REVIEW ARTICLE

Application and Modification of RT-LAMP for Rapid Detection of SARS-CoV-2 Viral Genome

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ABSTRACT

COVID-19 outbreak caused by the newly discovered SARS-CoV-2 has become a major public health threat around the world and has create a tremendous effect on the global economy. Hence, there is a high demand for rapid and accurate diagnosis to contain the spread of the disease. The Reverse-Transcription Polymerase Chain Reaction (RT-PCR), the current standard for diagnosis of COVID-19 however possesses certain drawbacks that limits its application to meet the high demand of the continually increasing COVID-19 cases. Conversely, Loop-Mediated Isothermal Amplification (LAMP) is another nucleic acid amplification method that shows a great potential as an alternative tool in rapid diagnosis of COVID-19 due to its simplicity and rapidity. This review summarized the recent published research articles related to the application and modification of RT-LAMP assay for the rapid detection of COVID-19 in comparison with other available diagnostic methods.
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INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 or well-known as SARS-CoV-2 is a novel coronavirus that was initially discovered in the city of Wuhan, China. The newly discovered virus is responsible for COVID-19 pandemic that has grown into a global public health concern, as it contributes to rapidly expanding number of illnesses with considerable morbidity and death over the globe. COVID-19 disease was later declared as a pandemic in March 2020 by the World Health Organization (WHO). The disease continues to spread, infecting millions of people around the world. This has resulted in an urgent need for a rapid diagnostic testing that could be swiftly expanded on a global basis (1). As COVID-19 disease is present with non-specific clinical symptoms which are similar to other diseases, molecular based-diagnostic method remains as the gold standard for detection and diagnosis of the disease (2).

Reverse Transcription Polymerase Chain Reaction (RT-PCR) is the standard molecular-based assay for detection of SARS-CoV-2 approved by the US Centers for Disease Control and Prevention (CDC) (3). Although the assay is proven to be a sensitive and reliable method for detection, the assay however possesses its own drawback. This nucleic acid amplification assay requires the usage of high-purity sample, well-trained individual and is a specialized and expensive instrument to run. On top of that, the assay is time consuming as it takes approximately up to 2 hours for the assay to be completed. Hence, these drawbacks restrict its extensive applicability to the present massive demand for COVID-19 detection in the current global pandemic situation (4).

LAMP, or isothermal nucleic acid amplification method is a great alternative to RT-PCR, in that it does not require the use of expensive instruments nor the change of temperature to perform the reaction nor to assess the results (5). This method amplifies DNA under isothermal conditions with excellent specificity, efficiency, and rapidity (6). Several previous studies have implemented RT-LAMP based assay which includes additional reverse transcriptase enzyme in the amplification and detection of SARS-CoV-2 viral RNA. Certain modifications were also made to further minimize the preparation steps, time of reaction and increase the efficiency and reliability of RT-LAMP amplification, especially to be used as a point of care test (POC). This review described the general principle of LAMP assay and summarized the previous studies that developed different LAMP-based methods for the detection of COVID-19.

REVERSE TRANSCRIPTION LOOP MEDIATED ISOTHERMAL AMPLIFICATION (RT-LAMP)

Loop-mediated isothermal amplification (LAMP) is a powerful alternative method for the amplification of nucleic acid, which can detect DNA even at a very small number of copies. This technology had previously been used in the detection and diagnosis of diseases caused by pathogens. LAMP is carried out in less than an hour under one constant temperature at about 60oC, obviating the need for thermal changes that is required by traditional PCR method (7). In LAMP, a set of four to six specifically designed primers is employed to bind to specific region on the target sequence of the DNA. This contributes to the high specificity of the assay. Likewise, DNA polymerase with strand polymerization activity is also included in LAMP reaction mixture to allow the extension of deoxynucleotide triphosphates on the newly synthesized DNA strand and accumulating many copies of target nucleic acid sequence in an isothermal condition (8).

On the other hand, amplification and detection of RNA virus, like SARS-CoV-2 becomes more effective by combining both reverse transcription and LAMP together in one single reaction. This reaction is known as the reverse transcription loop-mediated isothermal amplification (RT-LAMP). Single reaction can be done in RT-PCR by incubating the reverse transcriptase enzyme, nucleic acid sample, specifically designed LAMP primers, substrate and DNA polymerase in one same tube at optimal temperature of the reaction. Hence, the reaction time of the assay is significantly reduced and resulting in the rapid detection of disease caused by the virus (9).

Principle of RT-LAMP

In RT-LAMP, after the conversion of RNA into cDNA by reverse transcriptase enzyme, the reaction will proceed with the non-cyclic step and followed by the cyclic steps (Figure 1). This process employs a set of specific primers that bind to the target sequence that will later be amplified under isothermal condition with the help of DNA polymerase with high displacement activity (11). A total of six primers including two additional loop primer used in RT-LAMP accelerate the reaction in which enables the amplification to be achieved within 30 minutes (9,12).

