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Loop-mediated Iso-thermal amplification (LAMP) used for COVID-19 detection

Yixuan Wang

Shanghai University, Shanghai, 200444, China

13918220641@qq.com

Abstract: Loop-mediated isothermal amplification (LAMP) is a novel nucleic acid amplification technique, which can be efficiently amplified at constant temperature by using a *Bst* DNA polymerase with high chain replacement activity. Compared with traditional nucleic acid amplification technology, this technique has the advantages of high sensitivity, strong specificity, simple operation and low detection cost. In the outbreak of COVID-19 in recent years, LAMP technology has been successfully used to develop rapid domestic COVID-19 nucleic acid detection kits with the above advantages, which can achieve rapid detection of COVID-19 in only 20min, greatly improving the convenience and immediacy of nucleic acid detection. In this paper, the principle and development of LAMP technology were summarized by using databases such as NCBI PubMed, Web of Science, CNKI, etc., focusing on the application of LAMP technology in novel coronavirus detection, and the future development direction of LAMP technology was analyzed by describing the latest LAMP technology.

Keywords: Loop-mediated isothermal amplification (LAMP), Isothermal amplification, COVID 19, nucleic acid testing (NAT).

1. Introduction

In vitro nucleic acid amplification, or artificial replication of genetic material, has penetrated into various fields of life science research and has become a key technology in the development of molecular biology. Kary Mullis invented polymerase chain reaction (PCR) in 1983. Through three stages of chain denaturation, primer annealing and enzymatic extension, the artificial in vitro simulation is formed to form a continuous replication of variable temperature amplification system. The amplification system can make the single copy of nucleic acid grow exponentially in a short time, which is convenient for subsequent signal acquisition and analysis. Even though PCR technology is a creative invention, the strong demand for variable temperature systems makes it extremely dependent on expensive thermal cyclers, which largely limits the application of PCR in daily life.

The invention of isothermal amplification technology breaks through the above limitations and provides simplified conditions for the in vitro replication of artificial nucleic acids. The amplification efficiency of isothermal amplification is high, there is no need to repeat the heating and cooling steps in the established program, and multiple molecular reactions can be carried out asynchronously. And the technology does not require expensive variable temperature equipment, which further saves the cost of the experiment. Since the early 1990s, scientists have evolved a variety of isothermal amplification techniques using different principles, The most commonly used are loop-mediated iso-thermal

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amplification (LAMP) and Recombinase polymerase amplification (Recombinase polymerase amplification, RPA), Cross-priming Amplification (CPA), Strand Displacement Amplification (Strand Displacement Amplification) SDA), Rolling Circle Amplification (RCA), helicase-dependent amplification (HAD).

In the PubMed database, the results of 'loop mediated isothermal amplification OR LAMP' as the keyword were 965. 'Loop mediated isothermal amplification' in Web of Science, whose result is as high as 9835. The data show that the published papers in this field increase year by year from 2007 to 2023, indicating that the innovation of this technology is still the hot spot in the field. During the review process, it was found that most of the literature was about the application of LAMP technology in molecular detection, covering the detection of bacteria, viruses and parasites in many industries such as food safety, medical inspection, planting, animal husbandry, and aquatic products, indicating that LAMP technology has a wide range of applications and expansibility.

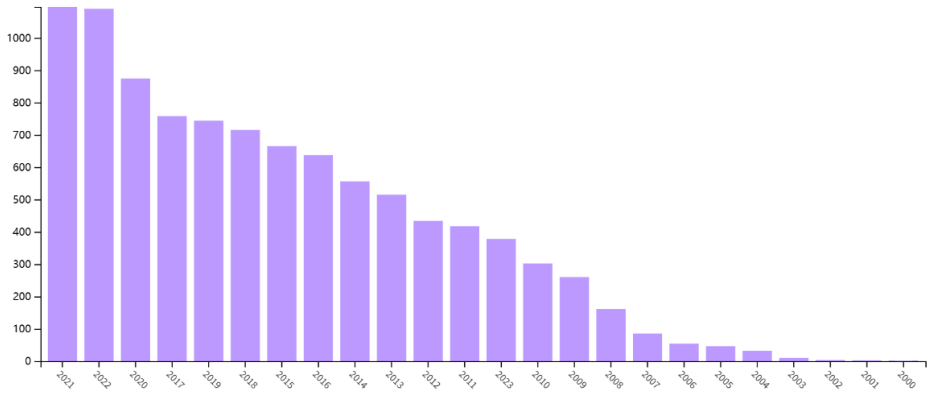

Figure 1. Annual publication volume of related literature on Web of Science.

