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Point-of-Care Diagnostic Platforms for Loop-Mediated Isothermal Amplification

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The loop-mediated isothermal amplification (LAMP) method is one of the Nucleic acid amplification tests (NAATs) that allows for the amplification of target regions without using a thermal cycle. With its unique primer design, LAMP ensures the rapid replication of the targeted DNA region with high specificity and high efficiency. LAMP technology is used for diagnostic purposes in pathogen detection due to its ease of use, low cost, and simplicity without requiring complex equipment. A wide range of LAMP diagnostic platforms have been developed for applications in bacteria, virus, and parasitic pathogen detection. Herein, the methodology of LAMP technology and its applications in pathogen detection and SNP genotyping and mutation detection are discussed. Point-of-care (PoC) LAMP platforms designed with the principles of microfluidic chip technology, including LAMP-on-a-chip, paper-based LAMP, and smartphone-based LAMP applications have been elaborated. LAMP technology represents a fast, robust, and reliable diagnostic platform for point-of-care testing.

1. Introduction

Nucleic acid analysis has relied heavily on nucleic acid amplification since the invention of polymerase chain reaction (PCR) in 1986,[1] which is the gold standard for nucleic acid amplification, as well as for many diagnostic tests, due to its high sensitivity and specificity.[2] However, PCR is associated with a high cost to operate due to the need for thermal cyclers and special equipment as well as a certain level of proficiency. Additionally, PCR is sensitive to inhibitors that can cause the tests to be invalid.[3] The emerging isothermal amplification techniques, especially loop-mediated isothermal amplification (LAMP), overcome the limitations of conventional PCR.[4–23] LAMP is a protocol for nucleic acid amplification that was first introduced in 2000.[24] It is not affected by inhibitors and operates at the same temperature throughout the assay run, eliminating the need for thermal cycling and high-cost equipment. Furthermore, LAMP facilitates ease of operation for non-professionals, and therefore requires less training. The minimal equipment allows for the development of portable LAMP-based tests.[25] The typical temperature for the LAMP process is between 60 and 70 °C. It requires the design and use of 4 primers that target 6 unique sequences of nucleic acid and takes between 30 and 60 min for the amplification reaction to take place, after which the amplification products can be detected in different ways, including optical detection with fluorescence, which requires the use of molecular dyes and a UV light source.[24]

One of the most important applications of LAMP is in pathogen diagnosis, especially for infectious diseases.[26] Its use in diagnostics has continued to increase since it is a rapid testing technique,[27] allowing the diagnosis of bacterial, viral, and parasitic diseases.[28] For example, several studies have demonstrated the effectiveness of LAMP in the diagnosis of *Mycoplasma synoviae*,[29] infectious bronchitis virus (IBDV),[30] and *Giardia duodenalis*.[31] Moreover, LAMP-based diagnostics have been

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adopted during different viral outbreaks, such as the Zika virus epidemic[32] and the recent COVID-19 pandemic.[33] LAMP is also useful for the detection of mutations and single nucleotide polymorphisms (SNPs), but it has some limitations that can result in false positives.[34] Therefore, different variations of the traditional LAMP technique have been developed for SNP genotyping and mutation detection, such as allele-specific LAMP (AS-LAMP),[35] probe-enhanced LAMP (PE-LAMP),[36] and peptide nucleic acid/locked nucleic acid-mediated LAMP (PNA/LNA-LAMP).[37] The advantages of fast results, ease of use, and portability allow LAMP to be adopted in PoC tests, giving rise to PoC-LAMP to provide diagnostic solutions for resource-limited settings.[38,39] Different manufacturing techniques used in various PoC devices have also been applied to PoC-LAMP devices.

In this review, the working principles of LAMP are described along with the operation parameters. The applications of LAMP, including pathogen diagnosis and mutation detection, are elaborated. PoC-LAMP devices show variations based on their manufacturing methods, working principles, and technologies used for operation and detection. Here, these variations are discussed under three categories: 1) microfluidic LAMP or LAMP-on-a-Chip (LoC), 2) paper-based LAMP, which is a special type of microfluidic LAMP that uses paper for passive liquid flow, and 3) smartphone-based LAMP, which utilizes smartphone cameras and image processing algorithms to enhance, digitize, and automate the detection step. This review demonstrates the recent advancements in LAMP technology and its applications in diagnostics and nucleic acid studies.

2. LAMP Methodology

2.1. Definition of LAMP

The LAMP technique, which is one of the nucleic acid amplification tests (NAATs), was first introduced in 2000.[24] LAMP has been used as an important diagnostic tool in pathogen detection and the fight against infectious diseases.[26] It has the capacity to rapidly amplify the targeted gene region with exceptionally high specificity in a short amount of time. Using this feature, various LAMP methods have been designed for amplifying target gene sequences. It is fast, practical, cost-effective, and remains promising for the development of advanced applications under isothermal conditions.[28] LAMP has gained popularity owing to its use in PoC platforms.[38,39]

2.2. Advantages of LAMP

LAMP is an amplification method that eliminates the complexity and some methodological difficulties associated with PCR.[40] Amplification takes place at a constant temperature without the need for thermal cycling in PCR. Therefore, complicated equipment and strong laboratory infrastructure are not needed for LMAP methods. Amplification can easily occur in a simple heating block or hot water bath.[40] Bst DNA Polymerase enzyme, isolated from *Bacillus stearothermophilus*, plays a key role in LAMP reactions.[41] This enzyme has strand-displacement activity and releases single-stranded DNA during synthesis.[42] Thus, the DNA denaturation step in PCR is skipped. At the same time, the enzyme has maximum efficiency at a constant temperature of 60–65 °C.[24] Due to these features of the enzyme, the nucleic acid amplification process can be performed under isothermal conditions without the need for cycles and steps involving time and thermal manipulations.[40] To perform the LAMP reaction, easily portable, small, field-friendly, low-cost devices operating under a single temperature value can be developed. Furthermore, such devices allow for the development of PoC diagnostic platforms for resource-poor settings.[38]

2.3. LAMP Primer Design and Reaction Components

The LAMP method requires a unique primer design. The method has high sensitivity and specificity; hence, primer design is of crucial importance when executing the LAMP reaction.[43] Online tools, such as LAVA and PrimerExplore, are available for the LAMP primer design.[44] According to the LAMP protocol, a gene region of 130–300 bp can be targeted. Typically, 6 primers targeting 8 different specific gene sequences are utilized within the gene region where amplification is planned. F3, F2, F1c, B3, B2, B1c, LF, and LB have targeted gene sequences on the template gene region.[43] The reaction can also occur with 4 primers targeting 6 specific gene sequences (without LF and LB).[24] LAMP reaction has 2 external primers: a forward external primer (F3) and a backward external primer (B3). These two external primers enable the targeted gene region to be synthesized and amplified as a straight strand. External primers function similarly to forward and reverse primers in PCR. Forward internal primer (FIP) and backward internal primer (BIP) are internal primers, and are responsible for the formation of amplificon in the loop structure. Each of the FIP and BIP primers includes 2 different gene sequences from the sense and antisense strands of DNA. FIP contains the primary F2 gene sequence and the F1 complementary (F1c) gene sequence in the $5'\rightarrow3'$ direction of DNA. Similarly, the BIP primer contains the B2 and B1c gene sequences on the reverse side. Loop primers (LF and LB) are designed complementary to the loop gene region and bind to the loop structures formed. They take part in accelerating the amplification reaction and stabilizing the loop structures.[43]

Template DNA, primers, Bst DNA Polymerase enzyme, betaine, MgSO4, deoxynucleotide triphosphates (dNTPs) and buffer for the enzyme are required for any LAMP reaction.[39] If the template nucleic acid is an RNA molecule (for diagnosis of RNA viruses), a second enzyme, reverse transcriptase, is used before LAMP amplification. Thus, the RNA molecule is converted into a double-stranded DNA (dsDNA) molecule. This protocol is termed the reverse transcriptase LAMP (RT-LAMP) method.[45]

