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# A Rapid and Specific Loop Mediated Isothermal Amplification Molecular Detection Method; Current and Future Perspective

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## Abstract

Loop-mediated isothermal amplification is a novel nucleic acid amplification method that relies on an auto-cycling strand displacement DNA synthesis, which is performed by Bst DNA polymerase. LAMP is a rapid and simple detection technique that can be used to diagnose several micro-organisms, including bacteria, viruses, parasites and fungi. It is a promising diagnostic and detection tool that could provide the results rapidly, and with high analytic sensitivity and specificity without the use of specialized instrumentation. Many researches and review articles have been published since the LAMP assay was established. However, there is lack of comprehensive review to cover LAMP primer design, mechanisms of amplification, detection methods, and applications together. This impeded and relegated its application for numerous types of disease diagnosis as a point care device. Therefore, this review mainly focuses on how LAMP assay can be designed, carried out starting from primer design to detection of the result, as well as the current approaches for diagnosis of COVID 19 pandemic; in comparison to commend nucleic acid amplification methods and immunological assay. This review exhibits how can be rapid and specific molecular diagnostic assay exploited for viral and bacterial disease detection with in resource limited area

**Keywords:** Clinical diagnosis • COVID-19 • LAMP • Primer design

## Introduction

The advent in the molecular biology has led to development of various types of molecular detection methods, such as Polymerase Chain Reaction (PCR), real-time PCR assay, reverse transcription PCR, quantitative PCR, immunocapture PCR, and PCR-ELISA are being used since the past four decades for diagnosis and molecular study by amplifying a number of relevant genes or DNA segment [1]. Thermo cycler PCR is the most common and gold standard method of nucleic acid amplification technique. Nonetheless, the need for trained staff, operating space and sophisticated instrument to maintain discrepancy, post amplification process like electrophoresis, takes more time (3-4 h) to know the result, requires extracted DNA from sample and sensitive for contaminants has impeded its usefulness [2-4].

To ravage the odd faced by PCR, different type of isothermal amplification of nucleic acid amplification methods like NASBA (nucleic acid sequence based amplification), 3SR (self-sustained sequence replication) and SDA (strand displacement amplification) along with rolling cycle amplification (RCA), signal mediated amplification of RNA technology (SMART), isothermal multiple displacement amplification (IMBA), helicase dependent amplification (HAD), single primer isothermal amplification (SPIA) and circle helicase dependent amplification (cHDA) have been devised [5,6]. Hence, there has been an increasing demand for simple and cost-effective molecular tests [7,8]. However, all of the above mentioned NAAT methods have disadvantages. For instance, 3SR, NASBA and SDA bypass from PCR by abolishing heat denaturation. The first two methods use a set of transcription and reverse transcription reactions to amplify the specific genomic material whereas SDA uses asset of restriction enzymes digestion and strand displacement DNA amplification with modified nucleotide as substrates. On the other hand, besides their advantage, they have short comes [9].

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Among the above isothermal amplification techniques, LAMP method which was firstly developed by and his coworker from Eiken Chemical Company, Japan is a popular, unique, and easy and an affective diagnosis assay [10]. As the name indicates, LAMP uses constant temperature, diminishes the use of different temperatures required for denaturation, annealing and extension of DNA amplification of PCR [8]. On the other hand loop means, the final products are mixture of stem loop DNA with various stem lengths and cauliflower structure with multiple loops due to hybridization between alternately inverted repeats in the same strand [9,11]. The basic principle of this technique is auto cycling strand displacement DNA synthesis using specific polymerase enzyme with high strand displacement activity like Bst polymerase, isolated from Bacillus stearothermophilus (Bst) and a set of six primers specially designed to bind to unique sites on the target sequence are used [9]. The added advantage of LAMP assay is the overall reaction completes within 30 min [12-15]. LAMP amplifies the greatest amount of DNA more than 109 DNA copies from negligible amount of DNA [9]. Thus, many researchers recommend the assay to use as an ideal microbial identification assay.

