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Review

Advances in Nucleic Acid Amplification-Based Microfluidic Devices for Clinical Microbial Detection

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Abstract: Accurate and timely detection of infectious pathogens is urgently needed for disease treatment and control of possible outbreaks worldwide. Conventional methods for pathogen detection are usually time-consuming and labor-intensive. Novel strategies for the identification of pathogenic nucleic acids are necessary for practical application. The advent of microfluidic technology and microfluidic devices has offered advanced and miniaturized tools to rapidly screen microorganisms, improving many drawbacks of conventional nucleic acid amplification-based methods. In this review, we summarize advances in the microfluidic approach to detect pathogens based on nucleic acid amplification. We survey microfluidic platforms performing two major types of nucleic acid amplification strategies, namely, polymerase chain reaction (PCR) and isothermal nucleic acid amplification. We also provide an overview of nucleic acid amplification-based platforms including studies and commercialized products for SARS-CoV-2 detection. Technologically, we focus on the design of the microfluidic devices, the selected methods for sample preparation, nucleic acid amplification techniques, and endpoint analysis. We also compare features such as analysis time, sensitivity, and specificity of different platforms. The first section of the review discusses methods used in microfluidic devices for upstream clinical sample preparation. The second section covers the design, operation, and applications of PCR-based microfluidic devices. The third section reviews two common types of isothermal nucleic acid amplification methods (loop-mediated isothermal amplification and recombinase polymerase amplification) performed in microfluidic systems. The fourth section introduces microfluidic applications for nucleic acid amplification-based detection of SARS-CoV-2. Finally, the review concludes with the importance of full integration and quantitative analysis for clinical microbial identification.

Keywords: nucleic acid amplification; microfluidic device; PCR; isothermal amplification; sample preparation; clinical microbial detection

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1. Introduction

Infections are usually caused by microorganisms such as bacteria, viruses, and fungi, which are transferred to humans from bodily fluids, water, food, and air and soil of the surrounding environment. Early and accurate diagnosis is crucial for some infections to reduce disease morbidity and mortality because appropriate treatment and treatment timing are critical [1,2]. Therefore, fast and precise diagnostic techniques are necessary [3]. Many diagnostic methods directly detect pathogens, such as culturing, microscopy, biochemistry, immunological detection, or specific gene detection. Generally, the conventional culture method is the standard method to detect infectious microorganisms [4–6]; however, its biggest disadvantage is the length of time to results. The immunological method offers a fast, simple, and affordable detection tool; however, it lacks high specificity and sensitivity. The nucleic acid amplification-based method is a promising candidate for pathogenic

identification because of its high sensitivity and specificity. However, its use is limited to high-resource facilities because of the need for sophisticated instruments.

Presently, microfluidic technology is leading significant changes in clinical microbial diagnosis, a trend supported by technological advances. Microfluidic technology automatically manipulates the flow of liquid inside microchambers connected by microchannels with the aid of other miniaturized components to analyze samples [7–10]. Microfluidic devices have been intensively used to screen pathogens [11–13]. Many techniques have been integrated into the microfluidic platform for clinical microbial diagnosis application. In this review, we focus on nucleic acid amplification-based microfluidic devices. The full integration of the process from sample preparation, amplification, and detection into one platform is crucial in the nucleic acid amplification-based method to compartmentalize these tasks. These advances open the door to a new level of diagnostic performance and efficiency, which reduce the time for test results and are highly automated, accurate, cost-effective, and simple [14–16]. The development of these platforms for clinical microbial detection offers an effective and timely response to outbreaks, especially in a pandemic period. Here, we provide an overview of some advances in the microfluidic field for clinical microbial detection, especially nucleic acid amplification-based microfluidic devices.

The nucleic acid amplification-based technique is a potential tool in clinical microbial detection given its high selectivity, sensitivity, and rapid results [17,18]. Nucleic acid amplification-based techniques amplify specific segments of nucleic acid. The primers can be designed to accelerate amplification reactions based on the specific gene sequences of the microorganism of interest. Various nucleic acid amplification-based methods have emerged in the development of microfluidic devices. Generally, nucleic acid amplification-based techniques are divided into polymerase chain reaction (PCR) and isothermal nucleic acid amplification (Figure 1).

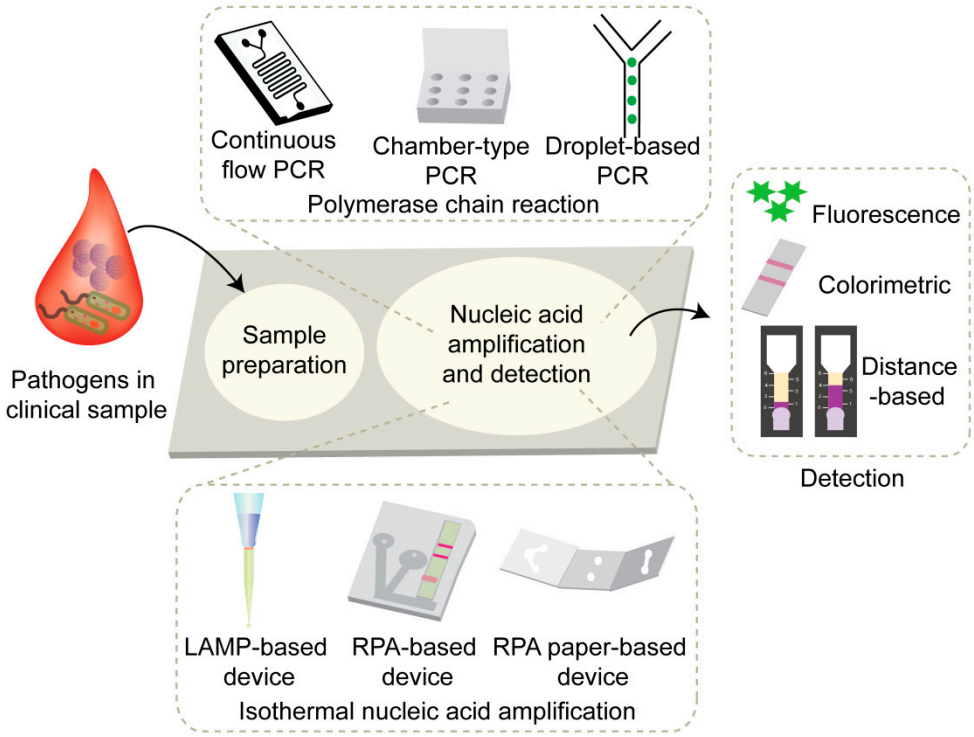


Figure 1. Microfluidic technologies for clinical microbial detection.

Since its development in the 1980s, PCR has emerged as a powerful tool to detect nearly every clinical microorganism. PCR is an in vitro enzymatic DNA amplification performed at three discrete temperatures. PCR consists of thermal cycling, including denaturation of double-stranded DNA at high temperatures, annealing of primers to the complementary

sequence of the genomic DNA, and extension with the aid of polymerase. The polymerase used in the reaction is Taq polymerase, which is tolerant of high temperatures [19]. The amplification process results in up to one billion copies of the original target genes. Because of the advances in microfluidic technology, PCR has been intensively integrated into microfluidic platforms for diagnostic purposes.

Isothermal nucleic acid amplification appears to be a promising alternative to conventional PCR because of its simplicity and low energy needs [20,21]. Various isothermal amplification techniques have been developed since the early 1990s. In this review, we focus on two major isothermal nucleic acid amplification techniques: loop-mediated isothermal amplification (LAMP) and recombinase polymerase amplification (RPA). LAMP was first developed by Notomi et al. in 2000. LAMP recognizes the target gene sequence using a set of four to six primers, including two outer primers (F3 and B3), a forward inner primer, and a backward inner primer. Loop forward primer and loop backward primer are optional primers to improve the reaction speed. LAMP reaction is catalyzed by Bst polymerase with strand displacement activity. LAMP offers a highly sensitive molecular diagnostic tool with a rapid reaction time of less than 1 h. The amplification results in billions of DNA copies in concatemer form. The high sensitivity and specificity of LAMP and lack of the need for sophisticated thermal cyclers make it an outstanding alternative to nucleic acid amplification-based methods [22–26].

RPA operates at temperatures of 37 °C to 42 °C with the aid of two enzymes (recombinase and DNA polymerase) and single-stranded DNA-binding (SSB) proteins. Recombinase assists primer hybridization with the template to form a recombinase–primer complex. The complex scans the target dsDNA for the homologous sequence to promote strand displacement. A D-loop structure is formed and stabilized by SSB proteins. DNA polymerase with strand displacement activity catalyzes the exponential amplification of the target sequence after binding to the 3′ end of the primer. RPA offers a sensitive and rapid tool within 1 h for molecular diagnosis; moreover, the RPA reaction can be conducted at one low temperature equivalent to the human body temperature, which makes it suitable for integration into the microfluidic platform. Amplifying nucleic acid at a constant temperature eliminates the need for complicated thermocyclers. Another advantage of isothermal nucleic acid amplification is the ability to integrate real-time detection of the amplicons via turbidity measurements or visual observation by colorimetric or fluorescent expression, offering ideal solutions for simple and compact nucleic acid amplification-based analysis [27–29]. Isothermal amplification methods vary based on the enzymes, proteins, and the number of primer and template types used. Because of their advances, isothermal amplification methods have been intensively applied and commercialized in point-of-care testing platforms [30].