2.4. LAMP Working Principle and Reaction Stages

In the LAMP amplification reaction process, there are two successive stages, noncyclic and cyclic.[26] The noncyclic stage covers the process of forming dumbbell structures from the beginning of the LAMP reaction (**Figure 1**A). This first dumbbell structure is characteristic of the LAMP method and provides a transition to the cycling stage. At the start of the LAMP reaction, one of the primers attaches to the complementary sequence in the template

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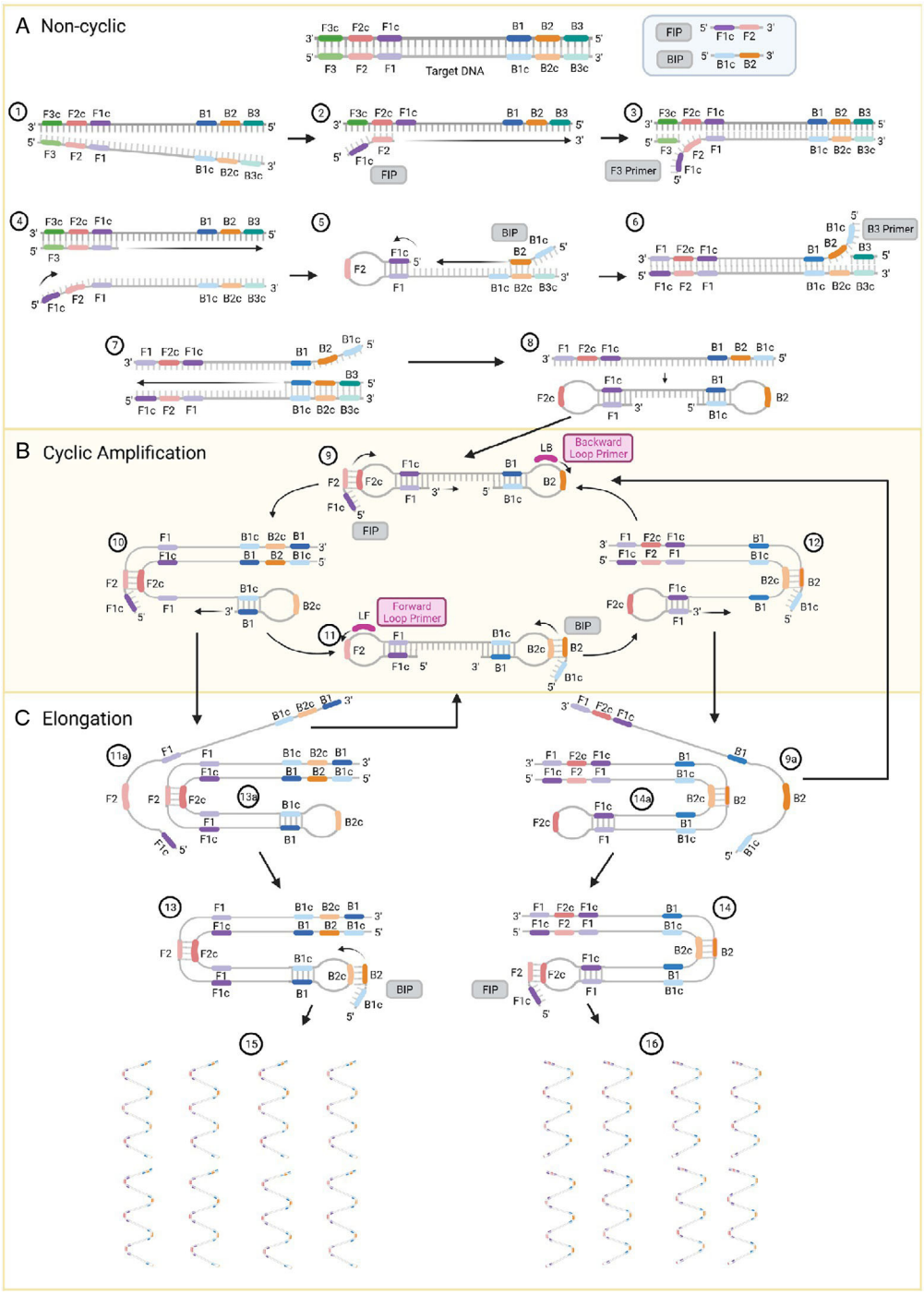


Figure 1. A) The initiation of the loop-mediated isothermal amplification (LAMP) process and the noncyclic stage are represented in detail schematically. The LAMP amplification process is initiated by annealing the complementary regions of the template DNA molecule of the F3, forward internal primer (FIP), and B3, backward internal primer (BIP) primers. In the first step, 4 different amplification products are created with LAMP primers. LAMP primers are reconnected to these amplification products, and new amplification products are formed which lead to the formation of loop structures. The noncyclic stage is completed with the production of dumbbell-shaped structures. B) In the cyclic stage, amplification products are synthesized in two different ways. Dumbbell-shaped stem-loop structures are formed, in which the first amplification product will enter the cyclic stage again. C) In the second type, amplification products of different sizes are produced, including multiple loop structures with repeated strand elongation. Reproduced with permission.[46] Copyright 2022, Frontiers Media S.A.

target DNA molecule. The template DNA molecule exists in a double-stranded form at a temperature of 60–65 °C (optimum temperature for LAMP). The Bst DNA Polymerase enzyme binds to the double-stranded template DNA molecule to which the

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primer is attached. The enzyme displaces the strand, releasing the single-stranded DNA molecule. Thus, contrary to PCR, the nucleic acid synthesis process starts without the need for a DNA denaturation step (at 98 °C). External primers (F3, B3) attach to their complementary sequences, allowing the synthesis of a straight amplification product similar to that of PCR. Consequently, a great number of copies of the targeted gene sequence are formed. Thus, the amount of targeted gene sequences to which internal primers can bind is increased. It is one of the reasons the LAMP method is able to generate a large number of amplification products rapidly. The FIP primer has the F2 region at the 3′ ends and the F1c region at the 5′ ends. F2 hybridizes with the F2c region of the targeted template gene sequence and a new DNA strand is synthesized in the 5′ directions. The 5′ end of this newly synthesized DNA strand no longer has the B3 region. Starting from the 5′ ends of this DNA strand, the F1c, F2, F1, B1c, B2c, and B3c regions are located, respectively. The F1c region at the 5′ end of this DNA strand hybridizes with the complementary F1 region, forming a hairpin fold. This structure takes the form of a single-loop structure. The synthesized DNA strand takes the form of a single-loop structure at one end. The B2 region of the BIP primer hybridizes with the B2c region of the single loop DNA strand, and a new DNA strand is synthesized. At the 3′ end of this newly synthesized strand, the B3 region is replaced by the B1c region. The B1c at the 3′ end hybridizes with the B1 region by forming a hairpin fold. The synthesized DNA strand takes the form of a dumbbell-shaped structure with loop structures at both ends. These stem loops synthesize into a dumbbell-shaped structure and lead to the initiation of the cyclic stage in the LAMP process.[24,26] Similarly, on the reverse side, the LAMP amplification process begins with the annealing of the complementary regions of the B3 and BIP primers to the opposite strand of the initially targeted Template DNA molecule. It continues on the reverse side as well as on the forward side until the stem-loop structure is formed (Figure 1A).

