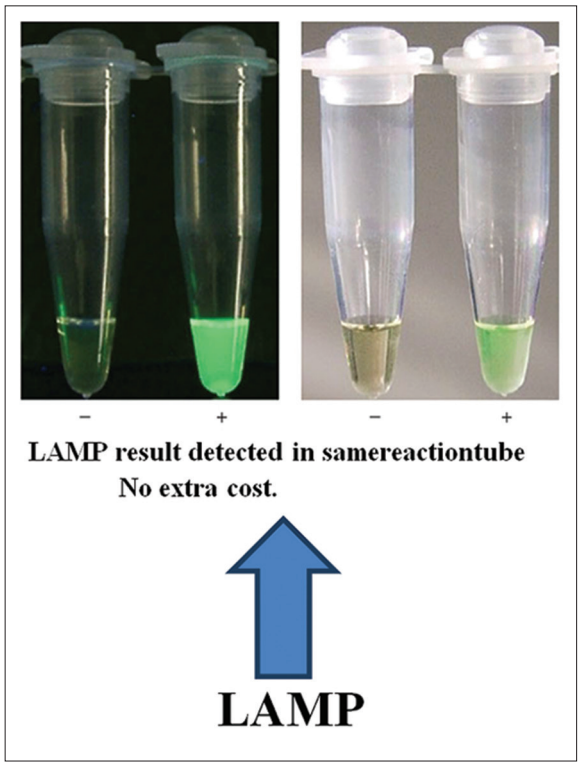

Figure 1: Amplification detection procedures (loop-mediated isothermal amplification)

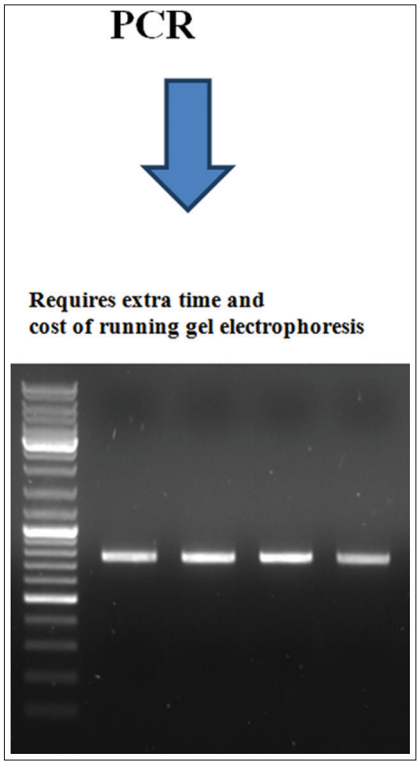

Figure 2: Amplification detection procedures (polymerase chain reaction)

Table 2: Comparative analysis of LAMP and PCR base on general principles of application

Property	PCR	LAMP	References
Amplification time	3-4 h	1 h	[1,3]
Specificity	High	Higher	[2,12]
Sensitivity	High	Higher	[18,19]
Simplicity	Sophisticated technique	Simple technique	[1,4]
Affordability	Expensive	Cheap	[1]
Accessibility of material	Readily available	Scarce	[2]
Purity of amplicon	Prone to carry over contaminations	Lower contamination risk due to closed tube system	[2]
Detection of result	By gel electrophoresis	Visual detection with naked eyes	[10]
Primers	Simple primer design (2 primers) involved	Complicated primer design (4-6 primers) involved	[2]
Stability	Inhibited by presence of impurities hence, only purely processed sample are amplified	Stable against sample impurities, can amply template DNA in a semi or unprocessed sample	[16]

LAMP: Loop-mediated isothermal amplification, PCR: Polymerase chain reaction

shows a 10-100 fold higher sensitivity than PCR with a detection limit of 0.01-10 Pfu of virus.[17,18] Accuracy of LAMP reaction is attributed to the nature of primers used.[3] Furthermore, high specificity of LAMP has been demonstrated through its ability to selectively amplify few copies of target DNA without interference of genomic DNA.[19] This remarkable specificity reflects on the strict adherence of four to six LAMP primers hybridizing to six or eight distinct regions of the target gene.[1-3]

Comparative Analysis of LAMP and PCR Base on Technique Essential Items and Protocols

PCR	LAMP
Thermocycler	Water bath or heat block
Thermo pol buffer	Thermo pol buffer
Taq polymerase enzyme	*Bst* DNA polymerase
DNA dye (optional)	DNA dye (optional)
Ethidium bromide	Not required
Electrophoresis setup	Not required

AMPLIFICATION PROTOCOL

PCR

The PCR amplifies DNA under three different temperature cycles, starting with the denaturation, followed by an annealing step and finally ends with the extension of the annealed primer sequence, annealing temperature varies according to the primer melting temperature (Tm), usually falls within the range below.

Denaturation ⟶ Annealing ⟶ Elongation

92°C-95°C 55°C-60°C 70°C-74°C

LAMP

Amplification begins and ends under the isothermal condition of about 60°C-65°C. The *Bst* DNA polymerase aids the annealing of outer primers to a double stranded template DNA, exhibiting its unique strand displacement activity.[1,12]

FUTURE PERSPECTIVE

Prompt and accurate disease diagnosis is a prerequisite for effective treatment and management of disease. The role of molecular diagnostic in health management is indispensable. The uniqueness of LAMP, which ranges from high stability, rapidity, simplicity, and cost effectiveness, has perhaps placed the novel technique above other molecular techniques. The high operational cost has been the most apparent limitation of PCR; LAMP promises to fill this gap limiting the use of PCR to only highly resourced settings. Several advancement has been made on LAMP since its advent, presently five versions of LAMP exist. They include the eLAMP, lyophilized LAMP, lateral flow assay LAMP, micro LAMP, and real-time monitoring and quantification LAMP. Pathologists and clinicians will benefit immensely from these advancements, due to the enhanced features introduced to the technology, making it more suitable in point of care diagnosis. Hence, continuous research is needed to fully explore and utilize the potentials of LAMP toward achieving a robust diagnostic tool. As enumerated in our previous review, unique principle of LAMP that made it very suitable can serve as a platform for improving on other techniques like the PCR.[67] The *Bst* DNA polymerase enzyme with strand displacement property is thought to be the most innovative features of LAMP.

CONCLUSION

The possible use of the powerful LAMP polymerase enzyme in PCR application will perhaps be a good area of research; this will no doubt lead to a major breakthrough in molecular diagnostic, by making the PCR much more simplified and cost-effective.

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