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MINIREVIEW

Loop-mediated isothermal amplification (LAMP): principle, features, and future prospects

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Loop-mediated isothermal amplification (LAMP), a newly developed gene amplification method, combines rapidity, simplicity, and high specificity. Several tests have been developed based on this method, and simplicity is maintained throughout all steps, from extraction of nucleic acids to detection of amplification. In the LAMP reaction, samples are amplified at a fixed temperature through a repetition of two types of elongation reactions occurring at the loop regions: self-elongation of templates from the stem loop structure formed at the 3′-terminal and the binding and elongation of new primers to the loop region. The LAMP reaction has a wide range of possible applications, including point-of-care testing, genetic testing in resource-poor settings (such as in developing countries), and rapid testing of food products and environmental samples.

Keywords: loop-mediated isothermal amplification (LAMP), nucleic acid amplification test (NAT), point of care testing (POCT)

Introduction

Since the inception and development of polymerase chain reaction (PCR) in the 1980s, this gene amplification method has been developed for widespread clinical use, particularly in genetic testing. This is especially true in the diagnosis of infectious diseases, such as hepatitis infections and tuberculosis, and hereditary diseases. The steps of genetic testing include nucleic acid extraction from the specimens, gene amplification, and detection. These steps require considerable skill and expensive equipment and facilities, making convenient testing at any given location difficult. To overcome these limitations, we have developed a new gene amplification method, the loop-mediated isothermal amplification (LAMP) reaction, which combines rapidity, simplicity, and high specificity (Notomi *et al.*, 2000, 2004). We have also developed simple tests based entirely on this technique. In this paper, we introduce the features of this technique and discuss many of its clinical applications.

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Mechanism of the LAMP reaction

In the LAMP reaction, gene amplification proceeds through repetition of two types of elongation reactions that occur via the loop regions (i.e., template self-elongation from the stem loop structure formed at the 3′-terminal and subsequent binding and elongation of new primers to the loop region). In this reaction, pairs of inner and outer primers are used. Each of the inner primers possesses a sequence complementary to one chain of the amplification region at the 3′-terminal and identical to the inner region of the same chain at the 5′-terminal (Fig. 1). The elongation reactions are sequentially repeated by DNA polymerase-mediated strand-displacement synthesis using the aforementioned stem loop regions as a stage. This method operates on the fundamental principle of the production of a large quantity of DNA amplification products with a mutually complementary sequence and an alternating, repeated structure (Fig. 1). A detailed description of the LAMP reaction mechanism is available on the Eiken Chemical Co., Ltd. website (http://loopamp.eiken.co.jp/e/index.html), which uses animation and other tools to explain the mechanism.

Features of the LAMP reaction

Several aspects of the LAMP reaction differ from those of other amplification methods. First, only a single type of enzyme is required, and the amplification can be carried out at a constant temperature. LAMP utilizes six gene regions for amplification. The fundamental characteristics of the inner primer provide the amplification with a specificity that is much greater than that observed in other methods. The amplification process is usually completed within 1 h, with sensitivity similar to that of nested PCR.

The rapid, sequential progression of the LAMP amplification reaction contributes to its high amplification efficiency. In addition, small quantities of a gene can be amplified within a short time. Specific loop primer designs can facilitate a

