Review Articles

Loop-mediated Isothermal Amplification (LAMP): An Alternative Molecular Diagnosis

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Abstract

This review article was focused on an innovative molecular diagnostic method based on nucleic acid amplification with simplicity, rapidity and high specificity, termed loop-mediated isothermal amplification (LAMP). LAMP is performed in a single tube based on a constant temperature condition (60 - 65 °C) by DNA polymerase with strand displacement. LAMP assay uses a set of minimum 2 distinct outer (F3 and B3) and inner (FIP and BIP) primers. Nonetheless, when it is applied with the other 2 loop primers, LoopF and LoopB, the sensitivity will be improved and the reaction time will be shortened by half. This technique does not require sophisticated thermal cyclers and the amplification duration is less than 1 hour. LAMP assay can be performed using basic laboratory tools, for instance, heating block or water bath. Several LAMP-based applications have been developed and showed higher specificity, sensitivity and rapidity over PCR. Therefore, LAMP is an alternative molecular diagnosis in the field or at the point-of-care with cost effectiveness.

Keywords: Loop-mediated isothermal amplification, molecular diagnosis

วิธี Loop-mediated Isothermal Amplification (LAMP): ทางเลือกหนึ่งของการตรวจวินิจฉัยระดับโมเลกุล

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บทคัดย่อ

การทบทวนงานวิจัยนี้กล่าวถึงวิธีวินิจฉัยระดับโมเลกุล ชื่อ Loop-mediated isothermal amplification หรือ LAMP วิธีนี้ดำเนินการเพิ่มปริมาณยีนด้วยอุณหภูมิเสมอ (ในช่วง 60-65 องศาเซลเซียส) มีความรวดเร็ว (ใช้เวลาน้อยกว่า 1 ชั่วโมง) อีกทั้งยังมีความจำเพาะสูง วิธีนี้ใช้ไพรเมอร์ 2 ชุดหลักสำหรับขยายยีนส่วนภายใน (FIP และ BIP) และภายนอก (F3 และ B3) หากใช้ร่วมกับไพรเมอร์พิเศษอีก 2 ไพรเมอร์ (LoopF และ LoopB) จะช่วยเพิ่มความไวและร่นระยะเวลาของปฏิกิริยาลงครึ่งหนึ่ง นอกจากนี้ LAMP ยังไม่ต้องการเครื่องมือพิเศษที่มีความซับซ้อนหรือราคาสูง โดยสามารถประยุกต์เครื่องมือพื้นฐานของห้องปฏิบัติการ เช่น เครื่องอังน้ำหรืออุปกรณ์อื่นที่สามารถสร้างอุณหภูมิเสมอได้ ปัจจุบันมีรายงานการพัฒนา LAMP เพื่อใช้เป็นเครื่องมือตรวจวินิจฉัยมากมายและแสดงให้เห็นว่า LAMP มีความไว ความจำเพาะ และความเร็วมากกว่าวิธี PCR ดังนั้น LAMP จึงเป็นทางเลือกหนึ่งของการวินิจฉัยระดับโมเลกุลที่มีต้นทุนประสิทธิผลสำหรับงานในภาคสนามและในห้องปฏิบัติการใกล้จุดดูแลผู้ป่วยหรือสัตว์ป่วย

คำสำคัญ : Loop-mediated isothermal amplification, การวินิจฉัยระดับโมเลกุล

Introduction

Molecular diagnostic methods are important to facilitate general practices and veterinarians to decide the effective treatment or prevention program. Basically, conventional diagnostic techniques are used, for example, *in vitro* culture and microscopy, due to easy to perform and inexpensive methods. However, such methods sometimes give unsatisfactory results and time consuming. Nucleic acid amplification methods are useful tool and can solve those limitations with high sensitivity and specificity. Nowadays, polymerase chain reaction (PCR), including gathering with other techniques, are commonly used molecular diagnostic that have demonstrated their applications in various fields (Yang and Rothman 2004; Moore 2005; Blomström et al. 2009; AbouLaila et al. 2010), including veterinary medicine (Johnson et al. 2008; Pal et al. 2008; AbouLaila et al. 2010). PCR-based technique can be applied to amplify DNA and RNA.