In the cyclic stage, the F2 region of the FIP primer anneals its complementary region in a single-stranded stem-loop structure, and the Bst enzyme binds to initiate amplification (Figure 1B). During amplification, the enzyme releases the stem-loop strand it uses as a template. This released stem-loop structure has a B1 region at the 3′ end. The B1 region anneals the B1c region by twisting on the strand. The enzyme binds and initiates another round of amplification, resulting in the release of the complementary strand to which the FIP primer is attached. This newly released amplification product creates a dumbbell-shaped structure. The newly formed dumbbell-shaped structure includes regions F1c, F1, B1, and B1c, respectively. When the cyclic stage begins, the "turnover" structures of the stem-loop strand, which are used as templates, are synthesized. In parallel, the same cycle takes place in the stem-loop structure, which is used as a template containing the complementary sequence to the B2 region of the BIP primer. These cyclic processes are repeated. As a result, structures consisting of transformative inverted repeats of the targeted gene region on the same strand are formed. These newly formed structures are of different sizes and contain multiple loop structures (Figure 1C).[24,26] For the LAMP protocol, 4 primers (F3, FIP, B3, BIP) are considered essential primers for amplification in a multi-loop structure. Loop primers participate in the amplification process to generate more amplification products rapidly. Loop primers (LF, LB) create new DNA synthesis regions by annealing the complementary regions in the loop structures. When loop primers are used in the reaction, cauliflower-shaped structures with multiple loop regions are formed.[26]

2.5. The Superiority of LAMP over PCR

In the LAMP method, many nucleic acid synthesis initiation sites are constituted using 6 primers.[40] This results in a higher amount of amplification product. LAMP method yields 109–1010 fold amplification products in a time period of 15–60 min. In ≈1 h, 1 bln copies of the targeted gene region are produced using the LAMP method, while only 1 mil copies are produced using PCR in which 2 primers are utilized.[40] In addition, the primer design in the LAMP method ensures tremendous specificity in the detection of the targeted gene region. The LAMP method can validate the presence of a targeted gene region in a sample successfully owing to its high specificity, practicality, and production of a high level of amplification product rapidly. Using the LAMP technology, the targeted gene region can be detected even at low concentrations.[47]

2.6. Different Methods of LAMP Product Detection

One of the reasons LAMP has become popular and applied to PoC platforms is that detection of the amplification product is easily achieved in different ways. LAMP products can be detected with the naked eye, without the need for any additional experimental procedures, after the reaction has ended or even while the reaction is in progress.[26] LAMP amplification can also be detected using imaging techniques for colorimetric, gel electrophoresis, or turbidimetric setups and reading in a real-time device can be obtained using fluorescence or end-point approaches.[48,49] Due to this variety of detection methods, the LAMP technique can be utilized both in PoC settings and in laboratory environments.[26]

Positive–negative LAMP reaction results are easily observed with the naked eye or via colorimetric imaging. Results are differentiated and analyzed based on the observed visual turbidity and visual fluorescence. In a visual turbidity evaluation, a white-transparent image indicates a negative, while a blurred image indicates a positive result. Positive results can also be detected using colorimetry by adding DNA dyes such as SYBR green or calcein, to the reaction.[50] Results can also be visualized using a UV LAMP when using intercalating DNA dyes.[51]

In turbidimetric imaging, it is essential to measure turbidity by means of turbidimetry to assess results. Turbidity is observed during the reaction as a result of the large amount of insoluble pyrophosphate released during DNA synthesis.[48] Pyrophosphates also allow analysis in real-time during the LAMP reaction using calcein, a fluorescent metal indicator that binds free Ca2+ ions.[49]

LAMP amplification products can be detected by plotting the real-time melting curve and dissociation curve using intercalating dyes such as SYTO-9 and SYBR green.[51,52] Quantification of the gene copy number or nucleic acid concentration of LAMP amplification products can also be achieved. To obtain the amplification signal at a given concentration, a standard curve with

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respect to the positivity time of known gene copy number and concentration can be plotted.[48] This method is often used to determine the degree of positivity of viral load in clinical samples.[48]

3. LAMP in Pathogen Detection

NAATs are important for the diagnosis of infectious diseases and pathogen detection.[26] Compared to other NAATs, LAMP is preferred for pathogen detection because it produces fast and practical results.[27] Additionally, amplification products can be obtained even from low concentrations of DNA using LAMP. This is particularly important for the detection of pathogens present in small amounts in body fluids.[53] LAMP platforms have been developed for the detection of many bacterial, viral, fungal, and parasitic pathogens.[27,28] There are LAMP platforms specially designed for the detection of pathogens infecting plants, animals, and humans.[27,54]

3.1. LAMP for Pathogens Detection in Animals

Many studies have been conducted to adapt the LAMP method for pathogen detection in animals. The effective use of the LAMP technique has been demonstrated in the diagnosis of infectious bronchitis virus (IBDV),[30] infectious laryngotracheitis virus (ILTV),[55] Marek's disease serotype 1,[56] *Mycoplasma synoviae*,[29] and *Mycoplasma gallisepticum*[57] in poultry. LAMP can be used as a reliable method to detect the presence of Eimeria species infecting chickens.[58] Research has been carried out for the diagnosis of blood parasites disease in cattle and sheep of the Theileria–Babesia lineage,[59,60] *Giardia duodenalis*,[31] *Cryptosporidium* species,[61] *Trypanosoma brucei gambiense*,[62] which cause. LAMP technique was also adapted for the diagnosis of *B. gibsoni*,[63] and *B. canis*[64] as well as parasitic agents in dogs and Plasmodium spp.[65] in intermediate host mosquitoes.

3.2. LAMP for Pathogens Detection in Humans

Since LAMP is a method that allows easy and fast detection of target gene regions in the obtained product, a variety of PoC-LAMP diagnostic platforms have been designed for human pathogen detection.[39] These PoC-LAMP diagnostic platforms have been modified for specific pathogen detection with the aim of diagnosing a particular disease.[38] PoC-LAMP diagnostic platforms are frequently used especially to detect infectious diseases that need to be controlled and can cause epidemics.[53] LAMP has, therefore, gained popularity with the emergence of the West Nile virus[66] and severe acute respiratory syndrome (SARS) coronavirus[67] and has been used in the diagnosis of viral pathogens. Furthermore, LAMP has been an important tool in the diagnosis of many DNA and RNA viruses. The LAMP method has been used for diagnostic purposes in detecting DNA viruses, Varicella-zoster virus,[68] Cytomegalovirus,[69] Herpes simplex virus,[70] BK virus,[71] Human papillomavirus (type 6, 11, 16 and 18),[72] Hepatitis B virus,[73] and Human herpesvirus (type 6, 7, 8).[74–76] Additionally, RT-LAMP diagnostic platforms were created to detect the RNA viruses, West Nile virus,[66] SARS,[67] Japanese encephalitis virus,[77] H5N1 avian influenza virus,[78] Chikungunya virus,[79] Dengue viruses (type 1, 2, 3, 4),[43] Zika virus,[32] and SARS-CoV-2.[33]

3.3. LAMP for Foodborne Illnesses Diagnosis

LAMP technology is also used to detect pathogens that cause foodborne illnesses in humans and animals. LAMP has been successfully modified as a sensitive and rapid test for the detection of food-derived *Clostridium botulinum* types A and B, which cause neurotoxicity in humans.[80] The rapid and effective detection of *Clostridium perfringens*,[81] *Salmonella* serotypes,[82] *Burkholderia pseudomallei*,[83] *Brucella spp.*,[84] and *Streptococcus pyogenes*,[85] which are foodborne disease agents, have been achieved using LAMP. Studies were also conducted to detect the serotypes of *Escherichia coli* (*E. coli*) O157, O26, and O111, which cause enterohemorrhagic infections, and the serotypes of *E. coli* O26, O45, O103, O111, O121, and O145, which produce Shiga toxin.[86–88]

3.4. LAMP for Infectious Diseases Diagnosis

Many modifications, including DNA and RNA extraction steps that break down proteins using high temperatures, can be applied to LAMP and RT-LAMP. The risk of contamination is low since all modified methods take place in a tube.[89] The reaction in the LAMP method is less affected by external factors causing contamination compared to that of PCR, and therefore clinical samples can be used even without special purification steps.[90] With pathogen-specific modified PoC-LAMP platforms, the sample can be analyzed right after collection.[53] LAMP eliminates the risk of contamination due to sample transfer.[38] It is important in the diagnosis of infectious diseases, especially in quarantine zones.[53] Recently, with the SARS-CoV-2 pandemic, the LAMP technique has lived its golden age.[38] Rapidly spreading globally, SARS-CoV-2 brought with it the need for PoC diagnostic platforms that would provide fast, high-efficiency, low-cost, and easy-to-use tests.[51] A wide variety of disease-specific LAMP-based PoC-diagnostic platforms have been developed for pathogen screening.[38,51,53] LAMP technology has been accepted for diagnostic purposes for many neglected tropical diseases, endemic diseases, and infectious diseases defined by WHO.[53,91,92] LAMP technology has been used for diagnostic purposes in Dengue fever,[93] Rabies,[94] Buruli ulcer,[95] Leprosy,[96] Chagas disease,[97] African trypanosomiasis,[62] Leishmaniasis,[98] Cysticercosis,[99] Echinococcosis,[100] Trematode infections,[101] Lymphatic filariasis,[102] Schistosomiasis,[103] malaria,[104] soil-transmitted Helminthiases,[105] tuberculosis,[106] and COVID-19.[33]