2. LAMP technology

2.1. Advantages of LAMP

1. LAMP technology does not need high and low temperature cycle, can achieve isothermal amplification (60℃~65℃), so the equipment requirements are low, only water bath pan or thermos bottle is needed
2. The amplification efficiency is high, and the exponential growth of several copies to 10^9 copies can be achieved within 60min
3. High sensitivity, the detection limit can reach 1.4×10^{-1} pg DNA
4. Strong specificity, 4 primers identify 6 sites of the target gene, any region does not match the primer can not be nucleic acid amplification, to ensure the specificity of amplification
5. Low cost, no need for accurate temperature control system and special reagents, suitable for clinical rapid detection
6. The template can be crude DNA, and the test results can be interpreted by the naked eye

2.2. Disadvantages of LAMP

1. The design requirements of primers are high, and all primers need to match for amplification
2. The amplified fragment is too small, and the length of the target sequence must be controlled below 300bp
3. The amplified products can not be used for cloning sequencing, only for judgment, and the reaction results can not be used for quantitative analysis
4. Nucleotide sites with single base differences are of poor applicability
5. It is easy to form aerosols and cause false positives
6. It is difficult to design internal and external comparison of reaction system

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3. New LAMP technology for COVID-19 detection

While LAMP is used to detect various pathogens, it is also gradually used for qualitative and quantitative detection of viruses, bacteria and parasites. This technology is simpler and more convenient than PCR technology both in terms of practical operation and instrument requirements [1]. In the 2019 novel coronavirus outbreak, LMAP has been successfully used to develop a rapid SARS-CoV-2 nucleic acid test kit for home use, which only takes 20 minutes to achieve rapid detection of the novel coronavirus [2]. In addition, some new technologies based on LAMP are also showing off in the novel coronavirus test.

3.1. RT-LAMP technology

RT-LAMP is reverse transcription-ring mediated isothermal amplification, which is generally considered to be between RT-qPCR and rapid antigen detection in terms of sensitivity and simplicity. RT-LAMP was found to be effective in detecting viruses in RT-qPCR amplified samples that were amplified under a quantitative cycle (Cq) of < 30. For samples with Cq values in the range of 32 to 35, the reliability of RT-LAMP was reduced [3-4].

Because the sample is RNA, which is easily degraded by RNA enzymes during extraction and amplification, higher requirements are put forward for the reverse transcriptional LAMP system. In the most direct form of detection, as the reaction heats up, viral particles release the RNA into the solution, exposing them to RNase. The RT enzyme must synthesize the first cDNA strand before the RNase degrades the viral RNA, so it can be described as a race between the RT enzyme and the RNase [5]. Work by Sun et al. using digital RT-LAMP shows that the RT step is significantly less than 100% efficient, so multiple RNA copies must survive to trigger exponential amplification and positive results [6]. It was found that the RNase can be saturated by adding large amounts of tRNA [7], or the RNase inhibitor can be incorporated to suppress the RNase activity in a stoichiometric manner, but the reagent price can be very expensive [8]. Pretreatment before mixing with the premix can effectively improve the above problems. Usually, RNA is treated with a strong alkaline solution, and the treated sample is diluted in the RT-LAMP premix, so that the pH is about 8.5~9.0[9]. Enzyme pretreatment with protease K is another technique to achieve RNase inactivation. Polymerase and RNase can be digested under high temperature and denaturation conditions, so inactivation is required before adding RT-LAMP enzyme. Thermally unstable protease K becomes an alternative to inactivation near the RT-LAMP reaction temperature. Lalli et al. demonstrated that regimens containing protease K had better performance [10]. Full extraction and purification of RT-LAMP was achieved by Rabe and Cepko using a bulk silica matrix [11]. Bokelmann et al. selectively enriched SARS-CoV-2 RNA using oligonucleotide-coupled magnetic beads, and purified the RNA using low-salt wash heating elution [12]. Kondo et al. also used antiboy-coupled magnetic beads to concentrate and purify relevant RNA [13].

Yang et al. applied RT-LAMP to the rapid detection of SARS-CoV-2, using different primers target the Orf1ab gene, S gene and N gene, and the detection limit was 80 copies per ml [14]. It has also been suggested that not all targets in the Orf1a and N genes have the same sensitivity [15]. During the COVID-19 pandemic, many teams simultaneously conducted RT-LAMP research for SARS-CoV-2, and the principle and conclusion were roughly the same [16-20].

3.2. LAMP-CRISPR technology

LAMP-based CRISPR technology is a new generation of molecular detection technology developed in recent years, through the combination of the two can effectively eliminate the limitation of their single operation.

In the selection of Cas enzyme, Cas12 endonuclease is preferred because of its collateral cleavage activity. Among them, Cas12a and Cas12b are most commonly used for LAMP-CRISPR detection [21]. The target of Cas13 is single-stranded RNA, and it is worth noting that since Cas13a protein is only triggered by the RNA target, an additional T7 transcription step is required to convert the DNA amplification into RNA after the RT-LAMP reaction [22-23].