Microfluidic systems, which have a portable size of a few centimeters and in which the fluid flow inside patterned channels can be controlled, have rapidly developed since the 1980s. Various sample analyses have been integrated into microfluidic devices, including sample preparation, reaction, and detection. The devices usually contain microchannels, chambers, and valves that manipulate the fluid inside the device for various reactions [31]. These devices can save analysis costs because they require ultra-low volumes of reagents, samples, and energy [7,8]. Silica or glass was first used to fabricate microfluidic devices. Microfluidic devices are also made from thermal plastic, e.g., polycarbonate (PC), polymethyl methacrylate (PMMA), and polydimethylsiloxane (PDMS). Recently, paper has been widely adopted to fabricate microfluidic devices. Microfluidic devices serve many functions in nucleic acid amplification-based techniques, including sample preparation, amplification, and detection for clinical microbial diagnosis.

2. On-Chip Sample Preparation

Sample preparation is a key step in clinical microbial identification. Sample preparation for the nucleic acid amplification process consists of the extraction of nucleic acid from other macromolecules [31,32]. Before nucleic acid amplification, the nucleic acid from the

microorganism in the clinical sample must be purified to eliminate amplification inhibitors. Clinical samples are composed of many components, such as blood cells, proteins, glucose, urea, lysozymes, or immunoglobulins, which can interfere with downstream analysis if the sample is not thoroughly prepared [33–35]. Sample preparation, especially for clinical samples, is complicated, time-consuming, and labor-intensive [36–39]. Different methods have been integrated into microfluidic platforms to achieve fast and on-site identification of microorganisms in clinical samples [40–43]. Microchannel systems are specially designed to separate different particles in the sample based on the difference in particle sizes. One study proposed a simple nucleic acid purification strategy from blood cells using the design of the microchannel system inside the microfluidic device [40]. In this study, the sedimentation method was applied to separate the blood cells from nucleic acid and separate particles based on size. Using the special microchannel design, only particles with a diameter over 1 mm were selectively removed. Consequently, DNA of methicillin-resistant *Staphylococcus aureus* (MRSA) was successfully extracted from red blood cells within 12 min using approximately 22 mL of plasma [40].

Biochemical separation relies on specific recognition [41]. Unlike physical methods, biochemical methods separate the pathogenic microorganisms of interest from other particles of the same size. One common method that is usually integrated into microfluidic devices for sample preparation is the use of magnetic beads. Pathogenic microorganisms or their nucleic acids can be captured onto magnetic beads via specific interactions such as antigen–antibody, enzyme–substrate, and electrostatic interaction; consequently, the target can be separated from other components in the matrix [42]. Magnetic beads have been used to extract the DNA of MRSA from two clinical samples, namely, sputum and serum [43]. The sample was first injected into the cell lysis/DNA hybridization/LAMP reaction chamber. DNA was lysed from bacterial cells by heating the chamber at 95 °C. Target DNA was immobilized on the beads by the interaction between DNA and the probe-conjugated magnetic beads. Magnetic bead–DNA complexes were captured inside the chamber using the magnet attached underneath the chamber. The remaining components from the sample were washed away, offering a simple and automated sample preparation method.

Recently, paper has become an attractive platform for nucleic acid extraction [44–46]. For example, an FTA card is one excellent application of paper in nucleic acid extraction [47]. One study proposed the use of FTA cards for DNA extraction from the multidrug-resistant Gram-negative bacteria *Acinetobacter baumanii* [47]. With the use of lytic chemical and protein denaturants embedded inside the FTA card matrix, bacterial cells were lysed, and DNA was released into the card. FTA reagent and TE buffer were applied, and the wastes from the FTA card were washed away. Only DNA remained inside the FTA matrix for further processing [47].

3. On-Chip Amplification and Target Detection

3.1. PCR-Based Microfluidic Devices

3.1.1. Chamber-Type PCR

The conventional PCR technique is widely applied to develop nucleic acid amplification-based microfluidic devices to detect clinical microorganisms. Figure 2 describes some features of PCR-based microfluidic devices. The first type of PCR microfluidic device mentioned in this section is a chamber-type PCR. A chamber-type PCR microfluidic device fully integrates nucleic acid extraction, PCR, and colorimetric detection to screen multidrug-resistant *A. baumannii* [47]. The microdevice is made of three foldable layers of PMMA to transfer the fluid within the chambers. The foldable all-in-one microdevice enables the simple operation of nucleic acid analysis without the need for valve control or micropumps. Clinical samples were collected from patients in a Thai hospital. An FTA card was used for nucleic acid extraction in approximately 40 min. To achieve a ready-to-use PCR reaction, filter paper stored with PCR reagents was embedded into the reaction chamber of the microdevice. Amplification was completed in 75 min. Silver nitrate was used for colorimetric detection of the PCR result in 45 s. The $bla_{\text{OXA-23-like}}$ carbapenemase gene was successfully

amplified in 116 min [47]. The platform offers a rapid, specific, sensitive, and accurate tool to analyze genotypic antimicrobial susceptibility tests. Chamber-type PCR was also used to develop a diagnostic platform for viruses. An integrated microfluidic device possessing the advances of both reverse transcription PCR (RT-PCR) and microfluidic technology has been developed for the nucleic acid analysis of the influenza virus, including the processes of sample preparation, RT-PCR, and detection [48]. For sample extraction, glycan-coated magnetic beads were applied based on the glycan-binding specificity of the hemagglutinin (HA) antigen on the surface of the virus. The viruses were captured onto the glycan-coated magnetic beads and separated from the waste in the sample. RNA was released from the virus by thermolysis in 5 min and transferred into the RT-PCR reaction chamber. The reagents were pumped into the chamber for one-step RT-PCR using the heat supply from the thermoelectric cooler. After 40 cycles of RT-PCR, the resulting fluorescence signal was analyzed using an optical detection module. The total reaction was less than 100 min. With this microfluidic system, up to 12 influenza A subtypes were simultaneously screened in a rapid, sensitive, and fully automated manner [48]. Another example of chamber-type PCR is the research to detect influenza A virus H1N1 in saliva [49]. The system consists of preconcentration and amplification chambers that can preconcentrate the virus in saliva samples and amplify the target genes. Using this device, H1N1 viruses were preconcentrated from saliva samples by magnetic nanoparticles conjugated with specific antibodies [49]. After preconcentration, purified RNA of the virus was amplified in an RT-PCR chamber. Using this system, the detection limit for the H1N1 virus significantly improved. Moreover, the system is a promising tool for the timely screening of infectious diseases.

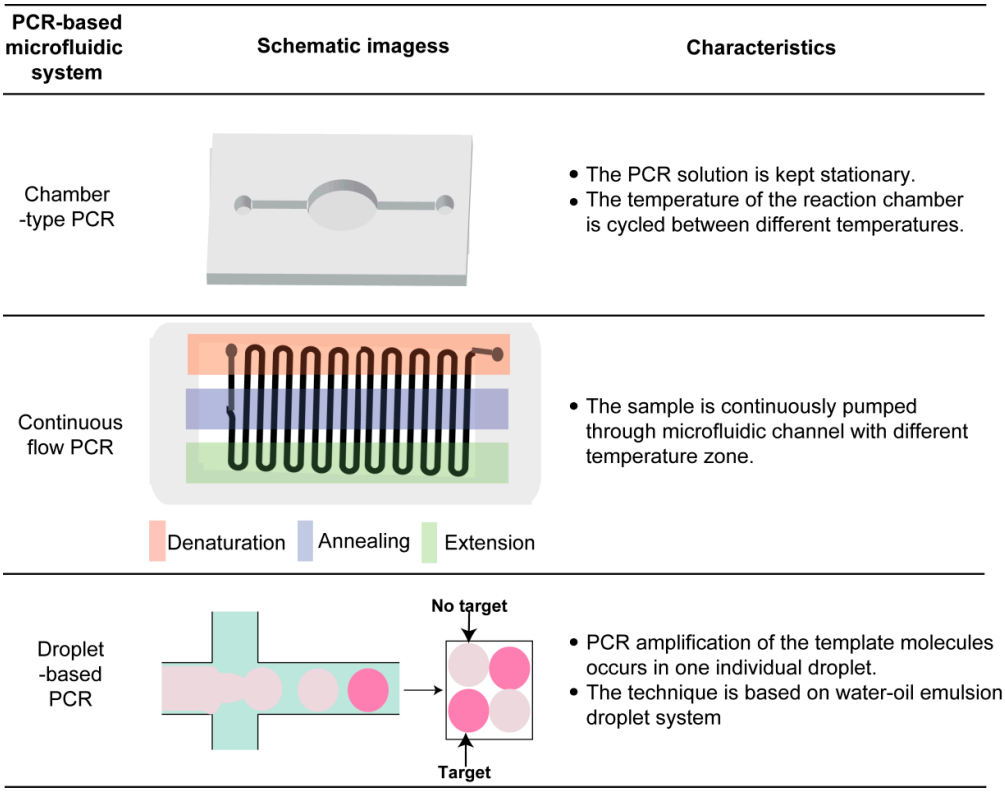


Figure 2. PCR-based microfluidic devices.