3.5. LAMP Technology Modifications

Additionally, multiplex-LAMP (M-LAMP) reactions can be designed for the simultaneous detection of many pathogens in a single sample tube. Rapid and accurate detection is important, especially in the diagnosis of pathogens that infect the same tissue and exhibit similar clinical symptoms.[53] Furthermore, the M-LAMP diagnostic platform was successfully applied to the detection of influenza A/H1, A/H3, and B viruses.[107]

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Minimizing the complex thermal cycling requirements of PCR methods, PoC-LAMP diagnostic platforms, where reactions can occur and be monitored at a constant temperature, are used as fast, accurate, and cost-effective methods for the detection of pathogens in the clinical diagnosis.[53] Moreover, LAMP technologies have been modified in several ways; lateral flow assays,[108] microfluidic techniques,[14] LAMP-on-a-chip platforms,[108] smartphone-based technologies,[109] techniques combined with nanoparticles,[110] and CRISPR-integrated methods[111] have been developed as PoC molecular diagnosis platforms.

4. LAMP for SNP Genotyping and Mutation Detection

Analysis of mutations and SNPs is important for the diagnosis of human diseases.[112,113] SNPs constitute ≈90% of the variations found in the human genome.[114] SNPs are inherited and contribute to genetic diversity among individuals. Particularly, SNPs are of great importance in the formation of complex phenotypes.[113] SNP genotyping is valuable in the early diagnosis and treatment of genetically inherited diseases.[113]

4.1. Advantages and Limitations of LAMP in SNP Genotyping and Mutation Detection

There are many NAAT-based and complicated analysis methods developed for mutation and SNP detection.[34] However, most of these methods require advanced laboratory infrastructure, tedious experimental procedures, and high-cost devices. The LAMP technique can be an ideal method for the detection of mutations and SNPs because it is fast, low-cost, and highly efficient. The results are rapidly obtained as compared to other NAAT-based methods without requiring a complicated instrument.[115] However, LAMP has some limitations, for example, it requires the use of 4 or 6 primers.[34] These primers must be located at certain distances on the target sequence, and the primers create a large number of nucleic acid synthesis initiation sites. LAMP also rapidly generates a high level of the amplification product. As a result, these limitations can give rise to false positives, nonspecific results, and primer-dimer formation.[116] These reasons make it difficult to precisely detect mutations and SNPs that occur with a single nucleotide change. To overcome this challenge in LAMP, many different modified LAMP methods have been developed for mutation and SNP detection.[34] Modified LAMP techniques were developed according to primer-based and probe-based strategies designed in a sequence-specific (SNP or mutation) manner.

4.2. Primer-Based SNP Genotyping or Mutation Detection with LAMP

For primer-based SNP genotyping or mutation detection, the AS-LAMP technique has been developed to detect amplification as present or absent depending on the relevant allele.[35] Two sets of primers, wild and mutant, are designed to target the last nucleotide SNP at the 5′ ends of the FIP and BIP primers. In total, two external primers (F3, B3) and four internal primers (FIPwild, FIPmut, BIPwild, BIPmut) are used, and the LAMP reaction is performed in two sample tubes (specific primers of wild type and mutant type are in different sample tubes). This modified method was utilized to detect C580Y and Y493H nucleotide changes that cause drug resistance in Plasmodium falciparum malaria.[35] Using this method, the N526K mutation, which plays a role in drug resistance in *Haemophilus influenzae*, was analyzed.[117] AS-LAMP-based PoC-LAMP platform was designed for the detection of CYP2C19 polymorphisms, which are important risk factors for drug interaction and drug toxicity in the pharmacogenetics.[118] A modified PoC-LAMP platform was developed to analyze the MTHFR gene C677T polymorphism, which affects folate metabolism, and the ALDH2 gene Glu504Lys (also called rs671) nucleotide change, which affects ethanol metabolism.[112] This platform also features a one-tube direct-LAMP strategy without the need for a DNA isolation step.[112] To perform ABO blood group typing, a PoC-LAMP ABO genotyping platform was constructed using the AS-LAMP strategy.[119]

To analyze the SNPs responsible for lactose intolerance, AS-LAMP with modified Au nanoparticles was developed.[120] Moreover, 2 primers specific for wild and mutant alleles were designed to be located at the 3′ ends of the F3 external primer. Two different noncomplementary ssDNA sequences and a mutant-specific Au-nanoprobe were designed. LAMP reaction was carried out with wild and mutant-type primers on different samples. After the LAMP amplification process was completed, Au-nanoprobe was added and incubated. Depending on whether the complementary sequence matched or not, the color change was monitored as a result of the Au-nanoprobe aggregation.[120]

4.3. Probe-Based SNP Genotyping or Mutation Detection with LAMP

The PE-LAMP method was based on designing internal and external primers to be located in the loop region of the SNP.[36] LF and LB primers were not included. Instead, two probes (11 bp) were designed to be complementary to the SNP region in loop structures and specific to the allele of interest. Amplification was observed by monitoring the colorimetric change that occurred at the optimized time.[36] Another modified method was termed peptide nucleic acid/locked nucleic acid-mediated LAMP (PNA/LNA-LAMP).[37] This method has been adapted to detect KRAS mutations used in the diagnosis of cancer cells. Primers (F3, FIP, BIP, B3) were designed to ensure that the mutation would be localized in the loop region. The PNA probe was wild-type-specific and blocked the progression of amplification once coupling occurred. The LNA primer was designed to be complementary to the mutant type and when bound, accelerated the amplification. Reaction results could be monitored using colorimetry and RT-PCR.[37]

For the KRAS mutation analysis, the LAMP method was modified by adding a second enzyme (RNase H2) that cleaves the RNA molecule.[121] In this modified method, the design was carried out using the targeted nucleotide change at the 3′ end of the BIP primer and by adding an RNA base (rA, rU, rC, rG). Amplification continued if a full match was achieved with the complementary sequence in the primary template DNA. If the other allele was present, or in case of a mismatch, amplification slowed down. At this stage, when

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the reaction slowed down, the RNAase H2 enzyme would bind, causing the DNA-RNA duplex to break down.[121]

Modified LAMP strategies have also been developed to detect sequence-specific nucleotide change (SNP or mutation) using DNA probes.[122] One of the probe-based modification methods utilized a reporter probe designed for the strand-displacement.[123] LAMP primers were constructed in such a way that the SNP region was localized in the loop regions. Probe F reporter and Q reporter consisted of a duplex of two strands. The F reporter was 10 nucleotides longer than the Q reporter, and this region was complementary to the SNP region. When the F reporter formed a hydride with the SNP region, the Q reporter was separated, and fluorescence was observed.[123] Another modified method was SNP detection using a loop-primer probe and an endonuclease IV.[124] The probe was designed to contain a quencher region, an abasic region, and an internal fluorophore specifically for the SNP region. In this protocol, the endonuclease would recognize the abasic region and would be included in the system, causing the displacement of the fluorophore and resulting in fluorescent radiation. While a high fluorescent emission was obtained for the mutant type, less fluorescent emission was detected for the wild-type.[124]