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The LAMP-CRISPR technology has shown high specificity and sensitivity in pathogen detection. Compared with qPCR, the LAMP-CRISPR test has a shorter turnaround time, making it suitable for rapid detection. However, LAMP-CRISPR still has some limitations. The amplification and CRISPR processes require different reagents and reaction conditions: the Bst enzyme used in amplification reacts at 60-65 ° C, while the Cas enzyme used in CRISPR reacts at around 37°C, so using two different reaction systems may increase the risk of contamination.

Broughton et al. developed a fast (≤12 minutes, easy to implement, and accurate CRISPR-Cas12-based side-flow assay for the detection of SARS-CoV-36 in respiratory swab RNA extracts [24]. Zhang et al report an RT-LAMP-CRISPR assay based on Cas12a for the detection of SARS-CoV-2 nsp8 and N genes [25]. Chandrasekaran et al. developed a DISCoVER technique involving dissolution of extraction-free samples by low-cost reagents, multiple isothermal RNA amplification and T7 transcription, and Cas13-mediated cleavage of quenching fluorophors with a sensitivity of 40 copies of μL^{-1}[26]. Verma et al. reviewed the mechanisms of action of different enzymes based on Cas9, Cas12, and Cas13, and elucidated the advantages of selection and improved methods [27].

3.3. LAMP-LFD

Lateral flow detect (Lateral flow detect) is a new method for detecting LAMP amplification products, It includes 3 steps: nucleic acid amplification (LAMP), molecular hybridization (hybridization of biotin-labeled LAMP amplification product with fluorescein labeled probe) and lateral flow detection (binding with fluorescein antibody, biotin antibody and fluorescein antibody labeled on the test strip through lateral chromatography to show specific colors on the detection line and quality control line). At present, LAMP-LFD technology has been used in various biological detection fields, and has the advantages of high sensitivity, high specificity, fast, convenient, low cost and easy product detection [28]. This technology has been applied to the detection of novel coronavirus. Simon et al. combined RT-LAMP with lateral flow technology (LFD), which can detect two gene amplification on a single strip, and is suitable for multiple detection. Chen et al. used a nanoparticle based side-flow biosensor to analyze mRT-LAMP products, and optimized conditions such as target RNA concentration, amplification temperature and time in MRT-LAMP-LFB amplification. In this study, mRT-LAMP-LFB assay was applied to detect SARS-CoV-2 virus from clinical samples and artificial sputum samples [29].

3.4. LAMP-Chip

In order to meet the needs of POC molecular diagnosis, Byung et al. proposed an integrated rotary microfluidic system. The chip is composed of three identical units, each of which consists of solid phase DNA extraction, LAMP reaction and lateral flow strip [30]. Fang et al. designed eight parallel reaction channels on the chip, each requiring only 0.4μL of sample, and a single channel combined with a photoconductive fiber can achieve a detection limit of 10 fg/μL [31]. Microfluidic chip technology can integrate sample processing, product detection and result output into one, so as to better adapt to the needs of field detection.

For COVID-19 detection, Torezin et al. developed an instant lab-on-a-chip instrument to diagnose SARS-CoV-2 using RT-LAMP. The microfluidics chip was kept at ~ (65.0±0.1) ℃ for 25 minutes and cooled for 5 minutes [32]. Colbert et al. combined RT-LAMP with particle imaging technology particle diffusion polarization to successfully detect SARS-CoV-30 on a portable chip with integrated heating in only 35 minutes, detecting as few as 2 virus particles per μL, and imaging a sample containing fluorescent beads using a smartphone device [33]. Song et al. proposed a fast MUSAL chip, powered by a simple LED for photothermal amplification operation and can detect 500 targets from a single swab sample with pollution-free amplification [34]. Rodriguez-Mateos et al. designed an integrated chip platform that combines surface tension-assisted immiscible filtration (IFAST) with RNA amplification and detection to provide a viable means of screening for SARS-CoV-2 infection in resource-limited settings [35].

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4. Conclusion

As one of the widely used thermostatic amplification technologies, LAMP technology is convenient, immediate, easy to operate, strong specificity, etc. Compared with gold standard PCR, it is more suitable for clinical diagnosis or field detection in public health environment. However, although LAMP can solve the temperature control problem of PCR well, it also brings other problems correspondingly, such as insufficient detection sensitivity, high difficulty in primer design, easy to cause contamination and false positives, and can not be accurately quantified. With the continuous improvement of technology and integration with new technologies, the shortcomings of LAMP have been corrected, such as DETECTR and SHERLOCK technology based on Cas enzyme, and immunoassay platform for pH change through engineering and optimized isothermal nucleic acid amplification. The future trend will be combined with microfluidic chip and computer program assistance to integrate nucleic acid amplification and result detection in a chip, to achieve integration of amplification and detection, automation, portability, so that LAMP technology becomes a supplement and replacement of PCR.

In the face of public health emergencies similar to COVID 19, we can also make LAMP technology into a rapid test kit, using its advantages of constant temperature detection to efficiently screen the outbreak point, so as to more effectively control the spread of the epidemic. This kind of testing method is also applicable to poor areas with poor medical conditions.

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