PCR requires repeated cycles of three different temperatures as denaturation, annealing and elongation steps. In addition, PCR is enzymatic amplification which needs thermostable DNA polymerase enzyme (Mueller et al., 1997; Yang and Rothman, 2004). *Taq* DNA polymerase is a good heat stable enzyme but its activity is probably inhibited by heme compounds, myoglobin, and immunoglobulin G(Akane et al. 1994; Bélec et al. 1998; Al-Soud et al. 2000; Al-Soud and Radstrom 2001). There are several limitations of PCR technique, especially requirement of expensive thermocycling device and skillful practitioners. For this reason, new molecular diagnostic methods are developed as alternative. Consequently, isothermal amplification is of interest and applying instead of PCR method which use multi-temperature, for instance, self-sustained sequence replication (3SR) (Guatelli et al. 1990), nucleic acid sequence based amplification (NASBA) (Compton 1991), strand displacement amplification (SDA) (Walker et al. 1992), loop-mediated isothermal amplification (LAMP) (Notomi et al. 2000) and helicase dependent amplification (HDA) (Vincent et al. 2004). Among these methods, LAMP is a good candidate to replace PCR for applying as molecular diagnostic tool because of simple, specific and rapid detection (Thekisoe et al. 2005, 2009; George et al. 2011). The comparison of some methods is summarized in Table 1.

Table 1. Comparison of DNA amplification techniques.

Property	PCR*	LAMP*	SDA*	NASBA*	3SR†	HDA‡
DNA or RNA	Both	Both	Both	RNA	RNA	DNA
Isothermal reaction	No	Yes	Yes	Yes	Yes	Yes
Reaction time (min)	120-180	15-60	60-120	120-180	60-120	60-180
Number of enzymes	1	1	2	3	3	1
Sensitivity (copies)	>10	>10	500	100	>10	500

* Applied from Walker et al. (1992), Notomi et al. (2000), Nagamine et al. (2002) and Yang and Rothman (2004). † Applied from Guatelli et al. (1990), Fahy et al. (1991) and Mueller et al. (1997). ‡ Applied from Vincent et al. (2004).

Loop-mediated isothermal amplification or LAMP is an ovel cost effective molecular assay with rapidity and high specificity. Since Notomi et al. (2000) established LAMP assay, this method is widely accepted and applied to several different fields because of the ease and speed of this technique. In addition, there are several reports showed the development of LAMP by applying with other techniques (Hataoka et al. 2004; Tani et al. 2007; Marciniak et al. 2008; Ahmad et al. 2011). This synopsis shows the principle and applications of loop-mediated isothermal amplification (LAMP) as an alternative to molecular diagnostics.

Principle of LAMP

LAMP amplification

LAMP is a new generation of nucleic acid, both DNA and RNA, amplification method which it is performed in a single tube under constant particular temperature, range between 60 - 65 °C, with a *Bst* DNA polymerase with strand displacement (Notomi et al. 2000). Basically, LAMP uses 4 different primers, forward outer primer (F3), backward outer primer (B3), forward internal primer (FIB) and backward internal primer (BIP). Nevertheless, Nagamine et al. (2002) demonstrated that addition of loop primers, forward loop (LoopF) and backward loop (LoopB), could reduce the reaction time down to half of the first initiative LAMP method and the method sensitivity was improved. Therefore, LAMP uses a set of 4 to 6 primers to amplify 6 to 8 regions in the target sequence (Figure 1) with high specificity, sensitivity and rapidity.

LAMP Primer designation

At least 4 different primers are required for LAMP, primer designation is quite complex. Nowadays, there are 2 versions, version 3 and 4, of LAMP primer design program, named Primer Explorer. This is web application based on Java technology which users can access at http://primerexplorer.jp/e/. The limitations of this application is acceptable only the target sequence with a length of less than 2,000 bp. It does not support multiple sequence alignment file format. However, there is a project of LAMP signature software development, named LAVA (LAMP Assay Versatile Analysis), with no limitation of length of target sequence (Torres et al. 2011). Primer Explorer can design basic 4 primers (F3, B3, FIP and BIP) of LAMP and use the information of selected primer set to further design loop primers. Furthermore, in version 4, users can design LAMP primer set using mutation gene.