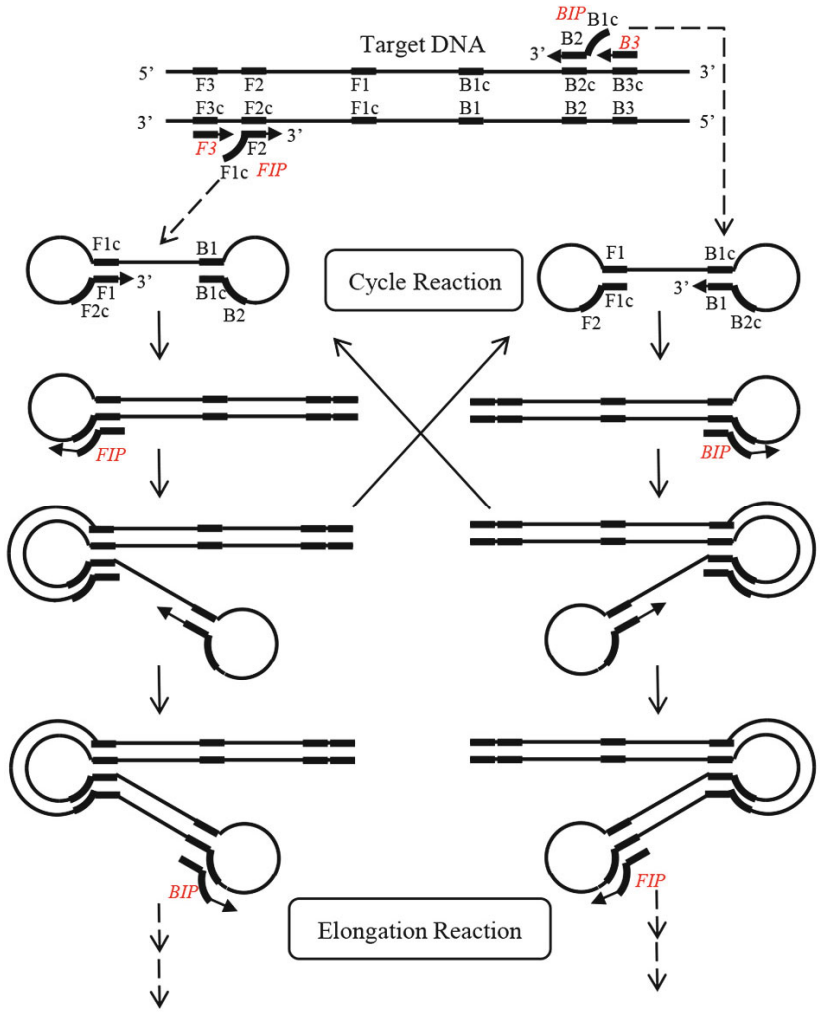

Fig. 1. Schematic representation of the LAMP mechanism. Design of the primers for LAMP. Forward inner primer (FIP): F2 sequence with F1c sequence at the 5′ end. Backward inner primer (BIP): B2 sequence with B1c sequence at the 5′ end. Outer primers are designed at the regions of F3 and B3. The LAMP method employs a single strand of DNA shaped like a dumbbell with loops at both ends. This starting material is used to initiate a cycle of amplification reactions. DNA having an inverse structure relative to the starting material is produced, and the starting material is formed again by the same reaction. This cycle produces amplified DNA products that are connected to an inverted repeat structure at the amplified region. The amplified products again pass through repeated elongation reactions, which generate amplified DNA products of various stem lengths.

reduction in the amplification time to half or one-third that of the original LAMP method (Nagamine *et al.*, 2002). Furthermore, this method can be utilized for the amplification of a target RNA sequence. In this case, a one-step amplification–the same as the amplification of DNA–can be performed by the simultaneous addition of a reverse transcriptase enzyme because the reverse transcriptase also exhibits strand displacement activity.

Simple detection methods, such as turbidity (Mori *et al.*, 2001, 2004) or fluorescence detection methods (Tomita *et al.*, 2008), can be employed because of the high specificity and large output of the amplification products. The turbidity detection method utilizes the turbidity of magnesium pyrophosphate, a byproduct of DNA synthesis, as an indicator, while the fluorescence detection method utilizes fluorescent chelation reagents. Both methods allow for real-time detection and visual inspection. In addition, detection using normal DNA probes is also possible.

Amplification products possess a repeating structure, where identical chains are linked in opposite directions. In addition, due to the resistance of the utilized enzymes to inhibitors in the specimen, it is possible to simplify the process of nucleic acid extraction.

By combining processes for simplicity, rapidity, and precision, the LAMP method could be employed in a wide range of applications, such as point-of-care testing, genetic testing in clinical settings, and rapid testing of food products and environmental samples.

Another feature that contributes to the simplicity of the method is the availability of primer design support software (http://primerexplorer.jp/e/), which facilitates the design of appropriate primers. This software can be operated online and can automatically produce candidate primer sets specific to the input target sequence.

Applications of the LAMP reaction

The LAMP reaction does not require any special reagents and it can be conducted in a general molecular biology laboratory. Recently, several reports have been published that used LAMP-based research (Mori and Notomi, 2009; Mori *et al.*, 2013). We describe two examples of such recently developed research methods below.