3.1.2. Continuous-Flow PCR

In continuous-flow PCR (CF-PCR), also called flow-through PCR, the fluid flows through a serpentine microchannel system with separate thermal zones required for PCR. For amplification reaction, the PCR mixture moves along the microchannel length and undergoes different heat zones to complete thermal cycling. In a CF-PCR system, the

seamless flow of the fluid inside the microchannel conveniently allows for the full integration of nucleic acid extraction, amplification, and detection [50]. There are several advantages in the CF-PCR platform, including miniaturization, portability, fast analysis, and high-throughput capacity.

A handheld, all-in-one system was proposed to detect periodontal pathogens [51]. This system consists of a pumping unit, two aluminum heaters, and a CF-PCR microfluidic device integrated with electrophoresis for detection. The system proposed rapid automatic sample injection for fast PCR and on-site detection. Using this system, three types of periodontal pathogens, namely, *Porphyromonas gingivalis*, *Treponema denticola*, and *Tannerela forsythia*, were screened from gingival crevicular fluid with a limit of detection (LOD) of 125 CFU/μL.

A continuous-flow reverse transcription PCR microfluidic device was developed to detect RNA viral pathogens [52]. The system consists of a microfluidic pump, heater, continuous-flow reverse transcription PCR microfluidic device, and an optical detection system. Using this system, RNAs of different viruses such as Ebola, Zika, and chikungunya virus were rapidly, conveniently, and sensitively detected.

A microfluidic system was previously proposed to perform CF-PCR-based multiplex detection for MRSA [53]. Pumps and valves were used to control the fluid inside the microchannel to different temperature zones for amplification reactions. The system can detect four strains, including methicillin-susceptible *S. aureus*, MRSA, methicillin-susceptible *S. epidermidis*, and methicillin-resistant *S. epidermidis* in less than 40 min.

3.1.3. Droplet-Based PCR

Droplet-based microfluidics have been intensively applied in PCR devices for clinical microbial analysis. Each created droplet becomes an individual chemical reactor, thereby offering reproducible and high-throughput reaction conditions [54,55]. Droplets in sizes as small as picoliters or femtoliters have been generated to conduct single-cell and single-molecule analyses. To benefit from these advantages, PCR has been conducted in droplets. First, in droplets, PCR is conducted in an isolated environment in which the reaction can avoid the intervention of inhibitors or carryover contaminators. Second, the small reaction volume not only requires less reagent and sample quantity but also enables high sensitivity. Finally, quantitative analysis can be achieved using this technique because each droplet contains either zero copies or one copy of the amplified target; droplets of the former category are defined as "one". Quantitative analysis can be achieved on the basis of this calculation.

An automated and multichannel digital platform was developed to detect the DNA of multiple clinical microorganisms, including MRSA, *Mycoplasma pneumoniae*, and *Candida albicans* [56]. Multiplex PCR assay was accomplished using electrowetting-enabled flow-through methods. The droplets are shuttled between two distinct temperature zones for PCR reaction; therefore, no pump or valve is needed for fluid manipulation. The system is a promising tool for simple multiplexed real-time PCR to screen pathogens in clinical samples.

A droplet PCR assay was proposed to test multidrug-resistant bacteria in blood [57]. In this system, PCR was directly conducted using blood samples without the need for sample treatment because of the use of PCR-enhancer cocktails and inhibitor-resistant Taq mutants. Inside the microfluidic system, the sample and reagents were mixed and encapsulated into millions of droplets. Direct nucleic acid amplification without DNA purification was achieved. Using this system, the antibiotic-resistant genes $bla_{\text{OXA-48}}$, bla_{KPC}, *vanA*, *nuc*, *mecA*, and the $bla_{\text{CTX-M-1}}$ and $bla_{\text{CTX-M-2}}$ families were identified in less than 1 h simply and sensitively [57]. Table 1 summarizes some pathogens, target genes, and primers used in mentioned studies. Features of the above-mentioned PCR-based microfluidic devices are also summarized in Figure 3.

Table 1. Target genes and primers used for pathogen detection.

Pathogens	Nucleic Acid Segments	Primer	Ref.
Multidrug-resistant *Acinetobacter baumannii*	$bla_{\text{OXA-23-like}}$ carbapenemase gene	FP: GATCGGATTGGAGAACCAGA RP: ATTTCTGACCGCATTTCCAT	[47]
Staphylococcus aureus	*nuc* gene	FP: ACACCTGAAACAAAGCATCC RP: TAGCCAAGCCTTGACGAACT	
Salmonella	*inv*A gene	FP: AAAACATATGCTGGACCAACTGGAAGC RP: TTCGCTTAACAAACGCTGCAAAACTT	
E. coli O157:H7	*eae*A gene	FP: GACCCGGCACAAGCATAAGC RP: CCACCTGCAGCAACAAGAGG	
Influenza A Virus (H1N1)	*M* gene coding matrix	FP: ATGAGYCTTYTAACCGAGGTCGAAACG RP: TGGACAAANCGTCTACGCTGCAG	[49]
Periodontal pathogens *Porphyromonas gingivalis*	Conserved regions of 16 S rDNA	FP: GTAGATGACTGATGGTGAAAACC RP: ACGTCATCCCCACCTTCCTC	[51]
Treponema denticola		FP: AAGGCGGTAGAGCCGCTCA RP: AGCCGCTGTCGAAAAGCCCA	
Tannerella forsythia		FP: GCGTATGTAACCTGCCCGCA RP: TGCTTCAGTGTCAGTTATACCT	
Ebola virus	Ebola virus L gene	FP: GTCCGTCGTTCCAGTCATTT RP: CCCTCTTGGATGCTGAGTTA TG	[52]
Methicillin-resistant *Staphylococcus aureus*	*mec*A gene	FP: TGGTATGTGGAAGTTAGATTGG RP: ATATGCTGTTCCTGTATTGGC	[53]
Methicillin-resistant *Staphylococcus aureus*	N/A	FP: GTCAAAAATCATGAACCTCATTACTTATG RP: GGATCAAACGGCCTGCACA	[56]
Mycoplasma pneumonia		FP: CTGTTTGAGCGTCGTTTC RP: ATGCTTAAGTTCAGCGGGTAG	
Candida albicans		FP: TTTGGTAGCTGGTTACGGGAAT RP: GGTCGGCACGAATTTCATATAAG	
Methicillin-resistant *Staphylococcus aureus*	*mec*A	FP: CCAATTTGTCTGCCAGTTTCT RP: GGTATGCAACAAGTCGTAAATAAAAC	[57]
Vancomycin-resistant enterococci	*van*A	FP: CCATGTTGATGTAGCATTTTCAGC RP: CAAGGTCTGTTTGAATTGTCCG	
Gram-negative extended spectrum β-lactamase-producing Enterobacteriaceae	blaCTX-M-1	FP: TTCTTCAGCACCGCG RP: CGAATTAGAGCGGCAGTC	
	blaCTX-M-2	FP: GGATTGTAGTTAACCAGGTCG RP: ATGTGCAGTACCAGTAAGGTGAT	
	blaCTX-M-9	FP: CCATAACTTTACTGGTACTGCAC RP: GTCGCGCTCATCGATAC	
Carbapenem-resistant Enterobacteriaceae	blaKPC	FP: ATAGTCATTTGCC GTGCCATAC RP: TGATTGGCTAA AGGGAAACAC G	
	blaOXA-48	FP: AAGACTTGGTGTTCATCCTTAACC RP: GAATGAGAATAAGCAGCAAG GA	
	blaNDM-1	FP: CCATCCCTGACGATCAAAC RP: GACCAACGGTTTGGCGATCT	