According to world health organization (WHO), the criteria of ideal diagnosis comprise those things: specificity (detect solely the targeted pathogen), sensitivity (detect the pathogen if the microorganism is found in small amount), considerably of low cost, rapidity (technique requires minimum time for detect or result), simplicity, adoptability of any sort of environment variation and easy availability of instrument. Perhaps, PCR fulfills some of WHO requirements for ideal diagnosis such as sensitivity and specificity. In case of Loop mediated isothermal amplification technique, satisfies other qualities which are stated by WHO [4]. Moreover, the rapidness of LAMP with its specificity would add upon the short time for physicians to manage patients.

### Features of lamp sensitivity and specificity

The specificity of LAMP for target sequence is very high compared to other nucleic acid amplification methods. This is achieved by the primers which can recognize six to eight sequences of the target gene at the beginning of amplification and later on four regions or sequences of the target gene [5]. This renders to amplify solely the target gene which is unique to that pathogen/specious in order to detect and discriminate from other pathogens. LAMP is very sensitive and able to detect a small amount of DNA (78 pg). This indicates that it is 100 times more sensitive than PCR; which can detect 7.8 ng DNA per test tube [16]. Hence, if there is a little amount of DNA in a sample, it easily amplifies DNA in to billions of copies [17,18]. Additionally, LAMP is less prone than PCR to the presence of irrelevant DNA and PCR inhibitors; so that DNA can be easily extracted through boiling method of DNA extraction [19,20].

## Principles of primer design

Unlike to that of PCR, LAMP has six to eight primers. The performance of lamp mainly relies on crafting of primer which is specific to the target gene. LAMP primers comprise asset of two inner primers, two outer primers and two loop primers. The inner primers are known as forward inner primer (FIP) which contains F1C and F2 hybridize with F2C and backward inner primer (BIP) hybridize with B2C and it contains B1C and B2. Outer primers are known as forward outer primer (F3) and backward outer primer (B3) and those comprise F3 and B3 which are complementary to F3C and B3C accordingly [21,22]. Incorporation of two more primers i.e. loop primers increase the specificity and specificity as well as reduce the reaction time [21,23]. This symbols (F1C, F2C, B1C, B2C, F3C, B3C) were assigned by and explain as follow, the nucleotide sequences [23-34] inside both ends (5'and 3') of the target region for amplification in a DNA are designed F2c and B2 respectively, and two inner sequences (23-24 ntd) 40 ntd from the ends of F2c and B2 are designed as F1c and B1 as well as two sequences (17-21) outside the ends of F2c and B2 are designed as F3c and B3 (Figure 1).

## LAMP reaction mechanism

LAMP reaction is carried out by auto-cycling strand displacement DNA synthesis which is done at 60-68 0c for 30-60 min with the aid of specific primers, dNTPs and template DNA and Bst Polymerase; used for both strand displacement and polymerase activity. The reaction mechanism has three stages; starting material producing stage, cycling amplification stage and elongation and recycling stage [5]. In the initial stage, FIP (inner primer) hybridizes with F2 region which is found in the target gene. Thereof, it initiates complementary new strand synthesis. F3 (outer primer) which is short length base and less concentration than inner primers. This render to diminish the hybridization of outer primer (F3) with the target region of the gene before FIP hybridizes. F3 slowly hybridizes with F3c on the target DNA strand and initiates strand displacement DNA synthesis, releases FIP linked complementary strand, which forms looped structure at one end. This single strand used as a template for BIP -initiated DNA synthesis and subsequent B3-primed strand displacement DNA synthesis leading to the production of a dumb-bell form DNA, which is quickly converted to a stem –loop DNA. Then this stem loop structure is used as a starting material for the next of reaction, LAMP cycling.

The second stage starts by hybridization of FIP with the loop in the stem-loop DNA. Then FIP primes strand displacement and DNA synthesis, generate an intermediate one gapped stem loop DNA with an additional inverted copy of the target sequence in the stem. BIP sequence forms Loop at the opposite end. Subsequent self-primed strand displacement DNA synthesis yields one complementary structure of the original stem-loop DNA and one gap repaired stem-loop DNA with a stem elongated to twice as long and a loop at the opposite end. Both of them are used as template for the next BIP-primed strand displacement in the subsequent cycles, the elongation and recycling step. Mixtures of stem loop DNA with numerous stem length and cauliflower-like structures with multiple loops are produced at end of the reaction [5]. Moreover, the reaction mechanism is illustrated in the underneath (Figure 2).

