Review Article

Annals of Clinical and Laboratory Research
ISSN 2386-5180

2022
Vol.10 No.8:429

Loop-Mediated Isothermal Amplification: A Rapid Tool for Microbial Diagnosis

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Received: 05-Aug-2022, Manuscript No. IPACLR-22-10697; **Editor assigned:** 09-Aug-2022, PreQC No. IPACLR-22-10697(PQ); **Reviewed:** 23-Aug-2022, QC No. IPACLR-22-10697; **Revised:** 26-Aug-2022, Manuscript No. IPACLR-22-10697(R); **Published:** 31-Aug-2022, DOI: 10.36648/2386-5180.22.10.429

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Citation: Agbaje S (2022) Loop-Mediated Isothermal Amplification: A Rapid Tool for Microbial Diagnosis. Ann Clin Lab Res. Vol.10 No.8:429

Abstract

The contributions of nucleic acid amplification to the field of medical science cannot be overemphasized. Polymerase Chain Reaction (PCR) has been the most preferred technique of nucleic acid amplification over the years as a result of its high sensitivity. However, PCR limitations which include high cost of set up and high level of expertise needed makes it a setback to many researchers. Loop Mediated Isothermal Amplification (LAMP) was introduced to fill these bridges. In this review, previous studies of LAMP are highlighted, laying more emphasis on its types, usage, detection of its amplicons as well as its merits and demerits. LAMP advantages include its simplicity, cost effectiveness, rapidity, specificity and direct use of sample from site of infection. The various application of LAMP is also discussed. We have concluded that Since LAMP conform to the criteria set by the World Health Organization for an ideal diagnostic tool, LAMP will continue to be a valuable and important tool for diagnostic and research both in developed and developing countries in the future.

Keywords: Amplification, Polymerase Chain Reaction (PCR), Primer, Pathogens, Protozoan, Bacteria, Viruses, Fungi

Introduction

In 1980s, the Polymerase Chain Reaction (PCR) was invented by Kary Mullis and his group [1]. Since then, Nucleic acid amplification tests have contributed immensely and have become essential in the field of life sciences [2]. As a result of its high sensitivity, polymerase chain reaction has become the Gold standard and widely accepted test for Nucleic acid amplification. However despites this, there are quite a number of setbacks tailback to many researchers which includes but are not limited to high cost of set up, high level of expertise and a high turnaround time [3]. Although there have been improvements to the Polymerase chain reaction over the years, there still exist some limitations [4].

The advent of loop mediated isothermal amplification has helped in bridging these gaps. Loop mediated isothermal amplification is an isothermal gene amplification method developed [5,6]. LAMP usage includes identification and detection of infectious agents, studying genes and other applications in research. LAMP is a molecular technique for gene amplification using multiple primers (usually 4-6 set of primers that binds to different regions, 8-10 of the target gene). The primer set consists of two inner primers (Forward Inner Primer (FIP) and Backward Inner Primer (BIP) and two outer primers (F3 and B3) [7]. It occurs at a constant temperature within range of 60°C-65°C LAMP reaction is done using DNA polymerase with high displacement activity. Detection of its amplicon is usually through monitoring with naked eye using stains such as SYBR green and Ethidium Bromide.

Simplicity, cost effectiveness, rapidity, specificity and direct use of sample from site of infection are among the advantages Loop mediated isothermal amplification has over other gene amplification techniques. Although, LAMP has its own demerit, the benefit surely trumps its disadvantages [8]. This article is aimed at reviewing previous studies of LAMP while shedding more light on its types, usage, detection of its amplicons as well as its merits and demerits.

Literature Review

Different forms of LAMP

Since the advent of loop, mediated isothermal Amplification. In 2000, several modifications have been made to it. Under this aspect, different modifications to LAMP will be explained including the conventional LAMP, Reverse Transcriptase LAMP, Multiplex LAMP and Real time LAMP [9].