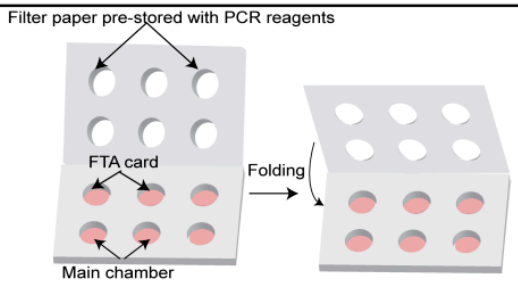
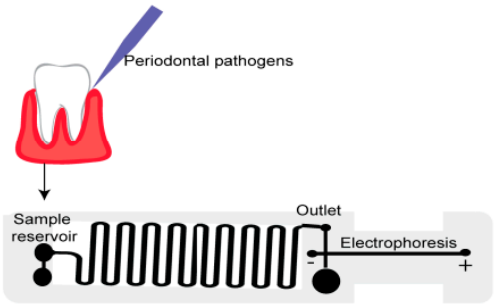
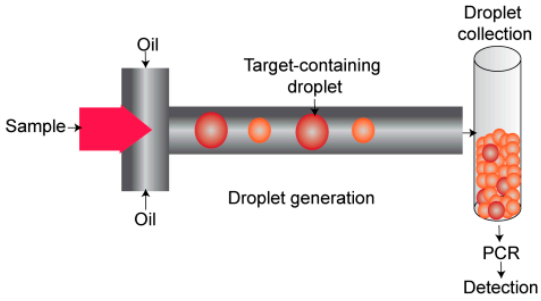
PCR-based microfluidic system	Examples	Characteristics of the system
Chamber-type PCR		• Sample: Clinical samples • Target: bla OXA-23-like carbapenemase of *A. baumannii* • DNA extraction using FTA card • Chamber-type PCR • Colorimetric detection using silver nitrate • Sample transportation by folding motion
Continuous flow PCR		• Sample: crevicular fluid • Target: *Porphyromonas gingivalis*, *Treponema denticola*, *Tannerela forsythia* • Continuous flow PCR • On-site detection of PCR products by a custom-made capillary electrophoresis system • Fluid transportation by pumping unit
Droplet-based PCR		• Sample: blood • Target: antibiotic resistant bacteria • Droplet digital PCR • Fluorescence detection

Figure 3. Examples of PCR-based microfluidic devices and systems used for pathogen detection. Reproduced from [47,51,57].

3.2. Microfluidic Devices for Isothermal Nucleic Acid Amplification

3.2.1. Loop-Mediated Isothermal Amplification

LAMP is a sensitive and highly specific isothermal amplification technique. One of the biggest advantages of LAMP-integrated microfluidic devices is the lack of a need for bulky, complicated external supplies for thermal control. Therefore, LAMP has been intensively used for developing microfluidic devices for clinical microbial detection. LAMP is a microfluidic platform integrated with magnetic bead-based DNA extraction and was proposed for screening the *mec*A gene from MRSA in two types of clinical samples (sputum and serum) [43]. After DNA purification using specific probe-conjugated magnetic beads, purified DNA was amplified using the LAMP method with LOD of 10 fg/μL.

A micropipette tip-based nucleic acid test was also developed for DNA and RNA detection in crude samples in a sample-in-answer-out system [58], which was integrated with an FTA card for nucleic acid extraction, LAMP of nucleic acids, and fluorescence detection using calcein. The research used a micropipette tip embedded with an FTA card for solid-phase nucleic acid extraction to achieve full integration of nucleic acid analysis. A pipette was used to manipulate samples inside the micropipette tip. The nucleic acid was extracted using an FTA card inside the micropipette tip through a 30 min incubation. Subsequently, the FTA card was washed, and the LAMP mixture was injected for the amplification reaction inside the tip. Calcein was used as an indicator for the LAMP

reaction. Visual determination was achieved using a handheld UV flashlight. Nucleic acid fragments of the Ebola virus were amplified in 45 min. Other samples, such as cytokeratin-19 mRNA, a cancer biomarker, were also successfully screened using such a method [58].

A diagnostic system was introduced to detect HIV-1 RNA within 80 min [59]. In this system, RT-LAMP was utilized without electricity using a robust heater. Moreover, RT-LAMP combined with nucleic acid lateral flow detection offered a suitable solution for point-of-care molecular assays in low-resource areas without complicated equipment or high power requirements [59].

Another study integrated RT-LAMP and portable commercial pregnancy test strips as a rapid, sensitive, and cost-effective diagnostic tool for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [60]. There are three pressure-vent pores and two zones for amplification and incubation in the device. In the amplification zone, The T-LAMP mixture was preloaded for the RT-LAMP reaction. The detection reagent, human chorionic gonadotropin (hCG) probes, was added to the incubation zone. The principle of the detection is based on the conjugation of RT-LAMP amplicons and hCG, the main target of the pregnancy test. RT-LAMP amplicons were incubated with hCG probes for hybridization reaction, after which the visual read-out was conducted with the pregnancy test strips. The formation of a massive amount of RT-LAMP amplicons and hCG probe complexes prevented the hCG probes from migrating on the paper strip; therefore, one control line was observed on the strip, indicating SARS-CoV-2 positivity. In contrast, hCG probes did not hybridize with RT-LAMP amplicons in negative samples; therefore, hCG probes freely migrated on the paper strip. This resulted in two lines (the control and testing line) on the strip. The system offers a promising solution for SARS-CoV-2 emergency detection.

Additionally, a digital LAMP microfluidic device has been proposed to quantitatively detect vancomycin-resistant *Enterococcus* (VRE) [61]. Using the system, VRE DNA was successfully amplified in 30 min with LOD as low as 11 copies. Moreover, the research introduced a microfluidic device that generated emulsion droplets of identical size for digital LAMP. LAMP reaction for *van*A amplification occurred in water-in-oil droplets.

To realize a simple and sufficient quantitative interpretation for diagnosis, a system combining LAMP and a distance-based paper analytical device was proposed to amplify the *mal*B gene of *E. coli* [62]. This method achieved semiquantitative determination based on the length of the color formed on the paper device [63]. The paper device was treated with a strong cationic polymer-polyethylenimine (PEI). Hydroxyl naphthol blue (HNB), a metal indicator, was used to realize the colorimetric signal in LAMP. After the LAMP reaction, the mixture was applied to the paper device. The negative charges of free HNB in LAMP reacted with the positive charges of PEI on the paper, resulting in blue color on the paper. Within 5 min, the visual distance appeared, which correlated to the concentration of DNA in the sample. Using this device, semiquantitative detection of pathogenic nucleic acid can be achieved in a simple, low-cost, disposable, and portable manner.

3.2.2. Recombinase Polymerase Amplification

Besides LAMP, RPA is also a promising isothermal nucleic acid amplification method that is suitable for integration with microfluidic devices. Because of the relatively low reaction temperature of 37 °C to 42 °C, RPA is usually applied to develop wearable devices for pathogen detection. A study reported a wearable device to detect HIV-1 DNA using RPA assay combined with a cell phone-based fluorescence detection system [64], using body heat as a heat source for RPA reaction. The device was made of PDMS to achieve maximum heat transfer and surface contact with human skin. With this device, the DNA of HIV-1 was successfully amplified and detected in 24 min with LOD of 100 copies/mL.

In another study, RT-RPA was integrated with lateral flow assay in a microfluidic device for rapid SARS-CoV-2 detection [65]. The chip was made of three layers of PMMA and consisted of one RT-RPA reaction chamber, a running buffer chamber, a mixing chamber, two inlet holes, and a test strip channel. The RT-RPA reaction occurred in the reaction

chamber by incubation in a heat block at 42 °C for 15 min. After the RT-RPA reaction, the chip was held upright and shaken to mix the running buffer and amplification products. Subsequently, the chip was held so that the mixture flowed through the mixing chamber and reached the lateral flow channel for a visual read-out. A colored test line indicated the presence of RT-RPA amplicons (positive result). A control test line indicated an invalid test strip. The assay can detect SARS-CoV-2 RNA with LOD of 30 copies per sample [65].

A digital RPA system has been introduced to quantitatively detect MRSA and HIV in blood samples [40]. Through the special design of the microfluidic device, HIV and MRSA nucleic acids were purified from blood samples via sedimentation. Because of a vacuum battery and lung-like vacuum component with an optional waste reservoir, the fluid flow inside the device could be easily manipulated without the bulky equipment, allowing for the rapid and simple quantitative diagnosis of pathogens through the integration of sample separation, digital RPA, and fluorescence detection using fluorescein. The total assay time was approximately 30 min, including 10 min of sample preparation and 20 min of nucleic acid amplification. The system quantitatively detected MRSA DNA from 10 to 10^5 copies/μL. Moreover, the system could screen HIV RNA spiked in human blood in approximately 18 min. Figure 4 summarizes the features of the above-mentioned isothermal nucleic acid amplification-based microfluidic devices. Some important aspects of microfluidic devices that must be considered include types of sample used, methods for extraction/amplification/detection, analysis time, and sensitivity, to mention a few; these are summarized in Table 2.

