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Applications of Loop-Mediated Isothermal DNA Amplification

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Abstract During the last 10 years, with the development of loop-mediated isothermal amplification (LAMP) method, it has been widely applied in nucleic acid analysis because of its simplicity, rapidity, high efficiency, and outstanding specificity. This method employs a DNA polymerase and a set of four specially designed primers that recognize a total of six distinct sequences on the target DNA. Expensive equipment are not necessary to acquire a high level of precision, and there are fewer preparation steps compared to conventional PCR and real-time PCR assays. This paper briefly summarized the applications of LAMP method in pathogenic microorganisms, genetically modified ingredients, tumor detection, and embryo sex identification.

Keywords Loop-mediated isothermal amplification · Pathogenic microorganisms · Detection · Application

Introduction

The loop-mediated isothermal amplification (LAMP) is a novel nucleic acid amplification method, which amplifies with high specificity, sensitivity, and rapidity under isothermal conditions, and employs self-recurring strand-displacement synthesis primed by a specially designed set of target-specific primers [1]. This method employs a set of four specially designed primers that recognize a total of six distinct sequences on the target. An inner primer containing sequences of the sense and antisense strands of the target DNA initiates LAMP.

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The following strand-displacement DNA synthesis primed by an outer primer releases a single-stranded DNA. This serves as template for DNA synthesis primed by the second inner and outer primers that hybridize to the other end of the target, which produces a stem-loop DNA structure. In subsequent LAMP cycling, one inner primer hybridizes to the loop on the product and initiates displacement DNA synthesis, yielding the original stem-loop DNA and a new stem-loop DNA with a stem twice as long. The cycling reaction continues with accumulation of 10^9 copies of target in less than an hour. The final products are stem-loop DNAs with several inverted repeats of the target and cauliflower-like structures with multiple loops formed by annealing between alternately inverted repeats of the target in the same strand.

LAMP method acquires three main features. Firstly, all reactions can be carried out under isothermal conditions. Compared to conventional PCR and real-time PCR assays, expensive equipment is not necessary to obtain a high level of precision, and there are fewer preparation steps [2–4]. Secondly, the amplification efficiency is extremely high, and tremendous amount of amplification products can be obtained [2, 5]. The third, the reaction, is highly specific [5, 6]. During the last 10 years, LAMP method has been widely used for nucleic acid analysis because of its simplicity, rapidity, high efficiency, and outstanding specificity. This review focuses attention on the applications of LAMP method in pathogenic microorganisms, genetically modified ingredients, tumor detection, and embryo sex identification.

Application in Detecting of Pathogenic Microorganisms

Detection of Bacteria Pathogenic Microorganisms

With the rapid development of molecular biology techniques, the identification of pathogenic microorganisms is no longer confined to the external morphology and physiological characteristics test based on the elements, in particular the detection of the nucleic acid level. Nucleic acid hybridization and polymerase chain reaction have been applied in pathogen detection. However, there are some drawbacks such as false positives, the formation of primer dimers. Apart from these, the repeated PCR experiments cannot be avoided during the diagnoses of multiplex pathogenic microorganisms. LAMP method has been used to detect various pathogenic microorganisms with simple, rapid, and cost-effective features in recent years.

LAMP method was first used to detect $stxA_2$ in *Escherichia coli* O157:H7 cells [7]. The mild permeabilization conditions and low isothermal temperature used in the in situ LAMP method caused less cell damage than in situ PCR. Higher-contrast images were obtained with this method than with in situ PCR.

Actinobacillus actinomycetemcomitans has been implicated in the etiology of aggressive periodontitis, and the LAMP assay was used for the rapid detection of *A. actinomycetemcomitans* in clinical specimens [8]. In addition, Iwamoto et al. [9] used the LAMP technology to specific detection of *Mycobacterium tuberculosis* complex, *Mycobacterium avium*, and *Mycobacterium intracellulure*. LAMP has also been successfully used for specific detection of pathogenic microorganisms such as *Streptococcus pneumoniae* [10], *Edwardsiella tarda* [11], *Edwardsiella ictaluri* [12], methicillin-resistant *Staphylococcus aureus* [13], and *Bacillus anthracis* [14].

Detection of the Virus Pathogenic Microorganisms

Various biochemical and serological tests, polymerase chain reaction, and probe-based nucleic acid detection have become increasingly popular in virus diseases diagnosis.

However, the time and expertise required for such diagnoses make these methods difficult to adopt under production conditions [15]. LAMP method can rapidly amplify the target sequence under isothermal conditions with good sensitivity and specificity and has been widely used in detection and diagnosis of DNA and RNA virus.

Up to now, there are many reports about LAMP method for detecting DNA virus. Fukuta et al. [16] applied LAMP method to detect tomato yellow leaf curl virus DNA. The detection sensitivity is 100 times higher than the PCR. Qu et al. [17] adopted LAMP method to diagnose porcine parvovirus (PPV). Their results demonstrated that the LAMP assay could be well-applied to laboratories, as a portable device and valuable tool for differential diagnosis of PPV in the countryside. Ihira et al. [18, 19] established LAMP method to detect human herpes virus 6. It can be applied directly in human serum HSV-6 type without the need for DNA extraction. LAMP technology was also successfully applied to detect the human herpes virus-7 [20], herpes simplex virus and varicella-zoster virus [21], and human influenza A virus Hl-H3 [22].