Conventional LAMP

The conventional LAMP is the initial assay developed. It amplifies gene with the help of multiple primers and at a constant temperature within the range of 60°C-65°C using Bst DNA polymerase with high displacement activity.

The conventional LAMP had a greater potential for developing quick and sensitive visuals detection methods, than other conventional PCR strategies in *Alternaria solani.* The conventional loop mediated isothermal amplification was used to diagnose the bacterium that causes meningitis [10]. LAMP was also used for detection of viruses, for instance adenovirus was detected in 2015 [11].

At the end of the surgery, after ensuring full asepsis the transversus abdominis plane block, and posterior rectus sheath block was administered by an ultra sound guided approach before extubation. Patients were randomised into two groups to receive either local anaesthetic infiltration of the laparoscopy port sites (n=40, Group A/standard group) and USAFBs (n=40, Group B/study group) using a total dose of 30 ml of ropivacaine 0.2% with sterile technique. Randomisation was done by flipping of coin method.

Reverse Transcriptase Loop Mediated Isothermal Amplification (RT-LAMP)

PCR is used in amplifying RNA by Reverse Transcription. In RT-LAMP, complementary DNA (cDNA) is synthesized from the RNA template followed by using the LAMP assay for amplification and detection [12, 13]. In addition to the reagent (the multiple primers, Bst DNA Polymerase with strand displacement activity), reverse transcriptase is added to the reaction mixture at a constant temperature between 60°C-65°C prior to detection.

RT-LAMP has been used in detection of wide range of viruses. The rapid diagnosis of SARS-CoV-2 using RT-LAMP, also in 2018, *Mycobacterium tuberculosis* using RT-LAMP.

Multiplex LAMP

Like Multiplex PCR, Multiplex LAMP is another modification to loop mediated isothermal amplification. This is aimed at simultaneous detection of multiple targets/genes in a single reaction, this enhance this specificity of the assay in 2015, [14]. Used multiplex LAMP in combination with Reverse transcriptase LAMP to detect Dengue virus, this result was compared with reverse transcriptase PCR and ELISA detection of multiple gene, the mcr genes (mcr-1 to mcr-5) contained by colisitin resistant bacteria using the multiplex LAMP assay.

Detection of LAMP products

Various methods have been adopted for the detection and visualization of LAMP reaction. Visualization of the turbid white magnesium pyrophosphate, agarose gel electrophoresis and precipitation titration of LAMP amplicons by adding Polyethylenimine (PEI) are the most common ways.

In agarose gel electrophoresis, the use of DNA intercalating agent such as stains like SYBR green and Ethidium Bromide (EtBr) are adopted [12-17]. The LAMP products is subjected to agarose gel electrophoresis, stained with Gel stain dye and visualized using chemi Image 5000 analyser. The appearance of ladder-like bands pattern on the agarose gel is marked positive amplification.

Also, the monitoring of the increase in turbidity of the reaction mixture or visualization using naked eye by noticing change in color negative reaction may remain orange while positive reactions turned green under UV-light [18]. In 2006 showed that addition of a low molecular weight PEI can be added to the LAMP product to form insoluble product-LAMP that contain fluorescently labeled probe which can then be visualized with UV-illumination.

Merits of LAMP

The novel diagnostic method has numerous distinct advantages which have been highlighted by researchers since its inception. According to the World Health Organization, the ideal properties of a diagnostic kit includes sensitivity, specificity, simplicity, adaptability to any climate condition, availability of instruments and affordability [19]. The LAMP method is famous for its high sensitivity and specificity. Other merits of this method include speed, safety easy detection and low cost [20]. Merits of LAMP include but are not limited.