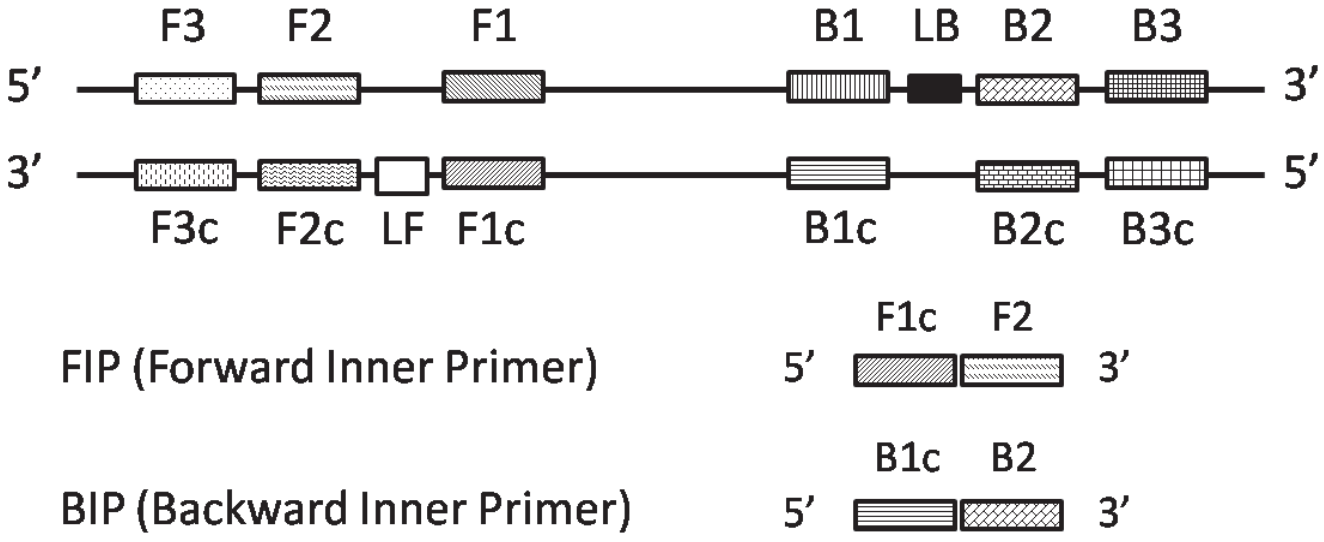

Figure 1. The location of a set of LAMP primers, F3, B3, FIP, BIP, LF (loop forward primer) and LB (loop backward primer), in the target sequence.

Schema of LAMP-primer designation is shown in Figure 1. The major step is alignment of the target DNA and mark regions for F3, F2, F1, B1, B2 and B3. The most important primers are FIP and BIP because the initial step starts from inner parts. FIP comprises of F2 and F1c that is sequence complementary to F1 while BIP consists of B2 and B1c sequences (Notomi et al. 2000). FIP and BIP primers employed a TTTT linker in the first publication of LAMP technique. However, a TTTT linker can be omitted and LAMP-primer design software, either new version or new applications, will not include a TTTT spacer in FIP and BIP primers (Torres et al. 2011).

Detection of LAMP result

The detection of LAMP product is very simple and there are several ways (Figure 2). Mostly, gel electrophoresis is used to confirm amplification of DNA. LAMP assay is also used it to show specific pattern of DNA amplification, ladder like bands (Figure 2A) (Notomi et al. 2000). LAMP is different from PCR because it does not use high temperature to perform DNA denaturation. As a result, the product of magnesium pyrophosphate, white turbidity, is not destroyed by high temperature and can be seen by naked eyes (Mori et al. 2001). Nevertheless, the visual assessment is limited by inspectors (Iwamoto et al.