In the non-cyclic step, RT-LAMP is initiated by binding the backward internal primer (BIP) to the target sequence on the 3′ end of the RNA template. After the binding, a new copy of DNA strand will then be synthesized based on the template RNA starting at the 5′ end of the BIP using reverse transcriptase enzyme (13). The copy of the DNA strand is termed as complementary DNA or cDNA. Then, the backward outer primer (B3) binds to region outside the BIP and start to synthesize new cDNA with the activity of reverse transcriptase, while releasing the previously form cDNA by the BIP (11).

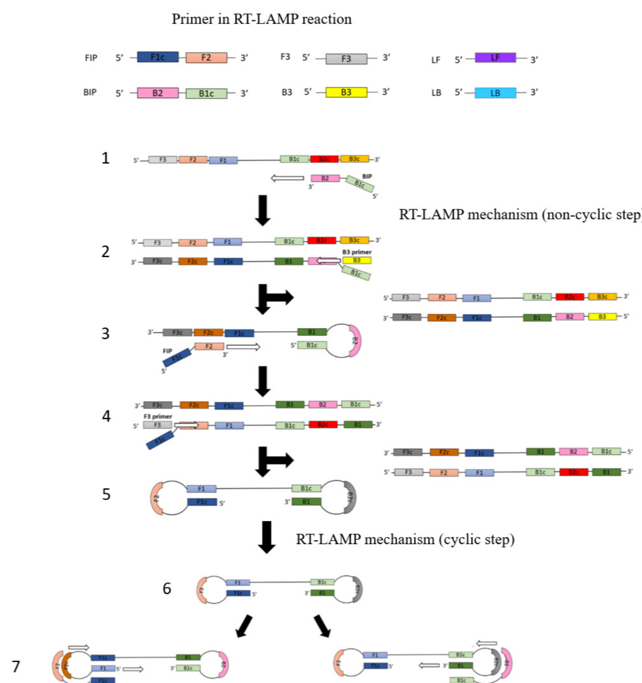

Figure 1: Illustration for primer and principle of RT-LAMP. Adapted from Salamin et al. (2017) (10).

The released single stranded cDNA forms a loop as it contains complementary sequence at the 3′end. Forward internal primer (FIP) then binds to the single stranded cDNA at the 5′ end accompanied by DNA polymerase and become a starting point to initiate the synthesize of a new complimentary DNA (6). The forward outer primer (F3) binds to the F3c region outside of FIP, generating new double stranded DNA while releasing the previously formed strand. The released single strand DNA forms a stem-loop at both 5′ end and 3′ end as the ends self-anneal to its complimentary F1c and F1 regions, resulting in a dumbbell-like structure. The dumbbell-like structure later serves as starting the structure for cyclic step in exponential amplification (14).

In exponential amplification, self-primed DNA synthesis is initiated at the F1 region with the help of DNA polymerase. At the same time, the FIP bind to the F2 region on the loop structure at one end of the structure and starts to synthesize new DNA strand. The new synthesized strand of DNA displaced the F1 self-primed strand that later self-bind at its complementary region to form a loop structure (15). Self-primed DNA synthesis is once again initiated, but at the B1 region of another newly formed loop, amplifying the template while displacing the previously formed FIP-primed strand. From this repeated cyclic process, various sized structures that consist of alternately inverted repeats of the template on the same strand are formed (11).

Application of RT-LAMP in clinical diagnosis

The LAMP or RT-LAMP method has piqued the interest of many researchers due to its simplicity, sensitivity, and rapidity (16,17). The characteristics and benefits of this amplification method are the key points that make RT-LAMP suitable to be applied in point-of-care diagnostic testing. Hence, scientists working on diseases that risk human life and health have been particularly interested in the LAMP approach to provide rapid clinical diagnosis of the diseases based on the target DNA sequences (18). For example, the diagnosis of periodontal disease based on LAMP method was first developed in 2005 (19). The method was developed to provide a rapid detection of three main periodontal pathogens that ware responsible for periodontitis including porphyromonas gingivalis, Tannerella forsythia, and Treponema denticola. This modified LAMP method by Yoshida et al. was able to provide qualitative and quantitative analysis of the infectious pathogen, giving rise to a detailed and more accurate diagnosis of the disease with corresponded result to conventional PCR assay (20).