Amplification of nucleic acid using LAMP is conducted under isothermal conditions (between 60°C-65°C). The method of nucleic acid amplification takes less time to perform when compared to other nucleic acid amplification method and attain a result [21,22]. There is no need for denaturation of the DNA template in its initial stage and thus, the reaction takes place rapidly. Another advantage of the LAMP method is its simplicity. It only requires the use of a water bath or heating block to attain an isothermic condition, unlike other amplification methods which requires the use of sophisticated instruments e.g. thermo cyclers. Also in the analysis of result, readings can be taken by mere visualization using the naked eye due to the presence of dye methods such as PCR however would require equipments such as electrophoresis to take the result. LAMPS possess a high specificity it is able to amplify a specific gene selectively from the human genome, ignoring the slightest nucleotide difference. The presences of unwanted DNA have little effect on LAMP as compared to other methods of nucleic acid amplification. The method is cost effective; it utilizes cost effective equipments for its reactions. For LAMP, Amplification and detection of nucleic acid sequences can be accomplished in a single step. The gene sample mixture, primers, substrates and DNA polymerase are all incubated at a constant temperature. Amplified products can be seen directly through its level of turbidity. LAMP is highly tolerant it is able to still work efficiently in the presence of some inhibitory materials (culture medium and other biological components) [23]. Only one set of primers is needed for the entire method of targeted DNA amplification. When DNA polymerase is combined with reverse transcription, LAMP can efficiently amplify RNA. LAMP is highly sensitive; it was reported that in a reaction mixture, this amplification method is capable of detecting at least 6 copies of DNA.

Demerits of LAMP

- One of the limitations of LAMP is that it can only be used in the laboratory evaluation in clinical settings is limited.

- The equipment and reagent used for LAMP are not readily available in some countries.
- The primer designs are complicated.
- Multiplexing is considered less effective.
- False-positive result; this is usually as a result of carry over
- contamination resulting to a control with false-positive result.
- LAMP is not versatile as it cannot be utilized for other biological process such as cloning etc.
- It is not automated; hence all processes are conducted manually.

Usage of LAMP

Due to its numerous advantages, LAMP is fast gaining the grounds as a reliable tool in infectious disease diagnostics in hospitals as well as in the rapid detection of food pathogens [24]. This method has been successfully used in detecting microbes (Fungi, Bactaeria, Viruses and Protozoans).

Application in the detection of microbes

Bacteria are of economic importance to man, plant and the environment, causing infectious diseases as well as food poisoning. LAMP has been reported to have successfully detected bacteria pathogens. In 2003, 3 *Mycobacterium sp*; *Mycobacterium tuberculosis, Mycobacterium avium* and *Mycobacterium* intracellulure using the LAMP assay. Also, reported the detection of *Mycobacterium tuberculosis*, a human respiratory pathogen, using LAMP method [25]. The detection of *Streptococcus pneumonaiae* using this LAMP technique was reported. The successful detection of food pathogens have been documented as follows; *Salmonella typhi*, 4 *Helicobacter pylori*, *Campylobacter jejuni* and *Campylobacter coli* [26]. Other pathogenic bacteria which have been successfully detected by this method include *Neisseria meningitides*, *Bacillus anthracis*, and Methicillin-resistant *Staphylococcus aureus* [27-29]. LAMP has successfully been able to detect bacteria rapidly and efficiently in a little amount of time and at a low cost.

Application in the detection of viruses

With LAMP, both RNA and DNA viruses have been successfully detected and diagnosed. Over the years, LAMP method for the detection of DNA viruses has been documented. In 2016, reported the successful detection of Hepatitis B virus [30-33]. The result from this works did not only detect the presence of the virus, but also showed that the LAMP method has potentials for the diagnosis of hepatitis, epidemiological studies, screening for a donor as well as for therapeutic monitoring of patients undergoing antiviral treatment. Also in 2003, the LAMP method in the detection of tomato yellow leaf curl virus DNA. In 2010, stated that LAMP assay could be used as portable devices in laboratories. This suggestion was derived from their results after the successful diagnosis of Porcine parvovirus using LAMP assay. In 2009, the successful detection of RNA virus. This was achieved by the addition of reverse transcriptase to the reaction mixture. The method has also been reported as an effective tool in the diagnosis and control of COVID-19 in developing countries [34]. LAMP assay has been used in various forms in the detection of corona virus RNA in patient's samples.