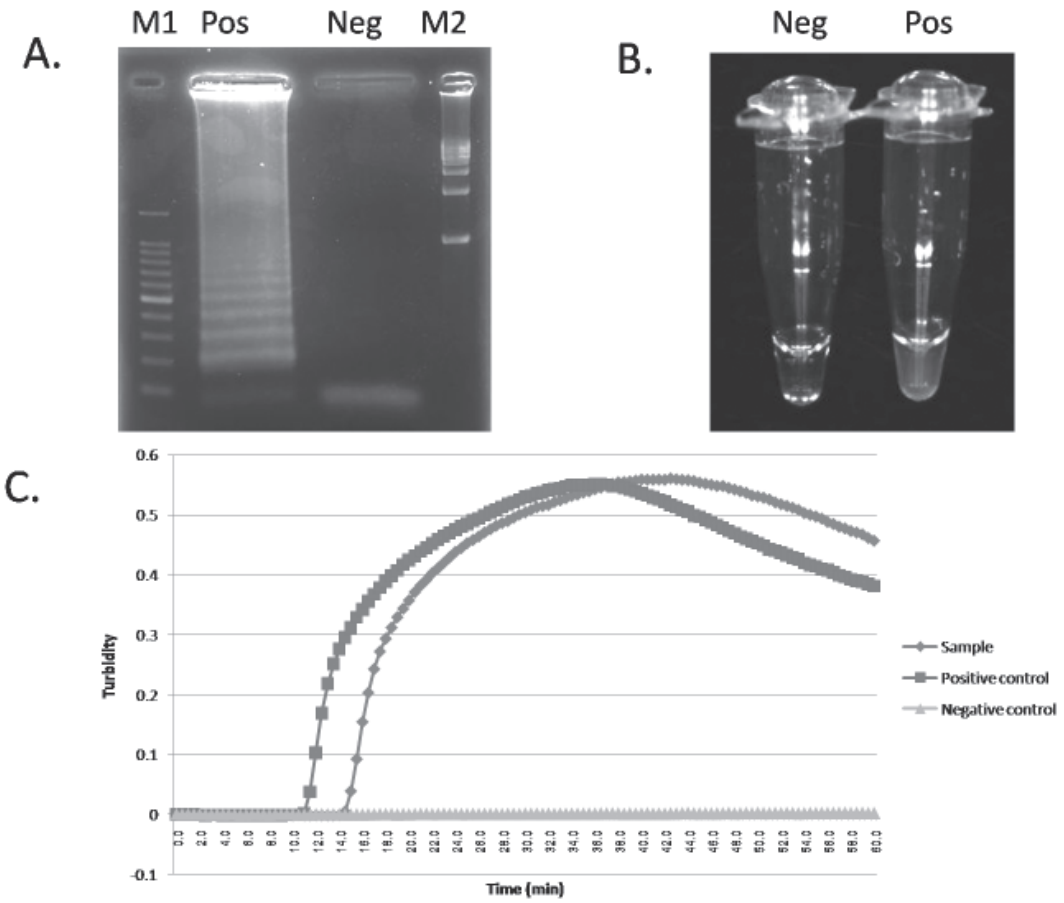

Figure 2. These are examples for the detection of LAMP result. (A) Detection of LAMP reaction by 2% agarose gel electrophoresis follows by ethidium bromide staining. LAMP produces many bands of different product size. M1: 100 bp ladder, Pos: positive control, Neg: negative control, M2: 1 kb ladder. (B) LAMP amplification as the turbidity of magnesium pyrophosphate can be detected by naked eyes. Pos: positive control, Neg: negative control. (C) Real-time monitoring by Loopamp real-time turbidimeter LA-200 (Teramecs, Kyoto, Japan). The positivity will be decided when turbidity threshold is greater than 0.1.

2003). The amount of magnesium pyrophosphate is related to amplified DNA in LAMP reaction, that has resulted in the development of turbidity detector in order to LAMP result and applies for quantification of nucleic acid (Mori et al. 2001, 2004). Accordingly, application of turbidimeter, end-point and real-time turbidimeter, can help to reduce detecting time and bias of analysts.

As mentioned in visual turbidity inspection, the detection of LAMP reaction is quite easy. However, there are several developments of alternative detection technique, for example, ethidium bromide, SYBR green, fluorescent, metallic ions and hydroxynaphthol blue (Notomi et al. 2000; Iwamoto et al. 2003; Aoi et al. 2006; Goto et al. 2009). These detection approaches of LAMP assist in decreasing diagnostic time which leads LAMP to be an alternative of point-of-care test.

LAMP applications

Applications in veterinary field

LAMP is suitable to be used in the field and also at the point-of-care because of simplicity, rapidity and specificity. LAMP becomes popular and has been recognized by researchers from the number of publications (Soliman and El-Matbouli 2005; Nogami et al. 2008; Tatibana et al. 2009; Thekisoe et al. 2009; Postel et al. 2010). The first application of LAMP that related to veterinary field is for *Mycobacterium tuberculosis* complex, *M. avium* and *M. intracellulare* (Iwamoto et al. 2003). This LAMP assay applied SYBR Green I for the detection of positive samples. In addition, the authors performed LAMP by using extracted DNA from sputum specimens and medium isolates. The study of Kono et al. (2004) demonstrated the LAMP for white spot syndrome virus in shrimp. For protozoan diseases, Kuboki et al. (2003) showed that LAMP can be used for detecting African trypanosomes, *Trypanozoon* and *Trypanosoma congolense*, which causes African Trypanosomosis in humans and animals. LAMP-based technology has successfully demonstrated to detect several pathogens, for example, *Edwardsiella ictaluri* in fish (Savan et al. 2004), West Nile virus (Parida et al. 2004), *Babesia gibsoni* (Ikadai et al. 2004), Newcastle disease virus (Pham et al. 2005), foot and mouth disease virus (Dukes et al. 2006) and Japanese encephalitis virus (Toriniwa and Komiya 2006). In addition, LAMP is also used as rapid method for sexing of bovine embryo (Hirayama et al. 2004, 2006; Nogami et al. 2008). Nowadays, Eiken Chemical Co., Ltd. has launched LAMP-based kit for several diseases, including tuberculosis complex and human African Trypanosomosis (news in the company's website at http://www.eiken.co.jp/en/index.html; accessed on Feb 2, 2012). Boehme et al. (2007) showed the clinical evaluation of the LAMP assay for the detection of tuberculosis in several countries. Moreover, Laohasinnarong et al. (2011) has demonstrated the application of LAMP-based method in order to the detection of African Trypanosomosis with cattle-blood and tsetse samples.