## LAMP amplified product detection methods

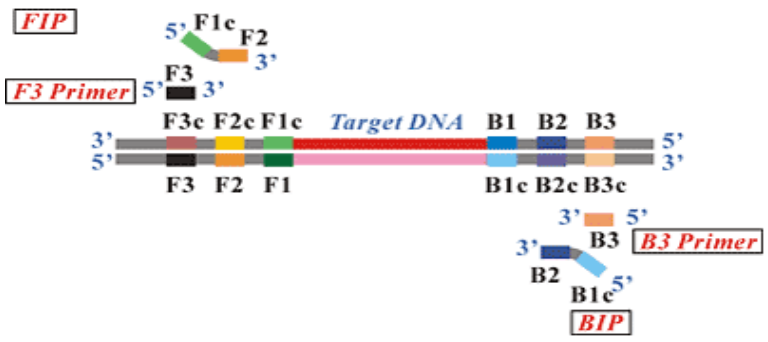
Several detection methods can be used for detection of positive and negative result of LAMP reaction. The most common method is agarose gel electrophoresis. Gel staining agent such as Ethidium bromide is used as intercalate in the DNA bases. Under UV illumination, the gel shows a ladder like structure from the minimum length of target DNA with the various length stem-loop products of the LAMP reaction. Amplified LAMP reaction mixtures contained magnesium pyrophosphate (as a by-product), a white turbidity, which could be observed by the naked eye, emerged in the positive tubes, and no turbidity in the negative tubes [24,25]. Naked-eye detection is performed using nucleic acid binding chemicals such as SYBR Green II and Calcine by adding in the reaction mixture, and the color change will be observed [26]. SYBR Green is one of a cyanine dye which closely binds to all double-stranded DNA present in the amplified product. SYBR Green dye binds to all double-stranded DNA, so in case of RT PCR the result is an increase in fluorescence intensity proportioned to the amount of PCR product produced [27]. SYBR Green also used as staining dye for southern analysis. Thus, it can be appropriate in primary clinics or field labs. Using instruments, the amplified DNA can be also detected as indicated in Table 1.

Moreover, based on the purpose, selection of Lamp detection method can be done; for instance endpoint turbidimetry and end point fluorimetry are inexpensive methods. For Sensitive, real time turbidimetry and real fluorescence can be used whereas for Sequence specific, lateral flow device and ABC-Lamp can be also used [28].

## LAMP applications

Loop mediated isothermal amplification (LAMP) is unique and easy assay for diagnosis of disease. According to WHO, the criteria of ideal diagnosis comprise those things: specificity (detect solely the targeted pathogen), sensitivity (detect the pathogen if the microorganism is found in small amount), considerably of low cost, rapidity (technique requires minimum time for detect or result), simplicity, adaptability of any sort of environment variation and easy availability of instrument [12,29]. Mostly published journals are talking about that application of LAMP on microbial detection but LAMP is also employed in diverse fields Table 2. It can be successfully employed for the identification of genetically modified organisms (GMOs), cancer cells, food adulterations, molecular diagnosis, drug resistance, identification of medicinal plants, and allergens [30,31].

LAMP assay was developed by [32] for diagnosis of cancer. As, LAMP can detect of carcinoembryonic antigen-mRNA as a marker for detecting tumor cells in patients with non-small cell lung cancer. RNA isolated from tumor and lung nodes were used for comparison of LAMP with RTPCR as diagnosis of cancer. The LAMP diagnostic assay was found to be 81% sensitive and 100% specific [33] also developed the LAMP assay for the diagnosis of chronic myeloproliferative neoplasms. This LAMP assay detected low levels of mutation which were undetectable by PCR. The other application of LAMP is GMO detection event and it was proved to be sensitive than the routine PCR. LAMP assay is target mainly exogenous elements or foreign genes of GMO [31].