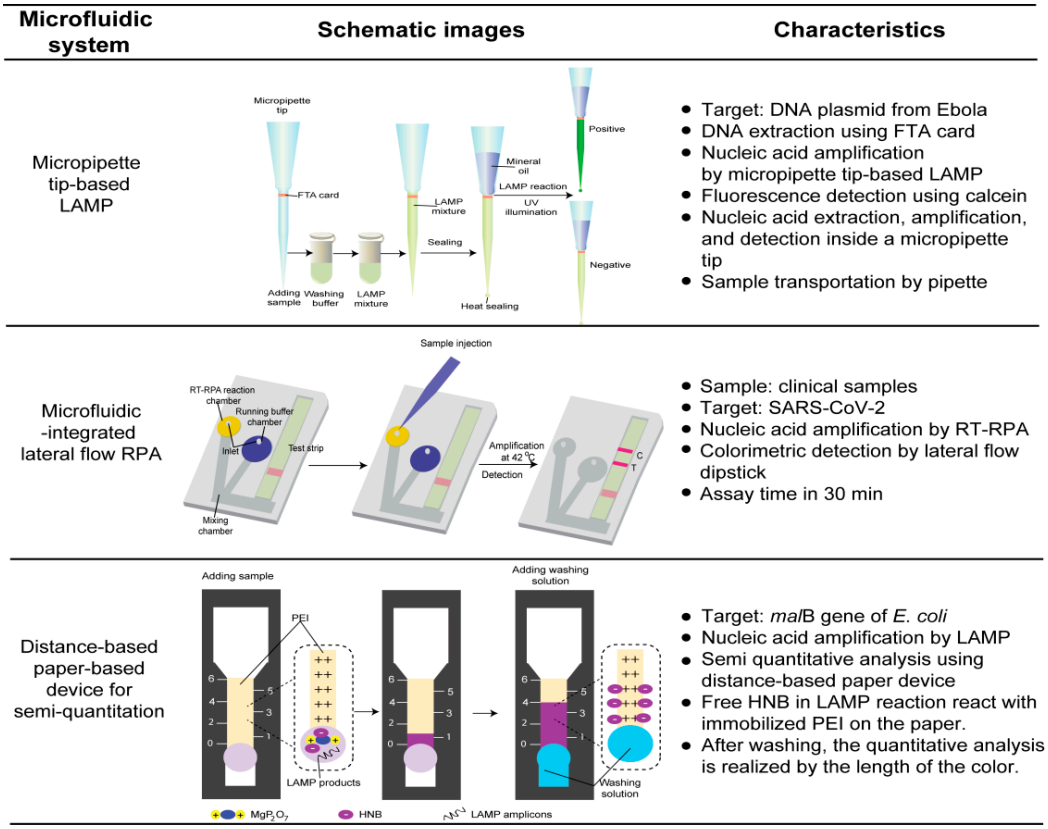


Figure 4. Examples of microfluidic devices used for isothermal nucleic acid amplification for pathogen detection. Reproduced from [58,62,65].

Table 2. Features of some nucleic acid amplification-based microfluidic devices.

Microfluidic Device	Sample Type	Extraction	Amplification Type	Detection	Operation Time	Sensitivity	Ref.
Foldable all-in-one point-of-care molecular diagnostic microdevice	Clinical sample	FTA card	Chamber-type PCR	Colorimetric detection	Within 2 h	3.0×10^2 for Gram-negative bacteria and 3.0×10^3 CFU for Gram-positive bacteria	[47]
Integrated microfluidic preconcentration and nucleic amplification system	Saliva	Virus precon-centration by magnetic nanoparticles conjugated with antibody	Chamber-type RT-PCR	Gel electrophoresis	Within 2 h	100 TCID50 (50% tissue culture infective dose) in saliva	[49]
All-in-one microfluidic device	Gingival reticular fluid	Off-chip sample preparation	Continuous-flow PCR	On-chip capillary electrophoresis	DNA amplification in 2′31″ Detection in 3′43″	125 CFU/μL	[51]
Continuous-flow, microfluidic, qRT-PCR system for RNA virus detection	Ebola virus L gene	Off-chip sample preparation	Continuous-flow RT-PCR	Fluorescence detection	30–50 min	10 RNA copies per microliter)	[52]
Micro-pipette tip-based nucleic acid test	Bacteria cell culture	FTA card	LAMP	Fluorescence detection	90–160 min	2 copies of plasmids containing Ebola virus gene 8 CFU of *Escherichia coli* carrying Ebola virus-derived plasmids	[58]
SARS-CoV-2 point-of-care (POC) diagnosis based on commercial pregnancy test strips and a palm-size microfluidic device	N gene of SARSCoV-2 full-length M gene, and the partial sequence of the N gene of SARS-CoV	Off-chip extraction	RT-LAMP	Pregnancy test strip	Within 2 h	0.5 copy/μL	[60]
Microfluidic-integrated lateral flow recombinase polymerase amplification assay	Clinical samples	Easy NAT nucleic acid extraction device	RT-RPA	Lateral test strip	30 min	1 copy/μL	[65]

4. Application for SARS-CoV-2 Diagnoses

Coronavirus disease 2019 (COVID-19) is an infectious disease caused by the SARS-CoV-2 virus. The COVID-19 pandemic is a persistent global challenge with high infectivity through direct, human-to-human transmission [66]. Rapid screening of infected patients is important for immediate treatment and efficient disease control. The gold standard to detect COVID-19 is RT-PCR, ensuring high specificity and sensitivity. However, the method has some limitations in on-site detection because it is time-consuming and laborious. Moreover, it requires sophisticated diagnostic equipment. To solve these issues, nucleic acid amplification-based tests have been integrated into microfluidic devices to realize simple and sensitive screening of diseases in low-resource settings. On one platform, all nucleic acid analysis processes which include nucleic acid extraction, amplification, and detection can be fully integrated. Numerous microfluidic devices have been introduced and applied for SARS-CoV-2 identification [67,68] since microfluidic platforms offer efficient,

rapid, simple, and reliable diagnoses. In this section, we describe some studies for SARS-CoV-2 diagnoses as well as commercial diagnostic kits which obtained the Food and Drug Administration's Emergency Use Authorization (FDA EUA) for SARS-CoV-2. These devices detect SARS-CoV-2 based on nucleic acid amplification.

4.1. Studies for SARS-CoV-2 Detection

The full-length M gene and the partial sequence of the N gene of SARS-CoV-2 were targeted for SARS-CoV-2 detection. A palm-sized microfluidic device was fabricated by integrating nucleic acid amplification and colorimetric detection to enable rapid, sensitive, and cost-effective screening for SARS-CoV-2 [60]. A clinical swab sample was used to prove the validity of the microfluidic system. The device amplified SARS-CoV-2 RNA by the RT-LAMP technique. A heat block was employed to provide constant heat for the amplification, and a lateral flow test strip was integrated into the system for colorimetric detection of the RT-LAMP product to realize an on-site detection of SARS-CoV-2. One control line on the test strip indicated the presence of SARS-CoV-2 in the sample. The detection was realized by the conjugation of RT-LAMP amplicons with hCG. The conjugation prevents hCG from moving along the strip, so nothing appears on the test line. On the contrary, in the absence of RT-LAMP amplicons, hCG can freely migrate along the strip and interact with the reagents in the test line, and therefore, the color signal appears on both control and test lines. The phenomenon indicates that the sample contains no SARS-CoV-2. The device analyzed the sample in less than 120 min, and the limit of detection of the test was two copies per reaction. The device also showed high specificity for SARS-CoV-2 detection in samples in the presence other types of pathogens.

A PMMA microfluidic device which integrated RT-RPA and lateral flow assay for detection was fabricated for the detection of SARS-CoV-2 [65]. FAM-labeled forward and biotin-labeled reverse RT-RPA primer set was employed for recognizing the nucleoprotein-encoding gene of the virus and expressing a colorimetric signal for result read-out. A throat swab preservation solution spiked with SARS-CoV-2-armored RNA particles was employed. RNA was extracted from an equipment-free nucleic acid extraction device. A heat block was used to supply a stable heat of 42 °C for 15 min for the amplification reaction. The result was read using the lateral flow strip. The assay can detect SARS-CoV-2 RNA in 30 min with the limit of detection of 30 copies per sample. The device was also tested with positive clinical samples. The performance of the device showed high sensitivity of 97% and specificity of 100%.

Another study developed a point-of-care device for molecular testing of SARS-CoV-2 [69]. The device featured RNA extraction, amplification, and naked-eye visualization. The device is made of two black polystyrene sheets and a cover made of PCR tape so that the device can be folded to transfer the sample. A nasopharyngeal swab was used as a sample, and lysis buffer was employed to lyse the virus and release RNA. RNA was captured on the extraction membrane, and washing reagents were used to wash away the waste. Purified RNA was then eluted using buffer and made ready for amplification. To perform RT-LAMP, stable heat of 65 °C was maintained using a hot plate. Fluorescent DNA intercalating dye, SYTO-82, was used for result read-out. The device analyzed the sample in 20–60 min with the limit of detection of one copy per reaction.