4.4. Molecular Beacon-Based SNP Genotyping or Mutation Detection with LAMP

The molecular beacon-LAMP (MB-LAMP) method for SNP detection has been developed as a new probe-based LAMP modification technique.[125] In this method, a hairpin structure, which has a fluorescent reporter at one end and a quenching reporter at the other, is characteristically observed. These two reporters were designed to form hydrides with each other at the end of the hairpin structure. LAMP primers were established to be located in one of the loop regions of the SNP. The molecular beacon was designed to contain a complementary array to the loop region of the LAMP amplification product. When the exact match was achieved, the molecular beacon hairpin structure would open, resulting in the separation of the F and Q reporters and thus leading to a fluorescent radiation.[126] Modified methods have been tested using two pairs of fluorescent dyes on MB-LAMP, and adding RNAase enzyme.[125,127,128]

5. LoC and Microfluidic-Based LAMP

LoC refers to the integration of microfluidics with LAMP technology, rendering LAMP applicable in PoC applications, which has the advantages of producing faster results and offering ease of operation at a lower cost.[129] Microfluidic assays are cost-effective and portable analytical devices that operate with a small volume of reagents and samples.[130–133] The applications of microfluidics range from lab-on-chip platforms to organ-on-chip devices.[134–148] Since DNA- or RNA-based diagnostic requires large numbers of nucleic acid to perform amplification, amplification of the original count of nucleic acid is essential for testing. In general, there are two types of nucleic acid amplification techniques: 1) temperature cycling and 2) isothermal amplification. PCR is the predominantly used technique that has adopted a temperature cycling.[149] PCR requires a continuous change of temperature to achieve the required amplification. This is achieved either by rapidly altering the temperature of the chamber that contains the sample, reaching a cycling time of less than 6 min, which is called stationary chamber PCR,[150] or by passing the sample into multiple chambers of varying temperatures with high speed, achieving even higher speeds for 8 to 20 s, which is called continuous flow PCR.[151] Even though the small size of microfluidic devices provides fast heat transfer to accomplish high speeds, the needed hardware for a PCR device limits its ability to be integrated with microfluidics and to be efficiently used as a PoC technology. In contrast, isothermal amplification uses simpler hardware without temperature cycling. Unlike PCR, it is not affected by polymerase inhibitors, but it usually takes 15 to 90 min to be operated. Various techniques can be used to achieve isothermal amplification that is suitable for microfluidics, including helicase-dependent amplification (HDA),[152] recombinase polymerase amplification (RPA),[153] nucleic acid sequence-based amplification (NASBA),[154] and LAMP. These techniques differ in operating temperature (41–65 °C), time (15–90 min), and cost, but LAMP is shown to be the most suitable for PoC applications because of its minimal hardware requirements, low cost, and simple working mechanism compared to other techniques, even though it requires a relatively higher operating temperature.[24]

5.1. Microfluidic-Based LAMP for Single Gene Detection

LAMP can be used for both DNA and RNA amplification and detection. When it is implemented as a microfluidic device, implementation approaches vary based on the target of detection. Therefore, LoCs can be categorized as being used for single-gene detection, multi-gene detection, or reverse transcription LAMP (RT-LAMP). One method of implementing a microfluidics-based LAMP device for single gene detection is called lab-on-a-disc (LoD), which was developed to perform sample preparation, DNA amplification, and gene detection altogether.[155] In this method, a pressure-sensitive adhesive (PSA) film was sandwiched between two layers of poly(methyl methacrylate) (PMMA), creating a disc that acted as the medium for LAMP. Microfluidics chambers and features were designed with computer-aided design (CAD) software, engraved with a computer numerical control (CNC) machine on the bottom PMMA layer, and then modified with a cutter plotter machine. Each set of those chambers had a specific purpose of mixing reagents, metering, isothermal amplification, or gene detection. The reagents and DNA samples within the solution were loaded onto the dedicated chambers. Next, disc rotation was performed by altering the rotation speed to ensure efficient mixing. A low-cost hot air gun was used to keep the sample at a constant temperature of 63 °C for 60 min, followed by 2 min at 80 °C to stop the amplification reaction. SYBR Green I was used as an intercalating dye to detect fluorescence change in the sample, achieving a detection limit of 5×10^{-3} ng μL^{-1}. This method enabled the detection of Salmonella—a major foodborne pathogen. Compared to previous studies, this method facilitated the flow of liquid into the destination chamber at lower spinning frequencies (less than 500 rpm) with precise metering (independent of the volume of the pre-loaded LAMP reagents). However, the main pitfall of this method, as a PoC tool, was the need for active

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disc rotation (**Figure 2**A). Another method for single gene detection was proposed using magnetic beads.[156] Specifically, magnetic beads were coated with specific nucleotides to detect methicillin-resistant *Staphylococcus aureus* (MRSA), and were loaded with the sample DNA, reaction mixture, and washing buffer inside chambers that are located on three separate chips. After DNA extraction with cell lysis, it was bound to the beads via the specific nucleotides and concentrated using an external magnetic field. Then LAMP was performed with the aid of an integrated temperature control module. A microvalve and a vacuum pump were used to assist the mixing and transportation of the fluids. A spectrophotometer was used for detection, reaching a detection limit of 10×10^{-6} ng μL^{-1}. Although the experiment was performed rapidly with a significant detection limit, external devices were used for some steps, limiting the PoC aspect of the approach (Figure 2B).

5.2. Microfluidic-Based LAMP for Multi-Gene Detection

Designing a microfluidic chip to detect a single gene is impractical, since detecting multiple genes is usually required in PoC