RT-LAMP on the other hand has been developed for diagnosis of virus infection in the previous year. This includes human infection due to H7N9 and H10N8 influenza virus that are highly associated with exposure to live poultry market (21,22). In 2014, Nakauchi et al. developed an RT-LAMP assay to provide rapid detection of avian influenza A (H7N9), a virus that once caused an epidemic in China (24). The assays were tested on in vitro transcribed RNA, clinical specimens and clinical isolates with sensitivity as low as 42.47 copies/reaction. The reaction of the assay can be completed within 30 minutes while retaining a high sensitivity and specificity. In 2015, one-step RT-LAMP based method was designed to detect H10N8 influenza virus in experimentally infected and clinical sample (25). This assay specifically targeting the hemagglutinin (HA) and neuraminidase (NA) gene of the virus and showed a higher sensitivity as compared to the RT-PCR. Hence, both developed RT-LAMP assays are seen as promising rapid diagnostic testing for detection of the influenza virus to monitor the spread of the virus and limiting human contact to infected fowl (26).

This rapid yet reliable isothermal nucleic acid amplification method was also applied in the clinical diagnosis of recent emerging diseases such as the Middle East respiratory syndrome coronavirus (MERS-CoV) infection. Rapid diagnosis becomes the key in the management of the virus spread due to limitation for specific antiviral and vaccine against the virus (27). In 2015, Bhadra et al. designed an RT-LAMP to amplify ORF1a, ORF1b and E gene of MERS-CoV with cooperation of one-step strand displacement probe (OSD) for verification of the LAMP product. The assay can detect at least 0.02 plaque forming unit (PFU) in infected cell culture supernatants. Additionally, completion of the assay was achieved within 50 to 30 minutes with high specificity as it does not cross react with another human respiratory pathogen (27). Nowadays, many researchers are focusing on the development of the RT-LAMP based method to meet the requirement for rapid diagnosis and detection of SARS-CoV-2, the virus responsible for the current ongoing COVID-19 outbreak (29–33). As of now, the RT-LAMP shows a great potential to become an alternative diagnostic tool for COVID-19 detection, replacing the current conventional RT-PCR.

COVID-19 DIAGNOSTIC METHOD

There have been many research laboratories and public health laboratories around the globe that implemented virus isolation using cell culture methods to detect on SARS-CoV-2 from respiratory sample of suspected patient (34). However, due to its low sensitivity, time-consuming nature, and requirement for specialized equipment and technical expertise to handle, virus isolation is not recommended as a routine diagnostic procedure (35). In contrary, Reverse Transcription Polymerase Chain Reaction (RT-PCR) is considered as the current gold standard for COVID-19 diagnosis as analyses using RT-PCR can detect SARS-CoV-2 RNA at low levels, with up to 98% specificity and 97% sensitivity using samples from upper and lower respiratory tract (36–38). Despite being the most widely used method in COVID-19 detection, this assay is expensive and complex. Thus, limit it uses especially in current pandemic situation with high demand for rapid detection of the infection and in resource-limiting setting (39).

Serology-based test is another method that is currently being used in detection of the disease. Enzyme-linked immunosorbent assays (ELISAs) and lateral flow immunoassay (LFA) are the two examples of the most popular methods that apply serology testing for detection of COVID-19 (40). Serology-based test is done through detection of viral antigen such as the spike protein or two primary antibodies against the virus, which are the IgM and IgG present in blood, serum or plasma of infected patients (41). Compared to nucleic acid amplification tests (NAATs) like RT-PCR, this method is considered faster and less expensive (42). Nevertheless, there are some evidences suggesting that virus-based IFAs and ELISAs have a high level of sensitivity (85–100%) but with a low degree of specificity (43). COVID-19 serological test is not effective at an early stage of viral infection as the targeted antibodies only produce after several days of infection (44)

APPLICATION AND MODIFICATION OF RT-LAMP IN DIAGNOSIS OF COVID-19

The RT-LAMP assay possesses great advantages over other molecular-based diagnostic methods, especially to be applied as point of care (POC) test for mass screening and detection of contagious infection like SARS-CoV-2. This method can be performed in a constant temperature

using simple machine example waterbath or heat block, obviating the need for expensive thermal cycler like in RT-PCR and can be completed under less than an hour (45,46). Moreover, Xin et al. in 2022 describe an inexpensive US$2 nucleic acid test kit based on RT-LAMP assay for self-administered at-home testing as compared to US$37.30 RT-PCR method (47,48). In terms of sample used, RT-LAMP requires the same samples as RT-PCR including nasal swabs, nasopharyngeal swabs and saliva (49). Additionally, in diagnosis of COVID-19 , the accumulative sensitivity and specificity of the RT-LAMP assay was recorded at 95.5% and 99.5%, respectively, based on meta-analysis (50). The characteristic of the RT-LAMP assay has piqued the interest of many researchers to concentrate on improving and modifying the RT-LAMP approach to meet the requirements of the COVID-19 diagnosis in order to achieve a rapid yet reliable result. In the following section, the current available modification and application of RT-LAMP for amplifying and detecting SARS-CoV-2 RNA genome in the diagnosis of COVID-19 will be describes. Table I summarized the platform of RT-LAMP in diagnosis of COVID-19.