FIP (Forward Inner Primer) BIP (Backward Inner Primer)

F3 (Forward Outer Primer) B3 (Backward Outer Primer)

**Figure 1.** Lamp primer location on the gene. Source: [9].

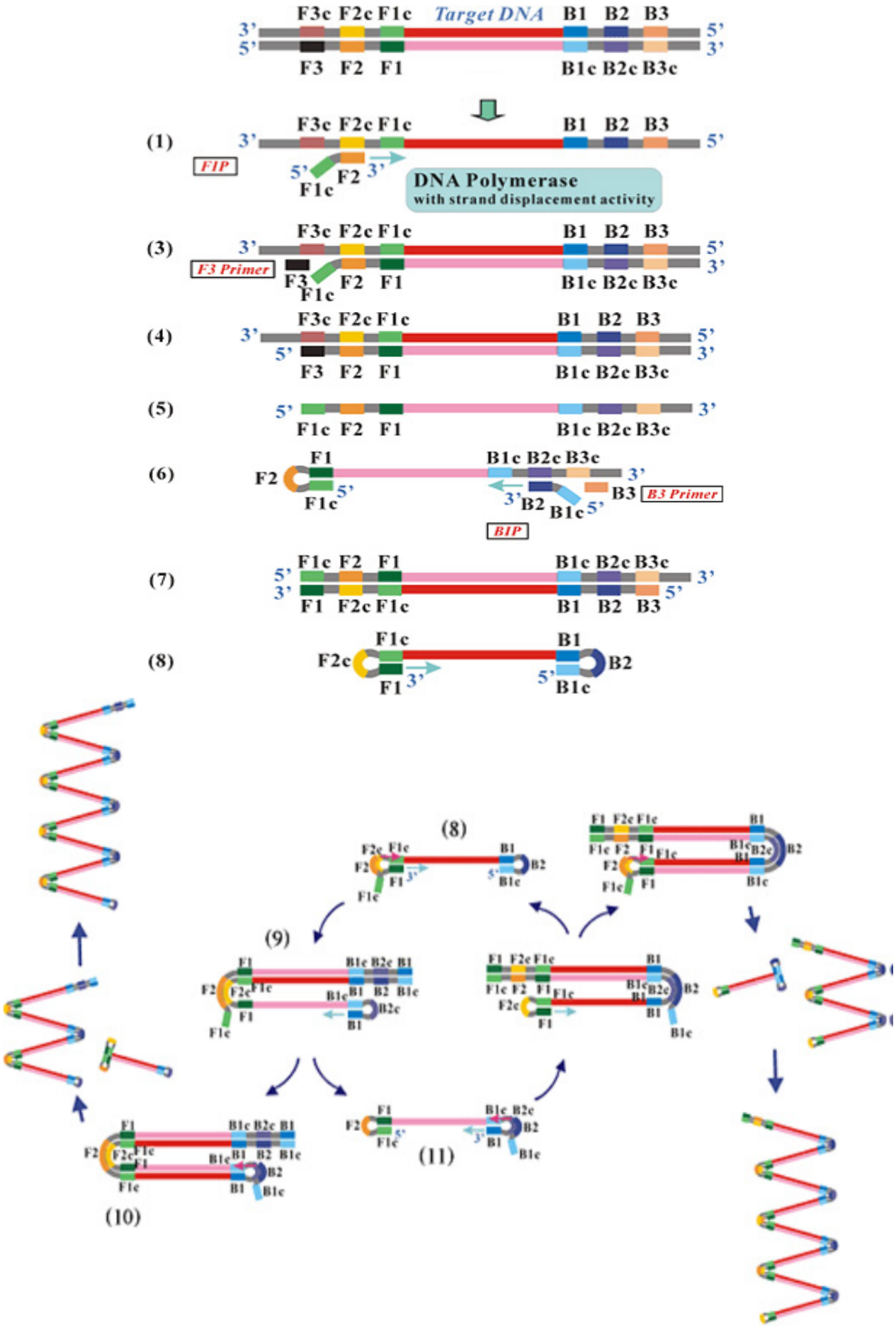



**Figure 2.** Mechanism of loop mediated isothermal amplification [9].

**Table 1:** various detection methods of LAMP results (28).

| Detection method | Instruments | Detects | Measurement | Cost | Result detects by necked eye | Time |
|---|---|---|---|---|---|---|
| End point turbidity | Spectrophotometry Or turbidimeter | Amount of Mgpyrophesphate | Qualitative | Inexpensive | Yes | Rapid |
| Real time turbidity | Spectrohtometer or turbisimeter | Amount of Mgpyrophesphate | Quantitative | Expensive | Yes | Very repaid |
| End point flourescence | Flourimeter | Amplified product (DsDNA) | Quantitative | Inxpensive | Yes | Rapid |
| Real flourescence | Flourimeter | Pyrophosphate salt | - | - | - | |
| Lateral flow device | Lateral flow device | amplified product | Non quantitaive | Inexpensive | - | sequence specifi, rapid |
| ABC-LAMP (Alternately Binding quenching probe Competitive LAMP) | Fluorimeter | Amplified product | quantitative | Expensive | - | Rapid |

**Table 2**: Diverse applications of LAMP: Beyond microbial identification Source: [31].

| Purpose | Detection | Gene |
|---|---|---|
| GMO | Maize | 35S Promoter |
| | Rice (TT51-1) | Sucrose phosphate synthase |
| | Soybean | Lectin, Nos: GTS 40-3-2: MON89788 |
| | Wheat | (B73-6-1) B73-6-1 |
| Diagnosis | Lymph node metastasis in lung | Carcinoembryonic antigen-mRNA |
| | Neoplasm (myeloproliferative neoplasm) JAK2V617F | JAK2V617F |
| | Gastric cancer cells | Cytokeratin-19 |
| Allergen | Celery (*Apium graveolens*) | Mannitol dehydrogenase |