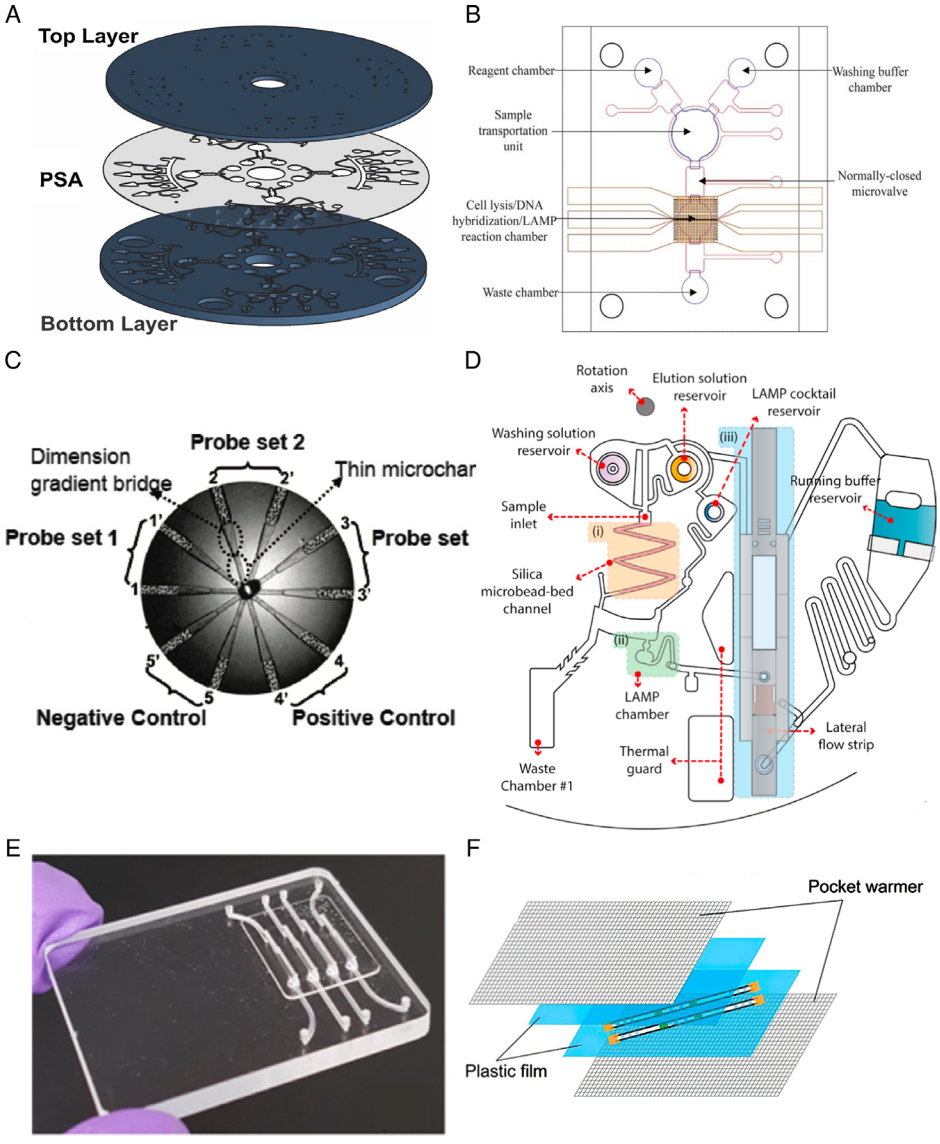


Figure 2. Different microfluidic-based LAMP platforms. A) Lab-on-a-disc assembly, consisting of a pressure-sensitive adhesive (PSA) layer sandwiched between two poly(methyl methacrylate) (PMMA) layers with the chambers engraved on the bottom PMMA layer. Reproduced with permission.[155] Copyright 2015, Elsevier B.V. B) Layout of the magnetic beads-based LAMP assay, consisting of chambers for reagent, washing buffer, and waste, a sample transportation unit, and a microvalve. Reproduced with permission.[156] Copyright 2011, The Royal Society of Chemistry. C) Schematic of the multiplexed LAMP assay, consisting of 10 microchambers divided into 5 groups of 2, where the first 3 groups are used for multiplexed samples while groups 4 and 5 are controls. Reproduced with permission.[157] Copyright 2010, American Chemical Society. D) Design of the multiplexed rotary microfluidic LAMP device, featuring DNA extraction, LAMP, and LFA units. Reproduced with permission.[158] Copyright 2016, Elsevier B.V. E) Microfluidic cassette used for RT-LAMP, featuring 4 independent LAMP reactors and an isolation membrane. Reproduced with permission.[32] Copyright 2016, American Chemical Society. F) Illustration of the multiplexed RT-LAMP device, consisting of microcapillaries sandwiched between 2 pocket warmers with plastic films to prevent contamination. Reproduced with permission.[159] Copyright 2014, American Chemical Society.

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applications. In such cases, designing a separate chip for each gene is inconvenient and increases the cost significantly. Introducing multiplex gene assays can overcome this issue. Furthermore, a diagnostic multiplex gene assay was developed to utilize LAMP technology at PoC to detect multiple viruses at once with high speed and specificity at low-cost.[157] The device featured a multichannel octopus-like microfluidic chip with 10 channels radiating from a common center, each ending with a microchamber. The sample would be deposited at the channels' hub to flow toward the microchambers, which were divided into five groups of two microchambers, and each group was precoated with a specific LAMP probe. Microchambers 1, 2, and 3 were used to detect viruses (H1N1, pandemic H1N1, and flu A), while groups 4 and 5 were used as positive and negative controls, respectively. After performing LAMP at 63 °C and using SYBR green I for fluorescence change detection, the detection limit of the assay was found to be less than 10 copies μL^{-1} for all three viruses, with detection sensitivity 100 to 1000 times higher than that of PCR (Figure 2C). The device was able to differentiate three viruses with low cross-reactivity and high reliability. Besides, as a result of using 10-fold serial dilutions, the corresponding TTP values could be recorded simultaneously. It is demonstrated that a minimum of 5 mins were needed to trigger the LAMP signal from the microchamber. However, due to the high-speed variability of the influenza A virus, screening suitable LAMP probes would be critical to avoid false positive/negative test results. For detecting multiple genes at once, a rotary microfluidic device was developed to integrate all steps of LAMP including DNA extraction, and isothermal amplification, followed by colorimetric detection, where each step was performed on a separate microfluidic layer.[158] The device consisted of three identical units to perform multiplexed detection. For each unit, the test started with DNA extraction with a solid phase matrix of glass microbeads. LAMP was performed in a dedicated chamber by mixing eluted DNA from the previous step with LAMP reagents and then incubating the mix at 66 °C. After the reaction was terminated, a lateral flow strip was used for the colorimetric detection of the result. The conjugate pad of the strip contained gold nanoparticles coated with streptavidin and two test lines with a control line at the detection zone to observe the result with the naked eye. The strip was also thermally isolated to prevent the high temperature of the LAMP step from affecting the reactions at the detection zone. The rotary system consisted of three heating units, a servomotor, and a camera to record the fluid's motion inside the device. The device was used to simultaneously detect *Salmonella typhimurium* and *Vibrio parahaemolyticus*, showing successful operation with a detection limit of 50 CFU (Figure 2D). The specificity and sensitivity of target gene amplification were improved using selective six primer sets to detect food-borne pathogens (*S. typhimurium* and *V. parahaemolyticus*). The achieved detection limit (10 CFU mL^{-1}) was adequate for the detection of intended pathogens, considering the infective doses of pathogenic bacteria in real samples range from 102 to 106 CFU mL^{-1}. Compared to previously developed devices that took 3≈4 h to complete the analysis, this device was able to perform the analysis in 80 mins. Despite the advantages of this method, further advancements, including large-volume sample pretreatment and lysis process, are still needed to realize a fully integrated genetic analysis system.

5.3. Microfluidic-Based RT-LAMP

Reverse transcription LAMP (RT-LAMP) method can be used for RNA-based detection. For Zika virus screening, RT-LAMP has been utilized for rapid and efficient RNA virus detection.[32] The developed device could handle the whole diagnosis process from sample deposition to virus detection. A disposable microfluidic cassette was customized to have four independent LAMP reactors. After collecting and lysing saliva samples, samples were filtered using isolation membranes located in each of the four reactors to extract the viral RNA. The cassette was then washed and the reaction mixture was introduced. It was then placed inside a heat cup to perform RT-LAMP at 68 °C. The reaction products were detected using leuco crystal violet dye and the colorimetric change was observed with the naked eye. The results showed high detection sensitivity of 5 PFU and the entire test took 40 min to conduct (Figure 2E). The ability to perform the amplification at a fixed temperature, without thermal cycling, considerably decreased the complexity of the setup and cost compared to PCR. Another advantage of this method was its ability to detect products with the naked eye using leuco crystal violet dye and the colorimetric change (eliminating the need for costly devices compared to conventional PCR). The results showed high detection sensitivity of 5 PFU and the entire test took 40 min to conduct. It was also shown that by increasing the "log Zika virus" from 1 to 3, the threshold time would decrease from 22 to 12 mins. Nonetheless, this study neglected the experimental verification of the absence of cross-reactivity with any other pathogens. Similar to DNA, multiplexed RNA detection is also possible using RT-LAMP. This was illustrated using an integrated PoC diagnostic device aimed at detecting multiple viral RNAs without relying on an external power supply.[159] The device consisted of a plastic microcapillary sandwiched between two pocket warmers that could provide the heat needed for the RT-LAMP protocol. The multiplexing capability of the device was validated by attempting to detect two different genes of HIV, where the viral RNA was extracted off-chip and then placed into the microcapillary. RT-LAMP was then performed with the aid of the pocket warmers at 68 °C for 1 h. SYBR green I dye was used to visually detect the reaction product with a portable UV LED, achieving high detection sensitivity of two copies of standard plasmids (Figure 2F). To decrease the evaporative loss/contamination of liquid samples during thermal reactions, water segments were introduced which successfully avoided the cross-talk between two reaction zones. In addition, this study demonstrated the possibility of parallelization of the LAMP platforms (8–10 capillaries in a single experiment). An application time of 15 min was achieved in this study (for 2×106 copies of DNA). It was also demonstrated that flexible polymers (PTFE and C-FLEX) were translucent (i.e., interfering with naked-eye result readout), whereas PMMA-based capillaries had acceptable transparency.