A capture and improve LAMP (cap-iLAMP) assay was developed for point-of-care testing of SARS-CoV-2 [70]. A total of 555 gargle lavage samples were collected from hospitals and nursing homes to test for the presence of SARS-CoV-2. A rapid bead-capture enrichment purification was employed, and RNA was isolated in 15 min. SYBR Green I was used for visual detection of SARS-CoV-2, which changed the sample color from orange to yellow. The color signal was quantified as numerical hue value and analyzed using smartphone apps. The point-of-care assay analyzed the sample in 55 min with the limit of detection of 100 copies per sample. This method offers simple, sensitive, accurate, and low-cost detection of SARS-CoV-2. Table 3 summarizes some studies performed for SARS-CoV-2 detection.

Table 3. SARS-CoV-2 diagnostic platforms.

Platform	Sample Type	Sample Preparation	Nucleic Acid Amplification	Gene Target	Detection
SARS-CoV-2 point-of-care diagnosis based on commercial pregnancy test strips and a palm-sized microfluidic device	N/A	Off-chip extraction	RT-LAMP	Full-length *M* gene and the partial sequence of the *N* gene	Lateral flow test strip
Microfluidic-integrated lateral flow recombinase polymerase amplification (MI-IF-RPA) assay	Throat swab preservation solution spiked with SARS-CoV-2-armored RNA particles	Off-chip extraction	RT-RPA	*N* gene	Lateral flow test strip
Point-of-care molecular testing for SARS-CoV-2	Nasopharyngeal swab	Virus lysis by lysis buffer RNA extraction by functionalized membrane and washing buffer	RT-LAMP	*RdRP* gene	Fluorescence
Point-of-care bulk testing for SARS-CoV-2 by combining hybridization capture with improved colorimetric LAMP	Gargle lavage	A rapid (15 min) bead-capture enrichment purification	RT-LAMP	Orf1a gene, *N* gene	Color

4.2. Commercialized Test Kit for SARS-CoV-2 Detection

4.2.1. Lucira Check It COVID-19 Test Kit

The Lucira Check It COVID-19 Test kit is used under FDA EUA for the qualitative molecular detection of SARS-CoV-2 causing COVID-19 [71]. The kit utilizes RT-LAMP for RNA detection of the virus. The target gene is the nucleocapsid (N) gene of the virus. RNA is extracted from the cell using elution buffer in a sample vial. After extraction, the treated sample enters reaction chambers for RT-LAMP, which is initiated by the resolubilization of lyophilized reagents in the reaction chamber. An internal electronic heating element applies a heat source for the amplification reaction. A pH-based detection method is used for downstream analysis. When the amplification is successful, the color of the pH indicator changes. The signal is collected and analyzed using optical and electronic elements and an onboard microprocessor. The result is displayed via LED indicators. Positive results are obtained in approximately 11 min, whereas negative results appear in a longer time.

4.2.2. Xpert Xpress SARS-CoV-2

The Xpert Xpress SARS-CoV-2 test is used under FDA EUA for the qualitative molecular detection of SARS-CoV-2 causing COVID-19 [72]. The test is based on the RT-PCR method for SARS-CoV-2 detection and is conducted on GeneXpert Instrument Systems, wherein sample preparation, amplification, and detection are integrated. The sample is transferred to a single-use disposable cartridge for nucleic acid analysis and provides a rapid result in approximately 45 min.

4.2.3. ID NOW COVID-19

The ID NOW COVID-19 kit is an instrument-based rapid test for qualitative molecular diagnosis of SARS-CoV-2. The kit is in use under FDA EUA [73] and is based on the isothermal amplification method, which utilizes nicking enzyme amplification reaction

to detect SARS-CoV-2. The test, conducted on the ID NOW instrument, is designed to target virus RNA, the unique region of the RdRp segment. The sample was treated with elution/lysis buffer for RNA extraction. To detect target amplicons, fluorescently labeled molecular beacons are used. The assay delivers rapid detection in 13 min or less for positive results.

4.2.4. Cue COVID-19 Test Kit

The Cue COVID-19 test is used under FDA EUA for the qualitative molecular detection of SARS-CoV-2 [74] and uses isothermal nucleic acid amplification to detect the N gene of the virus. The assay is automatically initiated upon inserting the Cue Sample Wand with the sample into the cartridge and takes 20 min to deliver the final result. Table 4 summarizes the features of some test kits for SARS-CoV-2.

Table 4. Test kits available for nucleic acid amplification-based detection of SARS-CoV-2.

Test Kit	Lucira Check It COVID-19 Test Kit	Cue COVID-19 Test	ID Now COVID-19	Xpert Xpress SARS-CoV-2
Company	Lucira Health	Cue Health	Abbott	Cepheid
Sample type	Nasal swabs	Anterior nasal swabs	Nasal, nasopharyngeal or throat swabs	Nasopharyngeal, oropharyngeal, nasal or mid-turbinate swab or nasal wash/aspirate
Sample preparation	Lysis	Lysis	Lysis	Lysis
Nucleic acid amplification	RT-LAMP	Isothermal amplification	NEAR	RT-PCR
Gene target	Nucleocapsid gene	Nucleocapsid gene	RdRP segment	N2-nucleocapsid gene E-enveloped protein gene
Detection	pH-based colorimetric method	Colorimetric detection method	Fluorescently labeled molecular beacons	Fluorescence

5. Conclusions and Perspectives

In this review, we summarized microfluidic advances for nucleic acid amplification-based detection of clinical microorganisms. First, we discussed the limitations of conventional methods and the advantages of microfluidic application for pathogen detection. Then, we described some nucleic acid amplification-based microfluidic platforms used for clinical microbial detection, including PCR-based and isothermal amplification devices. Finally, we introduced some commercialized systems for SARS-CoV-2 detection.

Nucleic acid amplification-based microfluidic devices have demonstrated promising potential for rapid, simple, high-throughput, automatic, and accurate clinical microbial detection. However, the issue of mass production remains a challenge before the devices can be readily commercialized. Additionally, fully integrated platforms that include sample preparation, nucleic acid amplification, and visual detection using clinical samples such as blood and feces are still limited. Moreover, most developed forms of devices still provide qualitative results. Although qualitative analyses can effectively identify the infection, quantitative analyses are important for monitoring disease treatments. Another issue is that the device sensitivity is limited when using real samples, unless the samples are not fully purified. Therefore, an urgent need still exists for fully integrated, sample-in-answer-out devices with the functionality of a quantitative result read-out.