LAMP has also been applied successfully for RNA virus detection with high efficiency by directly adding the reverse transcriptase to the reaction mixture, which is termed the reverse transcription LAMP (RT-LAMP) [23]. Fukuta et al. [24] first reported that the Japanese yam mosaic virus from the infected plants RNA could be directly detected by RT-LAMP. Imai et al. [25, 26] developed a rapid and sensitive detection of highly pathogenic H5N1 avian influenza virus through RT-LAMP method. This method is more sensitive and specific than conventional RT-PCR method. Kiatpathomchai et al. [27] developed a RT-LAMP method for detecting Taura syndrome virus (TSV) which is a causative agent of *Penaeus vannamei*. The nucleic acids of other shrimp pathogens (yellow head virus and white spot syndrome) were not amplified by this RT-LAMP system. The detection of TSV using RT-LAMP was ten times more sensitive than that of RT-PCR.

Detection of Fungi Microorganisms

The LAMP method has the advantage of speed and simplicity in detection of fungi microorganisms compared with the classic diagnostic methods such as the histopathological test and biological material culture. For instance, Endo et al. [28] and Tatibana et al. [29] successively used LAMP method to succeed in detecting the presence of the thermo-dependent dimorphic fungus *Paracoccidioides brasiliensis*.

Detection of Pathogenic Parasites

Protozoan parasites, common but important pathogens, are seriously harmful to human, animals, and fish, which severely endanger human health and the development of animal husbandry and aquaculture. LAMP-based technical characteristics and its detection of protozoan parasites have also been widely used, such as *Trypanosoma* [30–32], *Plasmodium* [33], *Babesia* [34, 35], *Cryptosporidium* [36], and *Tetracapsuloides bryosalmonae* in aquatic animals [37].

Detection of *Mycoplasma*

Mycoplasma infection can lead to a variety of infectious diseases. Laboratory testing methods such as *Mycoplasma* culture, antigen detection, and serological methods are generally adopted during clinical diagnosis of *Mycoplasma* infection. These detection methods are time-consuming and expensive. Therefore, Saito et al. [38] developed a LAMP

assay for the rapid detection of *Mycoplasma pneumoniae*, and no cross-reactivity was observed for other *Mycoplasma* species or respiratory bacterial species. It may be applied in the routine diagnosis of *M. pneumoniae* infection in the clinical laboratory. And Kawai et al. [39] developed a LAMP method to detect *Chlamydophila pneumoniae* infection. This assay exclusively amplified *C. pneumoniae* sequences, and no cross-reactivity was observed for other *Chlamydia* species. The detection limit for this assay was found to be ten elementary bodies in 25 min, as observed in a real-time turbidimeter and electrophoresis analysis. Among nasopharyngeal swab specimens from 120 patients with acute respiratory tract infections and 40 healthy individuals, the LAMP results showed total agreement with the results of real-time PCR assays.

Application in Detecting Genetically Modified Ingredients

Many detection methods have been developed and extensively used to detect genetically modified organisms (GMOs) based on the LAMP reaction. Cauliflower mosaic virus 35S promoter gene, a widespread genetic element, was amplified by a set of LAMP primers [40]. With this technique, GMO content ranging from 0.5% to 5% was found in Roundup Ready soybean. Lee et al. [41] applied the LAMP method to amplify GMO-related DNA sequences. Results showed that detection of 0.01% GMO in equivalent background DNA was possible, and dilutions of template suggested that detection from single copies of the template may be possible using LAMP. Guan et al. [42] reported one optimized visual LAMP method for the detection of exogenous DNA targets from two GM soybean events. This isothermal amplification could be performed within 40 min without PCR equipment, and the derived LAMP products could be directly observed by naked eye employing SYBR Green I dye instead of conventional gel electrophoresis analysis. The limits of detection of these established visual LAMP assays were about four copies of haploid soybean genomic DNA and which were much higher than those of reported conventional PCR assays.

Application in Embryo Sex Identification

LAMP method has also been successfully applied in identification of embryo sex. Hirayama et al. [43] developed a rapid sexing method for bovine pre-implantation embryos using LAMP. The results indicated that LAMP-based embryo sex identification accurately determined gender and are suitable for field application. Hirayama et al. [44] further identified a Y chromosome-specific sequence in water buffalo to establish an efficient procedure for embryo sexing by LAMP. The LAMP reaction required only about 45 min, and the total time for embryo sex identification was about 1 h including DNA extraction.

Application in Tumor Detection

Tailor-made surgeries for patients with tumor have been under consideration on the basis of the development of new approaches for minor metastatic foci of malignant tumors. Accurate and reliable methods to detect metastases in biopsy specimens with certain rapidity are essential for the performance of these surgeries. Horibe et al. [45] developed a rapid and practical method to detect metastasis in specimens from patients with gastric carcinoma using RT-LAMP to detect cytokeratin 19 (CK19) mRNA. The agreement rate of

CK19 expression detection by RT-LAMP and RT-PCR analysis was 31 out of 32 (97%). The RT-LAMP technique showed similar sensitivity to detect metastases as nested RT-PCR assay, with a rapidity comparable to that of intraoperative histopathological examination with frozen sectioning and hematoxylin and eosin staining.

Conclusions and Future Perspectives

LAMP method is a powerful innovative gene amplification technique emerging as a simple rapid diagnostic tool for early detection and identification of microbial diseases. Considering the advantages of rapid amplification, simple operation, and easy detection, LAMP has potential applications for clinical diagnosis as well as surveillance of infectious diseases in developing countries without requiring sophisticated equipment or skilled personnel. During the last 10 years, this method has been successfully applied in molecular detection and diagnostics for the detection of bacterial, viral, fungal, and parasitic diseases in both animals and plants.

In conclusion, LAMP method has been adopted in disease diagnosis, embryo sex identification, and genetically modified detection due to its simplicity, rapidity, high efficiency, and outstanding specificity. This method will be widely applied in clinical diagnostics, environmental monitoring, food safety, and health fields with a broader development prospects in the future.

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