CONCLUSION

The implementation of comprehensive and sustainable BE starting from the antenatal to postnatal period accompanied by peer or professional support has been proven to have a maximum effect or influence especially on exclusive breastfeeding. Secondary effects are early initiation of breastfeeding, breastfeeding self-efficacy, duration of breastfeeding, mother's knowledge and attitudes, breastfeeding problems, and other influences such as the length of hospitalization of the babies. An effective BE must pay attention to the target, important information that must be conveyed, the methods and media used, who provides education and the place education.

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Table I: Summary of platform for RT-LAMP based detection for COVID-19 diagnosis

Procedure (RT-LAMP platform)	Sample type	Target gene	Limit of detection	Need for RNA extraction	References
Placing sample into iSWAB™ Extraction-less buffer (direct RT-LAMP)	Oropharyngeal, nasopharyngeal swabs, anterior nasal and mid-turbinate nasal swabs, nasopharyngeal aspirates and bronchoalveolar lavage	ORF1 a/b, E and N gene	80 copies/µL	No	(44)
Two-color RT-LAMP (colorimetric RT-LAMP)	pharyngeal	N gene	N/A	No	(45)
Simple lysis and viral inactivation protocol (direct RT-LAMP)	Nasopharyngeal, nasal and saliva	N,E and OR-F1ab genes	93 virions per reaction	No	(48)
Heat inactivated swab sample (direct RT-LAMP)	Nasal swab	ORF1b	1.43 x 103 copies/mL	No	(50)
RT-LAMP readout based on Real-time turbidimeter, colorimetric and gel electrophoresis	Nasopharyngeal swab	N, E and OR-F1ab gene	1000 copies/ml	Yes	(51)
Addition of Lucigen Quickextract lysis buffer in Fluorescent-based detection RT-LAMP (direct RT-LAMP)	Nasopharyngeal swab	N, E and OR-F1a gene	625 copies/µl	No	(52)
Purification and optimization of alternative LAMP enzymes on heat inactivated samples (direct RT-LAMP)	Nasopharyngeal swab	ORF1ab	N/A	No	(53)
Pre-treated sample using Mucolyse™, chelating agent and followed up with heat treatment (direct-RT-LAMP)	Saliva	N/A	between 1×10^1 and 1×10^2 copies / µL	No	(54)
Sample purification using silica (direct RT-LAMP)	Nasopharyngeal swab and saliva	ORF1a	1 copy/ µL	No	(55)
Colorimetric RT-LAMP based on hydroxynaphthol blue (HNB) dye	Nasopharyngeal swab	N/A	1 copy/reaction	Yes	(56)
Dual RT-LAMP colorimetric	Nasopharyngeal swab sample	ORF1ab and N gene	100 copies/ µL	Yes	(57)
Addition of phenol red, pH sensitive dye (colorimetric RT-LAMP)	Saliva and swab	N gene	200 copies/ µL	Yes	(58)
Improvising colorimetric RT-LAMP by primer combination	Synthetic RNA control template	S gene	N/A	N/A	(59)
Pre-treated saliva sample with heat in multiplex colorimetric RT-LAMP	Saliva	ORF1ab and N gene	59 particle copies/ reaction	No	(5)
Increasing pH of inactivation buffer in pH-based colorimetric RT-LAMP	Nasopharyngeal swab and saliva	ORF1a and N gene	2 copies/ reaction (N gene) and 12.5 copies/ reaction (ORF1a)	No	(60)
Fluorescence RT-LAMP based on hydroxynaphthol blue (HNB) dye	Nasopharyngeal swab	E and N gene	100 copies/ reaction	Yes	(61)
Colorimetric RT-LAMP assisted by mobile application	Nasopharyngeal swab, aspirates and saliva	ORF1ab	between ~112,000 and ~22,000 copies/ µL	Yes	(62)
CRISPR-based DETECTR Lateral Flow Assay	Nasopharyngeal swab	N and E gene	10 copies/ µL	Yes	(63)
RT-LAMP-coupled CRISPR-Cas12 based on fluorescence detection	Nasopharyngeal swab	N and E gene	10 copies/ reaction	No	(64)
RT-LAMP linked to Cas13-based detection	Nasal swab and saliva	N gene	40 copies/ µL	No	(65)
One-pot visual RT-LAMP-CRISPR	Nasopharyngeal swab	S gene	5 copies/ reaction	Yes	(31)
Multiplex RT-LAMP combined with nanoparticle-based lateral flow biosensor	Oropharynx swab	ORF1ab and N gene	12 copies/ reaction	Yes	(66)
Multiplex RT-LAMP linked with gold nanoparticle-based lateral flow biosensor	Nasopharyngeal swab and artificial sputum	RdRp and N gene	20 copies/ reaction	Yes	(67)

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