6. Paper-Based LAMP Assays

Microfluidics has the potential to provide a rapid, cost-efficient, and highly specific technology to perform LAMP for PoC applications. However, the need for extra pumping equipment can

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limit the compactness, ease of use, and low-cost features of microfluidic devices. Paper-based microfluidics offers a promising solution for this challenge, since the fibrous nature of paper can provide wicking flow for the LAMP reaction fluids without relying on valves or pumping units.[160] To illustrate this, roll-to-roll (R2R) thermal molding technology has been utilized to fabricate a paper-based microfluidics device that integrated an isothermal amplification.[161] The device consisted of a PDMS chip and aluminum-coated paper. To fabricate the device, photolithography was used to create a mold that enabled coating with PDMS and replicating the microchannel pattern with the aid of Al-coated paper. Coating with Al can increase the detection sensitivity to reduce the auto-fluorescence of a conventional paper matrix. Additionally, the highly reflective surface of Al amplified the fluorescence of the LAMP products. The R2R technology allowed for the mass production of thousands of devices within an hour. RT-LAMP was used to validate the performance of the device, in which viral RNA from a commercial kit was used. A Peltier module was used to generate the required heat, while a thermocouple with two channels was used to regulate the temperature (70 °C). The same experiment was repeated without Al coating and results were compared, showing that coating with metal enhanced the fluorescence signal 2.7 times, which consequently improved detection sensitivity (**Figure 3**A).

A lateral flow assay (LFA) consisting of PDMS microfluidic chips and a paper-based shunt was developed to demonstrate the combination of PDMS and paper to improve the emission signal by optimizing fluidic delays.[162] The device incorporated four layers stacked over each other. The uppermost layer was made of polyvinyl chloride (PVC) and used for the lateral-flow assay. The second layer consisted of glass fibers and was used for isothermal amplification. The third layer was a Flinders Technology Associates (FTA) card for DNA or RNA extraction and sample deposition. The fourth and bottom layer was made of cellulose and was used as a disposal item for waste products resulting from washing the sample. Sample evaporation was prevented by packing the four layers within a small adhesive tape. To test the assay, Hepatitis B viral DNA-containing blood sample was deposited at the third layer, in which lysing chemicals were already added. The assay was then washed to purify it from waste produced after cell lysis, which was then absorbed by the bottom cellulose layer. After removing the bottom layer, the third and second layers were tightly mounted, and the LAMP mixture was added to the second layer to be heated by a portable heating module at 65 °C to perform LAMP. The first layer was then mounted to the rest of the assembly to perform the LFA. With a detection limit of about 5 pM, combining the shunt and the PDMS barrier improved the LFA signal 10 times as compared to results obtained using either PDMS or paper-based shunt. As this integrated device combines all the LAMP steps within a compact and user-friendly design, it is a suitable diagnostic platform for PoC applications (Figure 3B). This device illustrated the promising implementation of using FTA cards in paper-based LAMP applications.

Other studies have also used FTA to conduct LAMP. A multilayer design of a paper microfluidics strip that can slide into a fluidic path to introduce multiple elements to the assay including the sample, washing buffer, LAMP mixture, and detection chemicals was developed.[163] The paper strip was made of an FTA card between two magnetic sheets. The ports for the sequential deposition of elements were patterned on the paper and the compact design helped prevent fluid evaporation. Testing the device was accomplished by introducing a sample containing the *E. coli* malB gene, performing LAMP at 65 °C in 1 h, and using a

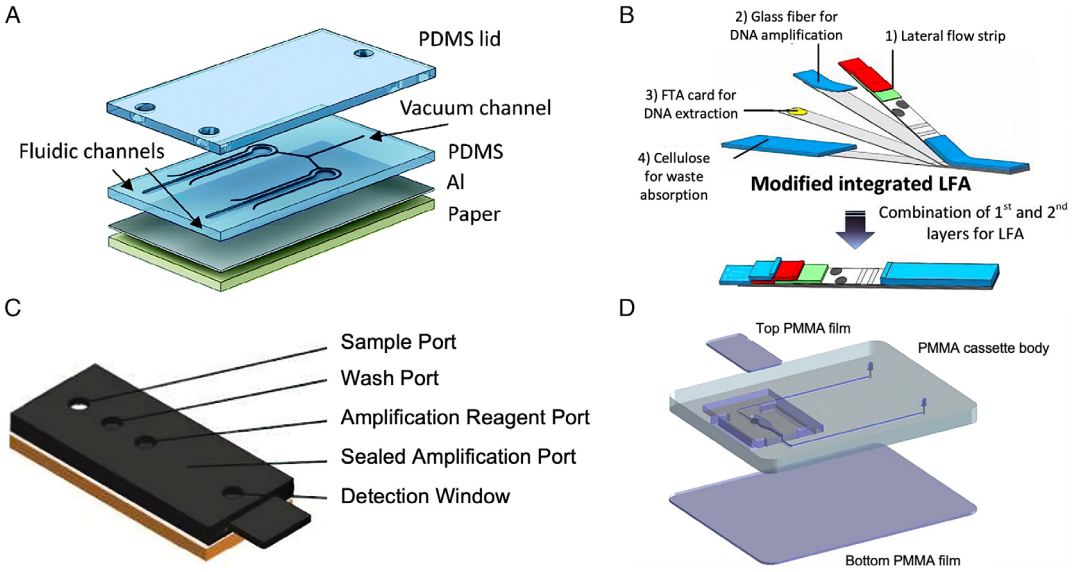


Figure 3. Different paper-based microfluidic LAMP platforms. A) PDMS–paper microfluidic chip fabricated using a roll-to-roll method, consisting of a PDMS chip on an Al-coated paper. Reproduced with permission.[161] Copyright 2018, The Royal Society of Chemistry. B) Lateral flow assay (LFA) modified with PDMS and shunted paper, with 4 layers to perform all steps of LAMP. Reproduced with permission.[162] Copyright 2016, American Chemical Society. C) Flinders Technology Associates (FTA)-based sliding strip after assembly of the 3 layers, namely the FTA strip and the upper and lower magnet sheets. Reproduced with permission.[163] Copyright 2015, American Chemical Society. D) Single-chamber FTA-based LAMP cassette, consisting of PMMA-based components. Reproduced with permission.[164] Copyright 2011, The Royal Society of Chemistry.

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portable UV light to detect fluorescent emission, which yielded a detection limit of 5 cells (Figure 3C). FTA cards were also used to create an easy-to-use and cost-efficient platform for the LAMP-based PoC diagnosis.[164] An HIV-containing sample was used to test the detection capabilities of the device. The device was a cassette that contained a chamber, where the LAMP reaction would take place. The chamber had an FTA membrane to isolate, concentrate, and purify the nucleic acid, as well as to filter out the inhibitors that significantly decrease detection sensitivity. An external film heater was used for temperature control, and a portable fluorescent detector was used for real-time monitoring of the reaction, which yielded a lower limit of detection than 10 HIV particles (Figure 3D). This study introduced a cost-effective LAMP assay with a total cost per assay of US $1.83. This device was able to process samples with a concentration as low as 1 double-stranded copy of the 200 bp dsDNA target. In addition, to investigate the effect of larger sample volume, it was displayed that the additional presence of cells in the larger volume samples was successfully lysed and their DNA was captured by the cellulose fibers of the paper reaction discs. Despite being low cost, the use of liquid amplification reagents can potentially prevent the application of this platform in resource-limited settings–used reagents have limited shelf life in the absence of refrigeration.