Application in the detection of Protozoans/ Parasites

In the detection of parasites, LAMP has been used in detecting Plasmodium. The LAMP assay significantly reduces the threshold of malaria detection and as such may serve as a prospective new tool in diagnoses, surveillance and screening of malaria in malaria-endemic regions [35]. He reported that the efficient detection of *Plasmodium faciparum* using LAMP method. The detection of plasmodium spp using the LAMP technique [36]. Other parasites detected with the use of LAMP technique include, *Trypanosoma*, *cryptosporidium* [37,38]. The efficient and rapid detection of *Neospora caninum*, a parasite which causes neurological disorders in dogs and also affects livestock [39]. The LAMP assay could be used in confirming the diagnosis of canine or bovine neosporosis [40]. This conclusion resulted from his successful detection of *Neospora caninum* using the LAMP method. Sensitivities of 80% to 100% and specificities of 94% to 100% have been reported in the use of LAMP in the diagnosis of human leishmaniasis.

Application in the detection of fungi

The application of LAMP was first documented in 2015 that successfully used it in the detection benzimidazole resistant isolate in *Sclerotina sclerotonium*. The detection of *Brettanomyces* in 2007. In 2004, successfully used LAMP technique to detect *Paracoccidiodes brasilliensis* [41-43]. Other successfully usage of LAMP to detect fungi has been reported as follows; *Candida albicans*, 44 and *pneumocytis jirovecii*.

Application in the identification of embryo gender

The use of LAMP in the predetermination of embryo gender has been reported in 2006, who established a procedure to determine the embryo gender of water bufolo.

Aquaculture

LAMP has been employed in the detection of viral and parasitic pathogens found in fish. This helps to screen infected fishes to avoid its consumption by human, thereby preventing illness and death.

Future perspectives

Due to its miniaturization and simple procedure, which would reduce contamination and automate complex analysis processes to become a one-in-all reaction, this review will provide us with a better understanding of the advantages of our most required analysis system, i.e., lab on a chip. Micro fluidic instrumentation chips are another potential trend in the usage of LAMP for on-site testing as virology has brought a proper insight to that. LAMP limitation in mycological research also would increase attempts made to apply LAMP to fungal organisms as time goes on, especially for diagnosis of medically important organisms [44-47].

LAMP-based diagnostics will not, in the near future, be able to substitute sequencing-based identification or traditional microbiological examination of microbial pathogens in clinical, food, or environmental laboratories. It will, however, be useful in all applications where rapid and sensitive identification of organisms is required to support decision-making regarding appropriate measures, such as hygiene and isolation testing, and food quality management. In addition, the development and deployment of LAMP-based systems for quarantine organism identification, particularly plant pathogens, will become a rising field in the future, as it will significantly speed up the release of plants and animals during import. Since lab-on-a-chip diagnostic equipment has been accepted, we should expect acceptance and advancement to LAMP.

Conclusion

Loop Mediated Isothermal Amplification (LAMP) is gaining more awareness due to its numerous advantages which include cheaper cost, easier detection, High sensitivity and specificity among others. LAMP have been reported to be useful in various field of Life and Medical sciences, this includes the detection of wide range of microbes, including viruses, parasites, fungi and bacteria. It has also been used in detection of embryo sex, aquaculture and detection of food borne pathogens. The direct use of samples or specimen for amplification without need for prior extraction gives LAMP the edge over other nucleic acid amplification techniques including the conventional polymerase chain reaction.

While LAMP technique has its own demerits which include primer design problem, the less availability of its reagents in some countries, it is certain and obvious that the advantages outweigh the disadvantages. Since LAMP passed the criteria set by the World Health Organization for an ideal diagnostic tool. LAMP will continue to be a valuable and important tool for diagnostic and research both in developed and developing countries.

Conflict of Interest

There is no conflict of interest among the authors.

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