Combination with other technologies

LAMP can be applied for both DNA and RNA. LAMP has shown the potential for amplifying nucleic acid in several ways, for instance, conventional LAMP, real-time LAMP, multiplex LAMP and SNP LAMP (Fukuta et al. 2006; Toriniwa and Komiya 2006, 2006; Iseki et al. 2007; Thekisoe et al. 2007; Laohasinnarong et al. 2011). LAMP is also able to combine with other technologies. Hataoka et al. (2004) integrate LAMP and electrophoresis analysis on microchips, termed on-chip LAMP, which it can detect gene within 15 - 20 min with high specificity and reproducibility. ABC-LAMP combines alternately binding probe competitive (ABC) assay and LAMP (Tani et al. 2007). This technique shows the similar accuracy to real-time PCR. However, it is easier and simpler as measuring at the beginning and the end of reaction. In recent times, LAMP is applied with microfluidic technique which enhances

LAMP to be used as point-of-care test (Fang et al. 2010, 2011; Ahmad et al. 2011).

Pros and cons of LAMP

Isothermal amplification methods have shown elimination of heat denaturation step and use multi-enzyme or enzyme with strand displacement to drive new round of DNA synthesis. Therefore, isotherm-based DNA amplification techniques do not need thermocycling tools. However, each assay has its own limitations, including LAMP.

LAMP has been thriving demonstrated that it can be applied to detect in a variety of samples (Iwamoto et al. 2003; Poon et al. 2006; Misawa et al. 2007; Laohasinnarong et al. 2011; Shao et al. 2011). Furthermore, it shows a very high sensitivity in order to detect trypanosomes DNA as little as 1 fg of DNA which is equal to 0.01 trypanosomes (Thekisoe et al. 2007). Numerous inhibitors for *Taq* DNA polymerase in sample may cause failure of PCR as previous mentioned. LAMP uses *Bst* DNA polymerase because it shows better performance of amplification and tolerates to those components (Notomi et al. 2000; Thekisoe et al. 2009). Additionally, LAMP is also resistant to various components in clinical samples than PCR, such as saline, plasma, serum and urine (Kaneko et al. 2007).

Although, LAMP can be an alternative molecular diagnostic, there is a concern of LAMP reagents storage. The manufacturer recommends keeping all reagents in -20^{o}C freezer. This makes it inconvenient to use in the field, especially tropical countries. On the other hand, LAMP reagents keep in lyophilized form, without need of cold-chain, which use in prototype LAMP kit for pulmonary tuberculosis, can be performed without effect (Boehme et al. 2007). The study of Thekisoe et al. (2009) has demonstrated of using temperature different from recommendation, 25^{o}C and 37^{o}C, LAMP can be performed as normal. This shows that LAMP buffer and *Bst* DNA polymerase are stable even store them in the ambient temperature. In addition, technicians with no experience of molecular techniques can perform after 1 week of training (Boehme et al. 2007).

A limitation of LAMP is inapplicable to amplify long sequence in comparison to PCR. PCR seems to be no limit on the length of the amplifiable sequence, while LAMP will work well when the length of the target DNA is less than 300 bp (Notomi et al. 2000). Another limitation is the detection by naked eyes from turbidity, fluorescent dye or hydroxynaphthol blue. In the real cases, there will be a slightly positive result for which LAMP method may not produce sufficient quantities of turbidity. Therefore, it is difficult to detect it. Overall, LAMP, however, it also has several advantages over PCR.

Conclusion

Loop-mediated isothermal amplification (LAMP) is a new molecular assay that amplifies nucleic acid by applying steady temperature with high specificity and rapidity. LAMP uses a set of 4 to 6 primers that will amplify 6 to 8 regions of the target sequence. In addition, LAMP does not require sophisticated thermal cycler tools and the amplification duration of less than 1 hour. And so, LAMP can be performed by applying basic laboratory tools, for instance, heat block or water bath. Several LAMP-based applications have been developed and showed higher specificity, sensitivity, simplicity and rapidity over PCR. In consequence, LAMP can be applied for field diagnosis.

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