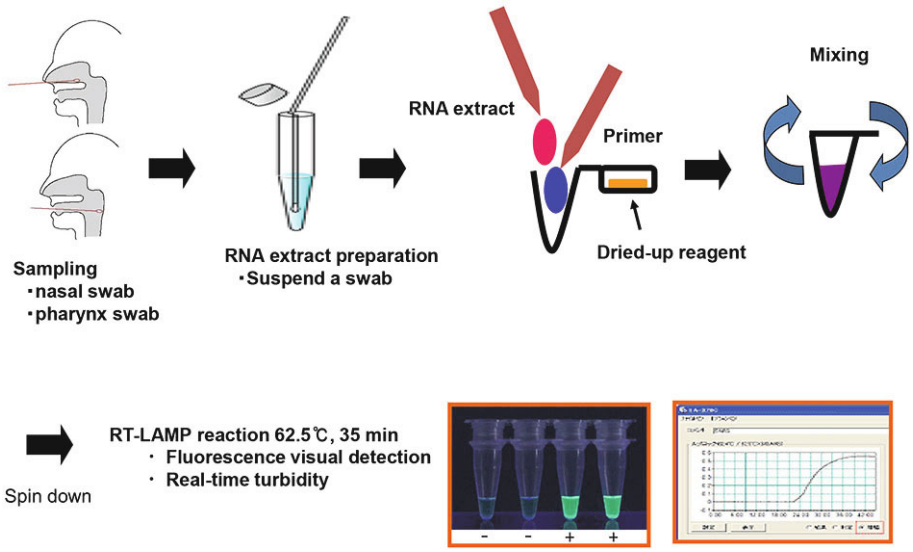

Fig. 2. Detection of influenza virus. The swab is soaked in the extraction reagent and transferred to a reaction tube containing the dried LAMP reagents. Primers are added, and the tube is inverted to mix the reagents, initiating the LAMP reaction. The results can be visually confirmed through fluorescence or by turbidimetry.

Development of detection reagents for influenza virus

The LAMP reaction is relatively resistant to the effects of inhibitors produced by specimens. However, nucleic acid extraction is often performed using previously developed methods. Thus, the simplification and acceleration of genetic testing is a considerable obstacle. Therefore, a simpler preprocessing technique is required in order to promote the use of genetic testing as a routine test rather than as a specialized test. Such advancements may lead to the development of an extremely simple extraction method for the detection of influenza virus in pharyngeal and nasal swab specimens.

With this method, it is possible to extract RNA from the influenza virus just by mixing a dedicated extraction reagent with the specimen fluid. The extraction fluid and the LAMP primer mix for detection of influenza virus is dispensed into a reaction tube to which dried amplification reagents are immobilized. This tube is then placed in the amplification device after mixing by inversion.

The amplification is conducted for 35 min at 62.5°C, and the products are detected using a real-time turbidity measurement device. Alternatively, the fluorescence detection method by visual inspection can also be employed (Fig. 2). The simplicity of this detection method lies in the use of dried amplification reagents that are dissolved in the extraction fluid. In addition, the reagents can also be preserved by refrigeration.

The clinical results obtained by the LAMP reaction have been shown to be similar to those obtained by real-time RT-PCR (e.g., the TaqMan Probe method) (Nakauchi *et al.*, 2011).

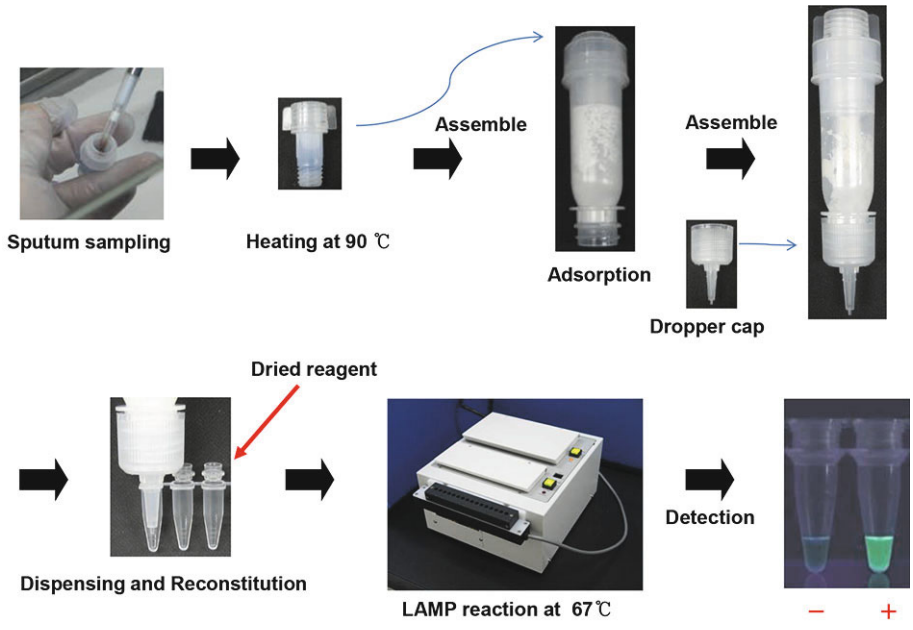

Fig. 3. PURE TB-LAMP procedure. The raw sputum is added into a heating tube and heated. This tube is then attached to an adsorbent tube, and a dropper cap is attached to the device. The purified DNA is then eluted by squeezing the tube. The LAMP reagents fixed in the lid of the tube are reconstituted and then incubated. The results of amplification are detected by observing the fluorescence using an LED light within the incubator.