| Pesticide | Organophosphorus in agroproducts | Monoclonal antibody against OP |
| Medicinal plants | Ginger (Zingiber officinale) | RAPD amplicon |
| Adulteration | Ostrich meat | Cytochrome b |
| Drug resistance | Multidrug resistance gene | NDM-126590244/ Cfr |
| Species and sex identification | Formosa landlocked salmon | Growth hormone GH 1 and OtY2m; |
| Epigenetic study | Hypermethylated DNA | Promoters of CDKN2A, GATA5 and |

**Notes:** GMO: genetically modified organisms, NDM-1: New Delhi Metallo-lactamase 1, cfr: chloramphenicol-florfenicol resistance, CDKN2A: cyclin-dependent kinase inhibitor 2A, GATA5: GATA binding protein 5, DAPK1: death-associated protein kinase1.

## Current perspective of LAMP assay for COVID 19 test

Numerous COVID19 cases have been confirmed and the spread is uncontrollable easily; conformed cases rise from day to day throughout the world. Early diagnosis plays a vital role to decrease the manifestation. For the diagnosis of COVID 19 pandemic, reverse transcription real time polymerase chain reaction (rRT PCR) is now being used as gold standard method in all over the world. According to the test procedure comprises: 1) specimen collection; 2) Storage and shipment; 3) Communication with laboratory and providing information; 4) Testing (laboratory); 5) Result report [34]. False negative results may be possible because of the viral sample is small and requires standard practice of RT PCR [35]. Additionally, this rRT PCR requires sophisticated instrument and time required to get the result relegate its usefulness. Therefore, in developing countries such as India, Brazil, Ethiopia and Nigeria, it's difficult to test large number of population using rRT PCR rather for COVID19 mitigation they need rapid and cost effective test method.