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References

1. Lee, J.S.; Ahn, J.J.; Kim, S.J.; Yu, S.Y.; Koh, E.J.; Kim, S.H.; Sung, H.S.; Huh, J.W.; Hwang, S.Y. POCT detection of 14 respiratory viruses using multiplex RT-PCR. *BioChip J.* **2021**, *15*, 371–380. [CrossRef] [PubMed]
2. Bowler, P.G.; Duerden, B.I.; Armstrong, D.G. Wound microbiology and associated approaches to wound management. *Clin. Microbiol. Rev.* **2001**, *14*, 244–269. [CrossRef] [PubMed]
3. Mason, M.G.; Botella, J.R. Rapid (30-second), equipment-free purification of nucleic acids using easy-to-make dipsticks. *Nat. Protoc.* **2020**, *15*, 3663–3677. [CrossRef] [PubMed]
4. Lagier, J.C.; Edouard, S.; Pagnier, I.; Mediannikov, O.; Drancourt, M.; Raoult, D. Current and past strategies for bacterial culture in clinical microbiology. *Clin. Microbiol. Rev.* **2015**, *28*, 208–236. [CrossRef]
5. Park, S.H.; Ryu, S.; Kang, D.H. Development of an improved selective and differential medium for isolation of *Salmonella* spp. *J. Clin. Microbiol.* **2012**, *50*, 3222–3226. [CrossRef]
6. Reller, L.B.; Weinstein, M.; Jorgensen, J.H.; Ferraro, M.J. Antimicrobial susceptibility testing: A review of general principles and contemporary practices. *Clin. Infect. Dis.* **2009**, *49*, 1749–1755.
7. Berlanda, S.F.; Breitfeld, M.; Dietsche, C.L.; Dittric, P.S. Recent advances in microfluidic technology for bioanalysis and diagnostics. *Anal. Chem.* **2021**, *93*, 311–331. [CrossRef]
8. Manz, A.; Graber, N.; Widmer, H.M. Miniaturized total chemical analysis systems: A novel concept for chemical sensing. *Sens. Actuators B Chem.* **1990**, *1*, 244–248. [CrossRef]
9. Whitesides, G.M. The origins and the future of microfluidics. *Nature* **2006**, *442*, 368–373. [CrossRef]
10. Patabadige, D.E.W.; Jia, S.; Sibbitts, J.; Sadeghi, J.; Sellens, K.; Culbertson, C.T. Micro total analysis systems: Fundamental advances and applications. *Anal. Chem.* **2016**, *88*, 320–338. [CrossRef]
11. Feng, X.; Du, W.; Luo, Q.; Liu, B.F. Microfluidic chip: Next-generation platform for systems biology. *Anal. Chim. Acta* **2009**, *650*, 83–97. [CrossRef] [PubMed]
12. Dou, M.; Dominguez, D.C.; Li, X.; Sanchez, J.; Scott, G. A versatile PDMS/Paper hybrid microfluidic platform for sensitive infectious disease diagnosis. *Anal. Chem.* **2014**, *86*, 7978–7986. [CrossRef] [PubMed]
13. Zhang, D.; Bi, H.; Liu, B.; Qiao, L. Detection of pathogenic microorganisms by microfluidics based analytical methods. *Anal. Chem.* **2018**, *90*, 5512–5520. [CrossRef] [PubMed]
14. Nasseri, B.; Soleimani, N.; Rabiee, N.; Kalbasi, A.; Karimi, M.; Hamblin, M.R. Point-of-care microfluidic devices for pathogen detection. *Biosens. Bioelectron.* **2018**, *117*, 112–128. [CrossRef]
15. Zhao, X.; Li, M.; Liu, Y. Microfluidic-based approaches for foodborne pathogen detection. *Microorganisms* **2019**, *7*, 381. [CrossRef]
16. Asghar, W.; Sher, M.; Khan, N.S.; Vyas, J.M.; Demirci, U. Microfluidic chip for detection of fungal infections. *ACS Omega* **2019**, *4*, 7474–7481. [CrossRef]
17. Lui, C.; Cady, N.C.; Batt, C.A. Nucleic acid-based detection of bacterial pathogens using integrated microfluidic platform systems. *Sensors* **2009**, *9*, 3713–3744. [CrossRef]
18. Gorgannezhad, L.; Stratton, H.; Nguyen, N.T. Microfluidic-based nucleic acid amplification systems in microbiology. *Micromachines* **2019**, *10*, 408. [CrossRef]
19. Zhu, H.; Zhang, H.; Xu, Y.; Lasakova, S.; Korabecna, M.; Neuzil, P. PCR past, present and future. *BioTechniques* **2020**, *69*, 317–325. [CrossRef]
20. Zhao, Y.; Chen, F.; Li, Q.; Wang, L.; Fan, C. Isothermal amplification of nucleic acids. *Chem. Rev.* **2015**, *115*, 12491–12545. [CrossRef]
21. Pumford, E.A.; Lu, J.; Spaczai, I.; Prasetyo, M.E.; Zheng, E.M.; Zhang, H.; Kamei, D.T. Developments in integrating nucleic acid isothermal amplification and detection systems for point-of-care diagnostics. *Biosens. Bioelectron.* **2020**, *170*, 112674. [CrossRef] [PubMed]
22. Notomi, T.; Okayama, H.; Masubuchi, H.; Yonekawa, T.; Watanabe, K.; Amino, N.; Hase, T. Loop-mediated isothermal amplification of DNA. *Nucleic Acids Res.* **2000**, *28*, e63. [CrossRef] [PubMed]
23. Tomita, N.; Mori, Y.; Kanda, H.; Notomi, T. Loop-mediated isothermal amplification (LAMP) of gene sequences and simple visual detection of products. *Nat. Protoc.* **2008**, *3*, 877–882. [CrossRef] [PubMed]
24. Zhang, X.; Lowe, S.B.; Gooding, J.J. Brief review of monitoring methods for loop-mediated isothermal amplification (LAMP). *Biosens. Bioelectron.* **2014**, *61*, 491–499. [CrossRef]

25. Mori, Y.; Notomi, T. Loop-mediated isothermal amplification (LAMP): A rapid, accurate, and cost-effective diagnostic method for infectious diseases. *J. Infect. Chemother.* **2009**, *15*, 62–69. [CrossRef]
26. Han, E.T. Loop-mediated isothermal amplification test for the molecular diagnosis of malaria. *Expert Rev. Mol. Diagn.* **2013**, *13*, 205–218. [CrossRef]
27. Shen, F.; Davydova, E.K.; Du, W.; Kreutz, J.E.; Piepenburg, O.; Ismagilov, R.F. Digital isothermal quantification of nucleic acids via simultaneous chemical initiation of recombinase polymerase amplification reactions on SlipChip. *Anal. Chem.* **2011**, *83*, 3533–3540. [CrossRef]
28. Crannell, Z.A.; Rohrman, B.; Richards-Kortum, R. Quantification of HIV-1 DNA using real-time recombinase polymerase amplification. *Anal. Chem.* **2014**, *86*, 5615–5619. [CrossRef]
29. Piepenburg, O.; Williams, C.; Stemple, D.; Armes, N. DNA detection using recombination proteins. *PLoS Biol.* **2006**, *4*, e204. [CrossRef]
30. Lutz, S.; Weber, P.; Focke, M.; Faltin, B.; Hoffmann, J.; Müller, C.; Mark, D.; Roth, G.; Munday, P.; Armes, N. Microfluidic lab-on-a-foil for nucleic acid analysis based on isothermal recombinase polymerase amplification (RPA). *Lab Chip* **2010**, *10*, 887–893. [CrossRef]
31. Lee, N.Y. A review on microscale polymerase chain reaction based methods in molecular diagnosis, and future prospects for the fabrication of fully integrated portable biomedical devices. *Microchim. Acta* **2018**, *8*, 285–307. [CrossRef] [PubMed]
32. Packard, M.M.; Wheeler, E.K.; Alocilja, E.C.; Shusteff, M. Performance evaluation of fast microfluidic thermal lysis of bacteria for diagnostic sample preparation. *Diagnostics* **2013**, *3*, 105–116. [CrossRef] [PubMed]
33. Mahalanabis, M.; Al-Muayad, H.; Kulinski, M.D.; Altman, D.; Klapperich, C.M. Cell lysis and DNA extraction of gram-positive and gram-negative bacteria from whole blood in a disposable microfluidic chip. *Lab Chip* **2009**, *9*, 2811–2817. [CrossRef] [PubMed]
34. Huang, Y.; Mather, E.L.; Bell, J.L.; Madou, M. MEMS-based sample preparation for molecular diagnostics. *Anal. Bioanal. Chem.* **2002**, *372*, 49–65. [CrossRef] [PubMed]
35. Pawliszyn, J. Sample preparation: Quo vadis? *Anal. Chem.* **2003**, *75*, 2543–2558. [CrossRef] [PubMed]
36. Lien, K.; Liu, C.; Kuo, P.; Lee, G. Microfluidic system for detection of α-Thalassemia-1 deletion using saliva samples. *Anal. Chem.* **2009**, *81*, 4502–4509. [CrossRef]
37. Chen, L.; Manza, A.; Day, P.J.R. Total nucleic acid analysis integrated on microfluidic devices. *Lab Chip* **2007**, *7*, 1413–1423. [CrossRef]
38. Niemz, A.; Ferguson, T.M.; Boyle, D.S. Point-of-care nucleic acid testing for infectious diseases. *Trends Biotechnol.* **2011**, *29*, 240–250. [CrossRef]
39. Zhang, L.; Ding, B.; Chen, Q.; Feng, Q.; Lin, L.; Sun, J. Point-of-care-testing of nucleic acids by microfluidics. *Trend Anal. Chem.* **2017**, *94*, 106–116. [CrossRef]
40. Yeh, E.; Fu, C.; Hu, L.; Thakur, R.; Feng, J.; Lee, L.P. Self-powered integrated microfluidic point-of-care low-cost enabling (SIMPLE) chip. *Sci. Adv.* **2017**, *3*, e1501645. [CrossRef]
41. Reinholt, S.J.; Baeumner, A.J. Microfluidic isolation of nucleic acids. *Angew. Chem. Int. Ed.* **2014**, *53*, 13988–14001. [CrossRef] [PubMed]
42. He, H.; Li, R.; Chen, Y.; Pan, P.; Tong, W.; Dong, X.; Chen, Y.; Yu, D. Integrated DNA and RNA extraction using magnetic beads from viral pathogens causing acute respiratory infections. *Sci. Rep.* **2017**, *7*, 45199. [CrossRef] [PubMed]
43. Wang, C.; Lien, K.; Wu, J.; Lee, G. A magnetic bead-based assay for the rapid detection of methicillin-resistant *Staphylococcus aureus* by using a microfluidic system with integrated loop-mediated isothermal amplification. *Lab Chip* **2011**, *11*, 1521–1531. [CrossRef]
44. Akyazi, T.; Basabe-Desmonts, L.; Benito-Lopez, F. Review on microfluidic paper-based analytical devices towards commercialization. *Anal. Chim. Acta* **2018**, *1001*, 1–17. [CrossRef]
45. Reboud, J.; Xu, G.; Garrett, A.; Adriko, M.; Yang, Z.; Tukahebwa, E.M.; Rowell, C.; Cooper, J.M. Paper-based microfluidics for DNA diagnostics of malaria in low resource underserved rural communities. *Proc. Natl. Acad. Sci. USA* **2019**, *116*, 4834–4842. [CrossRef] [PubMed]
46. Nishat, S.; Jafry, A.T.; Martinez, A.W.; Awan, F.A. Paper-based microfluidics: Simplified fabrication and assay methods. *Sens. Actuators B Chem.* **2021**, *336*, 129681. [CrossRef]
47. Trinh, K.T.L.; Stabler, R.A.; Lee, N.Y. Fabrication of a foldable all-in-one point-of-care molecular diagnostic microdevice for the facile identification of multiple pathogens. *Sens. Actuators B Chem.* **2020**, *314*, 128057. [CrossRef]
48. Shen, K.M.; Sabbavarapu, N.M.; Fu, C.Y.; Jan, J.T.; Wang, J.R.; Hung, S.C.; Le, G.B. An integrated microfluidic system for rapid detection and multiple subtyping of influenza A viruses by using glycan-coated magnetic beads and RT-PCR. *Lab Chip* **2019**, *19*, 1277. [CrossRef]
49. Kim, Y.; Abafogi, A.T.; Tran, B.M.; Kim, J.; Lee, J.; Chen, Z.; Bae, P.K.; Park, K.; Shin, Y.B.; van Noort, D.; et al. Integrated microfluidic preconcentration and nucleic amplification system for detection of influenza A virus H1N1 in saliva. *Micromachines* **2020**, *11*, 203. [CrossRef]
50. Kulkarni, M.B.; Goel, S. Advances in continuous-flow based microfluidic PCR devices—A review. *Eng. Res. Express* **2020**, *2*, 042001. [CrossRef]
51. Li, Z.; Ju, R.; Sekine, S.; Zhang, D.; Zhuanga, S.; Yamaguchi, Y. All-in-one microfluidic device for on-site diagnosis of pathogens based on an integrated continuous flow PCR and electrophoresis biochip. *Lab Chip* **2019**, *19*, 2663. [CrossRef] [PubMed]