Detection of tuberculosis

Tuberculosis is one of the greatest threats to global health, infecting approximately one-third of the world's population. It is especially prevalent in developing countries. The sputum smear method is the most widely adopted method for detection of tuberculosis. However, this method is not sufficiently sensitive or specific. Alternatively, the culture method is highly sensitive, but requires 4–6 weeks for the quantifiable detection of bacteria. In addition, detection by this method is limited to regions and facilities with the required equipment for conducting such tests.

Eiken Chemical has collaborated with the Foundation for Innovative New Diagnostics (FIND), a non-profit organization, and developed reagents for the simple and rapid detection of the *Mycobacterium tuberculosis* complex using the LAMP method. This method was designed to be conducted in the current testing environment in developing countries in order to contribute to the eradication of tuberculosis in the developing world (Boehme *et al.*, 2007). The genetic testing method for diagnosis of tuberculosis mainly uses sputum as a specimen. However, use of this sample necessitates a longer experimental time and the use of complicated processes, such as *N*-acetyl-l-cysteine (NALC)-NaOH treatment prior to nucleic acid extraction. It would be very difficult to conduct such processes in the resource-limited testing environments of developing countries.

Therefore, in order to develop a simplified, accelerated process, the components of the extraction fluid that inhibit amplification and detection, as well as inhibitory components derived from the specimen, were removed by preprocessing. This was performed in place of a purification process, in which the nucleic acid is adsorbed onto a carrier region. Specifically, the Procedure for Ultra Rapid Extraction (PURE) was developed in order to remove inhibitory components. This method involves inoculation of the specimen onto a porous material following mixing of the specimen with the extraction fluid and heat treatment (Fig. 3).

Additionally, dried LAMP reagents have been developed that can be preserved at normal temperatures. We have previously succeeded in developing a simple device in which the PURE treatment fluid could be added to a reaction tube without the aid of a pipette. Following this, the LAMP reaction could be initiated by dissolving the aforementioned dried reagents.

The specimen (i.e., sputum in the case of tuberculosis) is added to an alkaline extraction fluid and heated at 90°C for 5 min for bacteriolysis. The nucleic acid is extracted from this lysed sample, and the specimen treatment tube is placed in an adsorption tube, to which the LAMP reaction-inhibiting substances are added. A dropper cap is attached to the adsorption tube, and the nucleic acid extract fluid is dispensed into the reaction tube by squeezing the side of the tube. The dried amplification reagents, which are fixed to the inside of the lid, are dissolved by inversion mixing, and the reaction tube is inserted into the amplification device. Amplification is then performed at 67°C for 40 min. The products of this reaction are detected either by a turbidity detection method using a real-time turbidity measuring device or by visual inspection of fluorescence. The main advantage of this method is that it would be possible to obtain a result within approximately 1 h following the collection of sputum (Fig. 3).

Detection of the *M. tuberculosis* complex using a combination of the PURE method and the LAMP method is simple and rapid due to the lack of NALC-NaOH treatment. In addition, the minimum detection sensitivity of this test is 0.38 genome equivalents/test. In addition, the detection rate of the *M. tuberculosis* complex is comparable to that obtained by existing genetic tests using NALC-NaOH-treated specimens (Mitarai *et al.*, 2011).

Through the combination of simplicity (by the PURE method) and the ability to store dried reagents at room temperature, rapid extraction of nucleic acids and detection of amplification is possible, without the use of complicated pipette procedures and high-speed centrifugation, which are necessary aspects of currently used methods.

Additionally, the PURE method can be used with other specimens, such as blood serum and pharyngeal swab fluid.

Working towards further simplification

Microfluidic chips (Whitesides, 2006) have been attracting attention as a next-generation genetic testing device. This device consists of tiny canals that are constructed on platforms, such as a resin slab. In the case of genetic testing, a complete analysis, including extraction, amplification, and detection, can be performed on a single chip (Kopp *et al.*, 1998). These devices are called μ-total analysis system (μ-TAS) chips, or "lab on a chip". The merits of using such devices include the prevention of contamination of gene amplification products; the ability to automate complex systems according to the design of the device; the capacity for increased analytical speed for small samples; and the ability to conduct on-site testing due to miniaturization and simplification. This method may resolve a large number of problems associated with on-site genetic testing. Reactions that lack temperature cycling and amplification methods that further simplify extraction and detection are advantageous to the development of these devices. Importantly, one study has demonstrated amplification via LAMP, detection by electrophoresis, and subsequent analysis, all achieved on a single chip (Hataoka *et al.*, 2004), proving that the LAMP reaction is applicable in the context of next-generation devices. From this observation, we aim to develop simple devices based on the LAMP method that can be used by anyone, anytime, and anywhere.

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