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REVIEW

APPLICATION OF LAMP AS A POINT-OF-CARE DIAGNOSTIC ASSAY IN REMOTE AND LOW-INCOME REGIONS: MINI-REVIEW

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Abstract. *Loop-mediated isothermal amplification (LAMP) has received increased attention as a sensitive and fast molecular technique that could be applied at the site of need. The LAMP has been shown to specifically detect a variety of bacterial and viral infections as a promising point-of-care (POC) test. LAMP has recently demonstrated sensitivity and specificity comparable to or nearly equal to polymerase chain reaction in SARS-CoV-2 detection. The main advantages of LAMP are its application in low-income or remote areas where there are no specialized laboratories or molecular specialists. In the following mini-review we discuss the potential of LAMP as a POC molecular diagnostic method. Moreover, we summarized its validated application and future challenges.*

Key words: *LAMP, point-of-care, diagnostics*

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INTRODUCTION

As a DNA or RNA amplification technique with high specificity and sensitivity, PCR (polymerase chain reaction) is the gold standard. The technique necessitates the use of sophisticated laboratory equipment such as thermocyclers or real-time PCR machines, vortexes, centrifuges, heat blocks, etc. The targeted genomes, DNA or RNA, are exponentially amplified using specific primers at repeating cycles with different temperatures. Each cycle consists of reverse transcription (RT), if the targeted genome is RNA, and three repeating steps of DNA denaturation, primer annealing, and DNA elongation. The process and result interpretation require trained personnel, as well as expensive laboratory equipment and reagents.

Nonetheless, analyses at the point of need are in high demand in some regions with limited resources as well as in more distinct areas. Moreover, the COVID-19 pandemic has demonstrated that a large portion of the world's population has limited access to laboratory diagnostics. Therefore, using the gold standard PCR for global population diagnostics is not universally applicable. When widespread and regular testing is required, more rapid and easy to use diagnostic methods are needed.

Recently, a huge amount of scientific interest and efforts have been directed toward the development of point-of-care (POC) tests and remote digital analyses [1, 2]. The loop-mediated isothermal amplification (LAMP) is one of the methods suitable for POC testing in low-resource areas (Fig. 1). Furthermore, the shortage of diagnostic capacity, specialists and reagents during the COVID-19 pandemic, has urged improvements in the LAMP as an alternative to the PCR. Compared to the gold standard PCR, LAMP

is faster, cheaper and does not need highly trained personnel. Additionally, the LAMP method does not require expensive equipment because the nucleic acids are amplified at a constant temperature. Additionally, LAMP is less susceptible to inhibitors compared to the PCR technique.

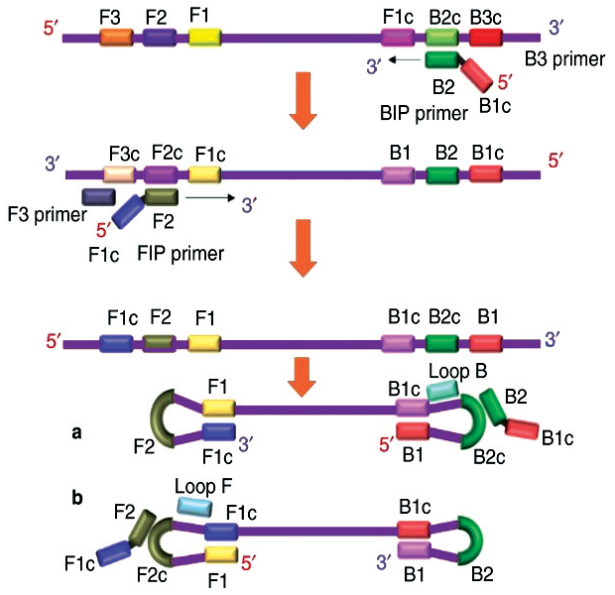

Fig. 1. LAMP reaction. FIP – Forward Inner Primer involves F2 region at the 3'-end and F1c region at the 5'-end; BIP – Backward Inner Primer involves B2 region at the 3'-end и B1c region at the 5'-end. F3 (Forward Outer Primer) involves F3 complement to F3c region of the target; B3 (Backward Outer Primer) involves B3 region complement to B3c region of the target. LF and LB (Loop forward/ backward primers) increase LAMP amplification

LAMP has been used for analyses of a variety of samples such as saliva, swabs, urine, blood, feces with the common steps of cell lysis, nucleic acid purification and elution. Interestingly, recent research has shown that LAMP strand displacing polymerase is tolerant to different sample inhibitors and reaction might be performed in crude samples [2, 3].

Bst DNA polymerase amplifies nucleic acids at a constant temperature of 60-70 degrees Celsius. Amplified matrix could be analysed using fluorescence reader, naked eye and lateral flow immunoassay. The visual interpretation of LAMP results using the naked eye has been largely studied and optimized for POC application, particularly during the COVID-19 pandemic.