52. Fernández-Carballo, B.L.; McBeth, C.; McGuiness, I.; Kalashnikov, M.; Baum, C.; Borrós, S.; Sharon, A.; Sauer-Budge, A.F. Continuous-flow, microfluidic, qRT-PCR system for RNA virus detection. *Anal. Bioanal. Chem.* **2018**, *410*, 33–43. [CrossRef] [PubMed]
53. Chen, Y.; Wang, H.; Hupert, M.; Soper, S.A. Identification of methicillin-resistant *Staphylococcus aureus* using an integrated and modular microfluidic system. *Analyst* **2013**, *138*, 1075–1083. [CrossRef] [PubMed]
54. Zhang, Y.; Jiang, H.R. A review on continuous-flow microfluidic PCR in droplets: Advances, challenges and future. *Anal. Chim. Acta* **2016**, *914*, e16. [CrossRef] [PubMed]
55. Kaminski, T.S.; Scheler, O.; Garstecki, P. Droplet microfluidics for microbiology: Techniques, applications and challenges. *Lab Chip* **2016**, *16*, 2168–2187. [CrossRef] [PubMed]
56. Hua, Z.; Rouse, J.L.; Eckhardt, A.E.; Srinivasan, V.; Pamula, V.K.; Schell, W.A.; Benton, J.L.; Mitchell, T.G.; Pollack, M.G. Multiplexed real-time polymerase chain reaction on a digital microfluidic platform. *Anal. Chem.* **2010**, *15*, 2310–2316. [CrossRef] [PubMed]
57. Abram, T.J.; Cherukury, H.; Ou, C.Y.; Vu, T.; Toledano, M.; Li, Y.; Grunwald, J.T.; Toosky, M.N.; Tifrea, D.F.; Slepenkin, A.; et al. Rapid bacterial detection and antibiotic susceptibility testing in whole blood using onestep, high throughput blood digital PCR. *Lab Chip* **2020**, *20*, 477–489. [CrossRef]
58. Lu, W.; Wang, J.; Wu, Q.; Sun, J.; Chen, Y.; Zhang, L.; Zheng, C.; Gao, W.; Liu, Y.; Jiang, X. High-throughput sample-to-answer detection of DNA/RNA in crude samples within functionalized micro-pipette tips. *Biosens. Bioelectron.* **2016**, *75*, 28–33. [CrossRef]
59. Singleton, J.; Osborn, J.L.; Lillis, L.; Hawkins, K.; Guelig, D.; Price, W.; Johns, R.; Ebels, K.; Boyle, D.; Weigl, B.; et al. Electricity-free amplification and detection for molecular point-of-care diagnosis of HIV-1. *PLoS ONE* **2014**, *9*, e113693. [CrossRef]
60. Yang, M.; Tang, Y.; Qi, L.; Zhang, S.; Liu, Y.; Lu, B.; Yu, J.; Zhu, K.; Li, B.; Du, Y. SARS-CoV-2 Point-of-Care (POC) diagnosis based on commercial pregnancy test strips and a palm-size microfluidic device. *Anal. Chem.* **2021**, *93*, 11956–11964. [CrossRef]
61. Ma, Y.D.; Luo, K.; Chang, W.H.; Lee, G.B. A microfluidic chip capable of generating and trapping emulsion droplets for digital loop-mediated isothermal amplification analysis. *Lab Chip* **2018**, *18*, 296–303. [CrossRef]
62. Hongwarittorrn, I.; Chaichanawongsaroj, N.; Laiwattanapaisal, W. Semi-quantitative visual detection of loop mediated isothermal amplification (LAMP)-generated DNA by distance-based measurement on a paper device. *Talanta* **2017**, *175*, 135–142. [CrossRef] [PubMed]
63. Cate, D.M.; Dungchai, W.; Cunningham, J.C.; Volckens, J.; Henry, C.S. Simple, distance-based measurement for paper analytical devices. *Lab Chip* **2013**, *13*, 2397. [CrossRef] [PubMed]
64. Kong, M.; Li, Z.; Wu, J.; Hu, J.; Sheng, Y.; Wu, D.; Lin, Y.; Li, M.; Wang, X.; Wang, S. A wearable microfluidic device for rapid detection of HIV-1 DNA using recombinase polymerase amplification. *Talanta* **2019**, *205*, 120155. [CrossRef] [PubMed]
65. Liu, D.; Shen, H.; Zhang, Y.; Shen, D.; Zhu, M.; Song, Y.; Zhu, Z.; Yang, C. A microfluidic-integrated lateral flow recombinase polymerase amplification (MI-IF-RPA) assay for rapid COVID-19 detection. *Lab Chip* **2021**, *21*, 2019. [CrossRef]
66. Sheridan, C. COVID-19 spurs wave of innovative diagnostics. *Nat. Biotechnol.* **2020**, *38*, 769–778. [CrossRef]
67. Escobar, A.; Chiu, P.; Qu, J.; Zhang, Y.; Xu, C. Integrated microfluidic-based platforms for on-site detection and quantification of infectious pathogens: Towards on-site medical translation of SARS-CoV-2 diagnostic platforms. *Micromachines* **2021**, *12*, 1079. [CrossRef]
68. Harpaldas, H.; Arumugam, S.; Rodriguez, C.C.; Kumar, B.A.; Shi, V.; Sia, S.K. Point-of-care diagnostics: Recent developments in a pandemic age. *Lab Chip* **2021**, *21*, 4517. [CrossRef]
69. Garneret, P.; Coz, E.; Martin, E.; Manuguerra, J.C.; Brient-Litzler, E.; Enouf, V.; Obando, D.F.G.; Olivo-Marin, J.C.; Monti, F.; van der Werf, S.; et al. Performing point-of-care molecular testing for SARS-CoV-2 with RNA extraction and isothermal amplification. *PLoS ONE* **2021**, *16*, e0243712. [CrossRef]
70. Bokelmann, L.; Nickel, O.; Maricic, T.; Pääbo, S.; Meyer, M.; Borte, S.; Riesenberg, S. Point-of-care bulk testing for SARS-CoV-2 by combining hybridization capture with improved colorimetric LAMP. *Nat. Commun.* **2021**, *12*, 1467–1474. [CrossRef]
71. The Lucira CHECK IT COVID-19 Test Kit—Instruction for Use. Available online: https://www.fda.gov/media/147494/download (accessed on 20 March 2022).
72. Xpert Xpress SARS-CoV-2—Instruction for Use. Available online: https://www.fda.gov/media/136314/download (accessed on 20 March 2022).
73. ID NOW COVID-19—Instruction for Use. Available online: https://www.fda.gov/media/136525/download (accessed on 20 March 2022).
74. Cue COVID-19 Test—Instruction for Use. Available online: https://www.fda.gov/media/138826/download (accessed on 20 March 2022).