Recently, other accurate and effective immunological assay based on immunoglobulins G (IgGs) has been proposed for epidemiological studies [36]. All patients can produce immunoglobulins G (IgGs) for SARS-CoV-2 with in two weeks after infection, but only some of patients do during the first week of post infection. Thereof, it does not effectively identify infected subject at the early stage of infection [37,38]. To improve novel corona virus test, since COVID 19 outbreak was occurred, a number of one-step reverse RT LAMP based COVID19 diagnosis have been devised and applied [39-42] deigned one step reverse RT LAMP in which the test results detected within 30 minutes after amplification starts. By targeting the fragment ORF1ab of COVID 19, was also developed and evaluated iLACO (isothermal LAMP based method for COVID-19) for rapid detection of the SARS-CoV-2 [43]. Before two years, in 2018 for MERS-CoV detection was established by combining RT-LAMP and a vertical flow visualization strip (RTLAMP- VF) as illustrated in [44]. Moreover, by using both recombinase polymerase amplification (RPA) and loop-mediated isothermal amplification (LAMP) techniques, two stage isothermal dsDNA amplification called Penn-RAMP was devised by Song's group [38]. This Penn-RAMP needs only a single tuber carry out the entire reaction process and the sensitivity was as the same as rRT PCR. The application of LAMP test is feasible for COVID 19 detection in order to reduce time and cost, and this test is affordable to exploit in clinics of developing countries (Figure 3). Thereof, LAMP has some advantages over RT PCR and immunological assay to be applied for point-of-care test.

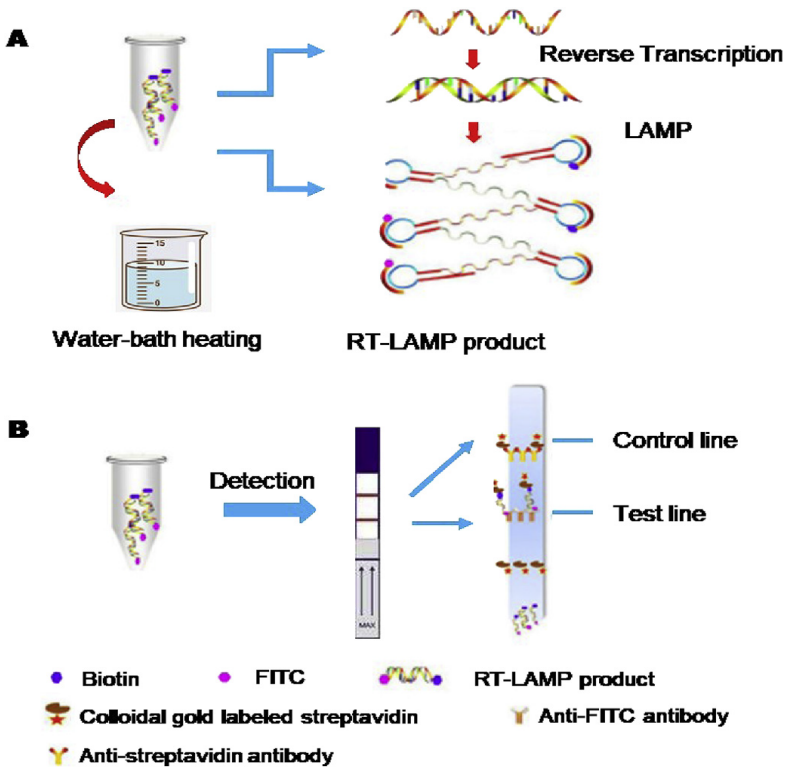



**Figure 3.** Schematic illustration of the RT-LAMP-VF assay. (A) Amplification reaction for RT-LAMP and (B) detection on visualization strip. Adopted from Huang et al., [44].

## Conclusion

In this century the best method of disease diagnosis is molecular diagnosis which is mainly done by PCR. However, this type of diagnosis is not rapid and affordable for developing countries to implement in hospitals, clinics and health center laboratories. For instance, in Ethiopia, before COVID 19 pandemic, PCR type of diagnosis was exploited in some hospitals and health research institutions merely. At this time developing countries are being facing by diagnosis problem of COVID 19 pandemic; they have been tested much less than they have to diagnosis regarding their population size. Domestication of cost effective, rapid, specific and sensitive diagnosis assay is beneficial. Therefore, LAMP assay fulfills the entire attributes of ideal diagnosis assay which recommended by WHO. So far, this assay can be used as an alternative molecular diagnosis of disease such as high prevalent and pandemic viral and bacterial disease in resource limited area.

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