LAMP ADVANTAGES

LAMP has been largely studied due to its advantage and application as a POC test in low-resource areas for infectious disease detection [4] and even as a home test. LAMP reaction might be visualized using colour or intercalating dyes, by chemiluminescence, turbidimetric detection as well with fluorescence. The presence of an amplified target causes colour change when using colorimetric visualization. This is a simple method to determine positive reactions without sophisticated equipment. In LAMP and RT-LAMP assays, pH-sensitive dyes such as phenol red, cresol red, and neutral red were used [5]. Compared to turbidimetry, colorimetric and intercalating dyes visualization of LAMP might increase the detection sensitivity and differentiate false and true negative results. Furthermore, some intercalating dyes applied for melt curve analysis might be used for multiplex LAMP assays as well as reducing open-tube carryover contamination [6]. The constant reaction temperature is the main advantage of the method, which can be achieved with basic equipment such as a water bath or heat block if a thermocycler is not available.

LAMP DISADVANTAGES

LAMP has some critical moments as a molecular technique. For example, the application of LAMP methods is associated with in-house primer design, which might be difficult because up to 8 regions on the target should be specifically recognized. Moreover, some LAMP primer sets might be non-specific or with low amplification capacity, thus, primer redesign and optimization might be time-consuming until the desired sets of primers are selected [4]. The need to design four or six primer sets for each target is one of the challenges for multiplex LAMP assay. Many free software have not been programmed for loop primers, which frequently need to be manually designed and adjusted. Another critical moment is the carryover contamination due to the high amount of amplified targets which might lead to false positive LAMP results [7] and frequently used open – tube analysis of results increases the rate of false-positive results [8]. Additionally, primer dimers, free energy, etc. need to be precisely calculated. Finally, LAMP is not applicable for therapy monitoring and quantification of infectious agents.

APPLICATION AND OPTIMIZATION OF LAMP FOR POC USAGE

LAMP application in infectious disease diagnostics

LAMP can be used for viral detection without isolation and purification of the targeted genome. Molecular POC that is high-throughput could be achieved by omitting the nucleic acid extraction and purification. Simplifying the isolation process, which could be used in resource-limited environments, is an area for future development.

Cell lysis is achieved using enzymes – proteinase K, or heat, or detergents in the same tube [8, 9]. Researchers should eliminate the need for sophisticated equipment such as centrifuges, allowing it to be used in resource-limited facilities in developing countries. For instance, RT-LAMP has been shown to detect HIV without RNA extraction and purification from plasma or blood using heat or detergent [10]. Authors have incubated the samples for 5 min at 117°C and have observed that this is enough for inactivating RNases and destroying viral envelope [10]. Priye et al. detected Zika virus directly from blood, urine, and saliva with the same sensitivity as if using purified viral RNA [11]. It has been demonstrated that SARS-CoV-2 can be detected using a colourimetric LAMP without the extraction step. Authors have achieved a limit of detection of 10 copies/μL and sensitivity and specificity of 86.7% and 98.4%, respectively just for 30 min [12]. Optimizing HIV diagnostics, LAMP evolved from open-tube assay [15] to more safety regarding contamination, closed-tube assay using a quencher. RNA viruses have been detected using multiplexed and closed-tube LAMP assay [16].

Interestingly, recent data has shown that small and portable devices such as smartphones might be implemented at the POC analysis. Researchers were able to detect differences in fluorescence signals in LAMP assays using CMOS cameras. Different fluorophores have been used for multiplex detection of Zika and Chikungunya virus, and Neisseria gonorrhoeae [17]. Moreover, smartphones have been further applied as point-of-care devices together with LAMP for detection of HIV [18], Herpes virus [19], Denga, Zika, Chikungunya [20].

LAMP has been used to detect tropical diseases in the world's poorest regions over the last decade. In these areas due to limited resources, LAMP has proven to be an effective technique for early disease diagnosis and monitoring. In 2021, the World Health Organization (WHO) has recommended a LAMP kit for diagnostics of Mycobacterium tuberculosis (TB-LAMP). Recently, LAMP has been applied for detection of hospital-acquired pneumonia pathogens – Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus and others [21]. Additionally, LAMP has been used for detection of gastrointestinal pathogens such as E.coli [22], Enterococcus faecalis [23], Campylobacter jejuni [24] etc. Field LAMP assay has been used for detection of urogenital schistosomiasis [25].

All above indicated that LAMP is an ideal rapid assay for field diagnosis of different pathogens. The convenience of the LAMP is that it could be easily adapted to different infections with primer set change and might be used for low-cost and field diagnosis of global public health infectious diseases.