7. Smartphone-Based LAMP

One of the limitations of PoC LAMP platforms is their relatively low detection sensitivity compared to gold standards, which require large equipment and special operation, resulting in a high cost. Smartphone cameras can be integrated into PoC diagnostic systems such as portable magnetic levitation-based devices,[165,166] sperm analysis devices,[167] cerebral edema diagnosis,[168] and microscopy.[169] Another advantage of using smartphones in PoC diagnostics is their availability and accessibility, where a smartphone can be used as an alternative to a custom-made device such as a processing unit, a camera, or a data transfer unit. These advantages have been utilized in PoC LAMP diagnostics, primarily focusing on using the smartphone camera in either fluorescence detection or colorimetric detection. Furthermore, smartphone-integrated PoC LAMP diagnostics offer processing data, in particular analyzing images. A device was developed based on immiscible phase filtration assisted by a surface tension (IFAST).[170] It simplifies impurity filtration using interfacial tension characteristics without centrifugation and washing steps, leading to lower cost and rapid DNA or RNA extraction.[3] A microfluidic chip was designed to have three regions: a nucleic acid extraction region with three chambers where IFAST is performed, a mixing and microdroplet formation region, and a droplet tiling section. After DNA extraction with IFAST, the extracted DNA was mixed with LAMP reagents in a mixing channel, followed by oil transportation to form microdroplets, and LAMP was performed with a Loopamp kit at 62 °C for 40 min. The smartphone-based detection system consisted of a 10× lens, a detection filter, an excitation filter, a Blu-ray flashlight, a dichroic mirror, and a smartphone. After capturing an image of the LAMP results using the smartphone, cloud computing was performed using Image-J software to process the image and provide the quantitative analysis (**Figure 4**A). The device was

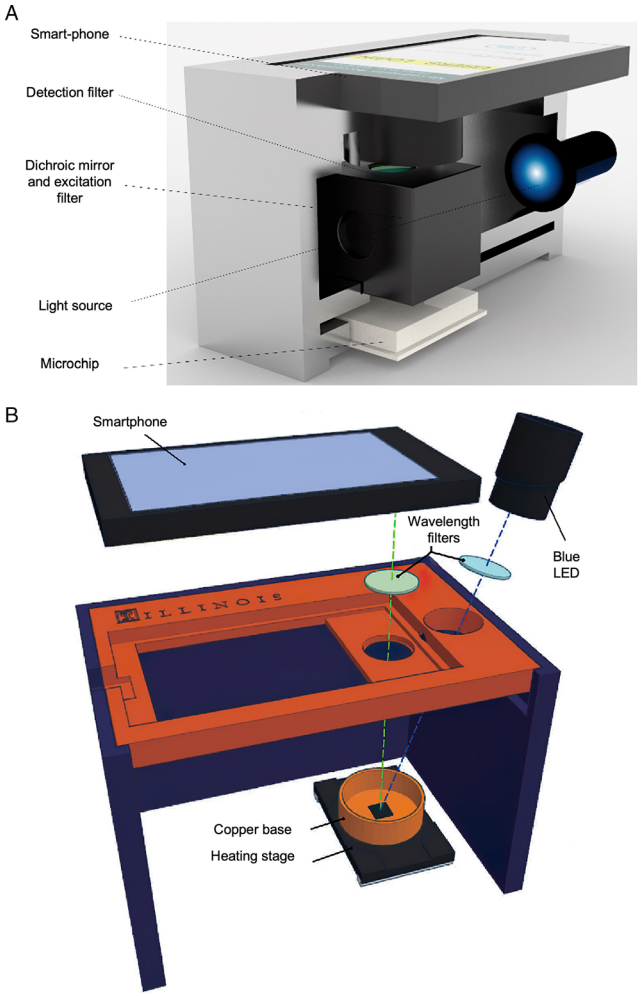


Figure 4. Two different smartphone-based LAMP platforms. A) Immiscible phase filtration assisted by a surface tension (IFAST)-based LAMP platform, consisting of a microchip holder to place the sample after performing LAMP, a smartphone, and a set of optics including a light source, a mirror, and filters. Reproduced with permission.[170] Copyright 2019, American Chemical Society. B) Integrated smartphone-based reverse transcriptase LAMP (RT-LAMP) setup, consisting of 3D-printed parts to hold the different modules, a LAMP unit that includes a heating stage and a copper base, a smartphone, wavelength filters, and a blue LED. Reproduced with permission.[171] Copyright 2015, Elsevier B.V.

able to yield high detection sensitivity even with samples of low-abundance DNA. It was demonstrated that the passage of PMPs through the liquid wax does not inhibit the succeeding amplification by RT-qPCR or interfere with the binding of the NA to the PMPs. Moreover, as a result of a fourfold increment in the weight of Ambion PMPs (from 40 to 160 µg), the liquid carry-over increased from 0.06 to 0.35 µL. The purification efficiency of this platform for chlamydia and gonorrhea DNA from urine was comparable to that of manual extraction methods (≈87.9%), indicating the potential application of this platform in DNA studies.

Integration of smartphone technology can be used for RNA-based detection using RT-LAMP. This application is particularly

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significant when the target of detection is viral RNA. To develop a PoC device for the detection of HIV, a microfluidics-based LAMP assay and a smartphone were integrated for fluorescence measurements.[171] Whole blood was collected from healthy individuals and mixed with whole HIV particles to prepare a testing sample. A microfluidic chip was employed as a mixing module, where whole blood lysis was performed to extract the viral RNA and demonstrated that lysis could be achieved efficiently with a simple microfluidic setup. In the next step, a silicon microchip platform was used to perform RT-LAMP, where the sample was added to the platform in the form of individual droplets, each with a volume of about 60 nL. The microchip was then heated on a heating stage inside a copper container at 65 °C. To assess the measurement performance, a traditional fluorescence microscope was used to image the RT-LAMP result, followed by imaging with a smartphone camera. The smartphone was not modified, yet a 530 nm filter was placed between the microchip and the smartphone's camera. A custom apparatus was designed and 3D printed to hold the smartphone in place, and another 3D-printed holder was used to fit a blue LED and a 500 nm filter (Figure 4B). After imaging with the smartphone, the data were transferred to a computer to perform image processing and analysis via MATLAB. The assembly was able to achieve a high detection sensitivity of 3 viruses in a 60 nL droplet.

8. Conclusion

NAATs are routine laboratory techniques for the determination of disease agents. LAMP technology as a NAAT method allows for the amplification of target regions under isothermal conditions without the need for thermal cycling. LAMP has proved to be more advantageous than other NAATs methods, as it can amplify target DNA rapidly and allows easy and fast detection of the target product. In this review, we discussed the LAMP methodology, its uses, its purposeful modifiability, and its convertibility into PoC-Lamp diagnosis platforms. The LAMP method is an effective method for the detection of pathogens that cause disease in plants, animals, and humans due to its high specificity, performance, and low cost. Despite some limitations, modified LAMP strategies are developed for SNP genotyping and mutation detection. LAMP has become an alternative method to PCR in the diagnosis of infectious diseases caused by viruses, bacteria, and parasites. Compared to PCR, there is no need for complicated instruments and high-cost laboratory infrastructure. LAMP allows for the design of portable PoC-LAMP diagnostic platforms. Microfluidic chips have been designed in PoC-LAMP diagnostic platforms, where DNA extraction and LAMP reaction can be performed, where the sample can be loaded directly. These microfluidic chips are LoC and paper-based LAMP technologies, enabling easy-to-use, low-cost, and rapid diagnostic testing. Visual/colorimetric, turbidimetric, and fluorescent monitoring of the amplification results in the LAMP method has also contributed to the creation of different types of PoC-LAMP diagnostic platforms. PoC-LAMP diagnostic platforms that can be monitored on a smartphone are particularly promising. PoC-LAMP diagnostic platforms represent a new generation of molecular genetic diagnosis technologies. The utilization of 3D printers can also facilitate the development of LAMP platforms, as 3D printing enables fast prototyping with minimum knowledge of the micromanufacturing.[172–176] Moreover, quantification of LAMP assays can be a labor-intense task that is prone to human bias. The adoption of machine learning-enabled image analysis methods can increase the LAMP analysis speed and accuracy. Machine learning refers to the ability of machines to learn based on labeled data or previous experiences with minimal human intervention.[177–180]

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

Keywords

LAMP-on-a-chip, loop-mediated isothermal amplification (LAMP), paper-based LAMP, pathogen detection, point-of-care LAMP (PoC-LAMP) diagnostics, smartphone-based LAMP

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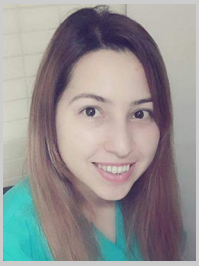
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