DISCUSSION

In recent years, the need for rapid, sensitive and low-cost molecular tests at the point of sampling or point of care has largely increased. Before the SARS-CoV-2 pandemic, LAMP has been implemented mainly in distant and low-income regions such as Africa and South America for diagnosis of malaria and Leishmania [26, 27]. Although certified molecular testing laboratories are available in many high-income cities and countries, the SARS-CoV-2 pandemic has demonstrated that they are unable to meet the required volume of analyses at affordable prices. Furthermore, the centralized and fully equipped laboratories possess sophisticated technologies and trained specialists, both of which are lacking in low- and middle- income regions. LAMP has proven to be a revolutionary diagnostic technique in remote low-income areas without access to lab settings and the lack of trained personnel. Due to these obstacles and inability for an appropriate education the analysis procedure and result interpretation should be easy to use. LAMP is a suitable technique for POC detection in areas without resources for screening purposes and qualitative results. The constant reaction temperature is one characteristic that makes LAMP a suitable POC test. As an isothermal amplification method, it could be performed using an inexpensive water bath or block heater. Contrary, a PCR analysis needs expensive thermocyclers, trained personnel for interpretation of biased results, and prolonged time to final results limit its application as a rapid POC diagnostic method (tabl. 1).

Table 1. Comparison between LAMP and PCR

Characteristic	LAMP	PCR
Temperature	Constant	Different
Time of analyses	Rapid 30-45 min	> 2 h; up to 8 h
Visual detection	Yes	No
Ease of use	Easy and low-cost	Expensive, sophisticated equipment, trained personnel
Inhibition sensitivity	Tolerant	Sensitive

POC tests should be with simple design, low-cost and easily applied in a field or clinical labs.

Antigen tests and lateral-flow dipsticks are rapid, cheap, and user-friendly making them suitable for massive point-of-care testing. Rapid antigen tests are suitable for screening of symptomatic patients. However, they have shown high rates of false results, especially when testing patients with low viral loads [28].

Due to the LAMP robustness and accessibility this technique might be applied in a variety of molecular diagnostics. It could be a valuable detection and screening technique during the flu season [29] as well as for other viruses [30, 31]. Recently, an increasing number of LAMP assays have been approved by the FDA Emergency Use Authorization policies for COVID-19 detection [32, 33, 34]. The in vitro diagnostics (IVD) approved LAMP include assays for detection of Severe Acute Respiratory Syndrome (SARS), influenza virus type A, human papillomavirus (HPV), Mycoplasma pneumoniae, Legionella species, Mycobacterium tuberculosis (TB) and others [35].

Although there is increasing interest and research in LAMP technology as an alternative to PCR for diagnostics, there are some pitfalls that need to be overcome when translating LAMP into clinical diagnostics. For example, some pH-dependent dyes, such as widely used phenol red, hydroxynaphthol blue could be affected by buffering conditions potentially leading to biased results [36]. Moreover, patients with low viral load can give ambiguous results that might be crucial for adequate diagnosis.

Due to the existing LAMP issues, improvements and modifications have been developed. For example, other isothermal amplification molecular analyses are strand displacement amplification (SDA), helicase-dependent amplification (HDA), rolling circle amplification (RCA), recombinant polymerase amplification (RPA), and nicking enzyme amplification reaction (NEAR) has demonstrated better performance regarding specificity, sensitivity, stability, overall time, ect. [37]. Furthermore, attempts have been made for simplifying the isolation process that could be used in resource-poor environments. The need for sophisticated equipment, such as a thermocycler, centrifuge etc, should be removed. The turnaround time and LAMP sensitivity has been improved by adding guanidine hydrochloride in colorimetric detection [38]. In an equipped laboratory with trained personnel, some intercalating dyes could be used for multiplex and closed-tube LAMP detection [39], as well as discriminating specific from nonspecific amplification in melt curve analyses [40].

In 2003, WHO issued an urgent call for development of POC assays for use in low-resource areas, using the acronym ASSURED that stands for affordable, sensitive, specific, user-friendly, rapid, equipment-free, and deliverable [41]. The validation of POC tests in remote areas and the construction of portable POC devices are critical steps in POC test development. POC testing could make use of smartphones, thermoses, and water baths. Smartphones have been used in LAMP detection of viruses such as Zika, Chikungunya, Dengue [42], and herpes simplex virus type 2 [43]. Actually, accessibility of smartphones, their high quality cameras and improvement in digital health and data analyses make smartphones suitable portable POC devices.

Using water baths or block heaters could be applied for LAMP diagnostics in remote and low-income areas with limited access to stable electricity. Recently, a chemical heater and hot water incubated in a thermos bottle have been used for detection of HIV [44] and Taenia tapeworms, respectively [45]. They have reported performance comparable to PCR analyses. Further improvement was using dry LAMP reagents that increase the storage period, and test simplicity as well as sensitivity, and stability.

All above explain the accelerated attempts for transition of rapid, and low-cost nucleic acid-based detection technologies from laboratories to points of sample collection.

CONCLUSION

In the era of high-tech medicine, rapid and reliable tests are still in huge demand especially in developing countries with resource-poor facilities. POC tests such as LAMP are becoming more and more popular not only in the low-income countries but for purposes of home testing. We think that evolution of molecular testing in the direction of decentralization and development of POC molecular analyses that are user-friendly, inexpensive, specific, and also sensitive would significantly improve disease screening and prevention.

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