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Review

Recent advances in microfluidic-based biosensors for nucleic acid detection: From amplification strategies to point-of-care diagnostic devices

Madhusudan B. Kulkarni [a,*], Vineela [a], Naresh Mandal [a], Yashwanth N [a], Panwala Fenil Chetankumar [b], Sergio O. Martínez-Chapa [c], Ricardo Izquierdo [d]

[a] Manipal Institute of Technology, Manipal Academy of Higher Education, Manipal, India
[b] Department of Electronics and Instrumentation Engineering, Siddaganga Institute of Technology, Tumakuru, 572103, Karnataka, India
[c] School of Engineering and Sciences, Tecnológico de Monterrey, Av. Eugenio Garza Sada 2501 Sur, 64849 Monterrey, NL, México
[d] Department of Electrical Engineering, École de Technologie Supérieure (ÉTS), 1100 Notre-Dame St. W, Montréal, QC H3C 1K3, Canada

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ABSTRACT

Nucleic acid detection has gained significant attention in healthcare because of its crucial role in diagnosing infectious and non-infectious diseases. Conventional molecular diagnostics are predominantly performed in centralized laboratories using sophisticated instrumentation and automated analyzers, resulting in increased analysis time, cost, and limited accessibility in decentralized settings. Consequently, there is an increasing demand for rapid, cost-effective, portable, and user-friendly diagnostic platforms capable of providing reliable results at the point of care. Target nucleic acid detection commonly relies on amplification strategies, including thermocyclic amplification such as polymerase chain reaction (PCR) and various isothermal amplification techniques. Although these methods provide high sensitivity and specificity, challenges associated with instrumentation, thermal control, sample preparation, assay integration, and accessibility remain. Microfluidic integration provides a promising approach for addressing these limitations by enabling miniaturized sample handling, reduced reagent consumption, rapid amplification, and integrated detection within compact analytical platforms. This review critically examines recent advances in microfluidic nucleic acid biosensors, including PCR and isothermal amplification platforms, emerging amplification and detection technologies, device integration, and point-of-care applications. Further, emphasis on comparative analytical performance, technological limitations, and the remaining challenges associated with translating microfluidic nucleic acid detection systems from laboratory demonstrations to practical point-of-care diagnostics applications.

1. Introduction

Recently, the microfluidic platform has materialized in both innovative technology and scientific research for several applications, including biological, biomedical, pharmaceutical, and biochemical domains [1,2]. Microfluidics is an interdisciplinary field that integrates microbiology, biochemistry, medical physics, engineering, nanotechnology, and biotechnology [3,4]. It deals with minuscule volumes of reaction samples using microfluidic channels with a resolution up to a hundred μm. Manz et al. [5], initiated microfluidic technology in the early 1950s for life sciences applications, which led to a colossal appreciation for the design and development of this technology. Microfluidics began after the original attempt to generate a microfluidic device in the early 1950s. Subsequently, different mechanisms were investigated and developed to drive and standardize the fluid flow in microfluidic microchannels. Numerous microfluidic devices based on lab-on-a-chip applications were produced, as stated by Haeberle et al. [6], These platforms were categorized as droplet-based microdevices with continuous and digital flow methods, capillary-based test strips, pressure-driven devices, and centrifugal microfluidic systems. In the end, microfluidics focused on producing chemical analysis and gas chromatography microdevices. Since the mid-1990s, the research and development protocol in microfluidics has augmented and begun unveiling a variety of potential to modernize the current laboratory customs, predominantly in the life sciences [7–9]. Because these can perform essential laboratory tasks on a tiny chip, microfluidic devices, known as Micro-Total Analysis System (μ-TAS) or Lab-on-a-chip (LOC), speed up various investigations and analyses [10]. Fig. 1 shows the

* Corresponding author.
E-mail address: madhusudan.kulkarni@manipal.edu (M.B. Kulkarni).

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schematic representation of Numerous applications of Microfluidic technology.

Additionally, it can reduce reagent mixture and response time, providing innovative and advanced resolutions for the chemical, pharmaceutical, food, and healthcare sectors. Microfluidics often deals with combining, moving, separating, and processing fluids. Developing systems that process tiny amounts of fluids to accomplish automation, screening, and multiplexing has practical applications for microfluidics [11–13]. Microfluidics later became one of the key instruments for creating technologies such as nucleic acid microchips, inkjet print heads, micro-propulsion, microbial/enzymatic fuel cells, and micro-thermal. The most basic microfluidic device now available consists of microchannels made of a polymer that is adhered to a planar surface. It concerns the precise management, control, and conduction of solutions geometrically constrained to a micro-range at which capillary flow controls the mass transfer. Furthermore, inertial microfluidic systems passively control particles, atoms, molecules, and cells by employing hydrodynamic forces in microchannels with various cross-sections [14, 15]. These features have contributed significantly to advancing the microfluidics field towards large-scale commercial production, especially in the biomedical sector, for detecting numerous biomarkers [16, 17]. Additionally, a transducer converts the reaction into an electric signal that electrochemical sensors can detect once a receptor binds the sample. In these electrochemical sensors, the electrode serves as a transducer. Microfluidic-based devices may manage fluid microchannels by drastically lowering the reaction volume due to their high surface-to-volume ratio [18,19]. This allows them to demonstrate the movement of thermal mass and temperature gradients more clearly and efficiently [20]. Fig. 2 shows the schematic representation of microtechnology with key beneficial parameters.

Point-of-care testing (POCT) utilizes customized medical equipment to conduct testing on-site or close to the site where care is to be provided. Due to its potential application to the POCT, portability has gained considerable significance in recent years. A microfluidic device with an integrated readout module is one of the most essential elements of POCT [21]. Very little human interaction is needed for POCT-based microdevices, which reduce human mistakes and use the least power. Most of the automated and miniaturized microfluidic POCT devices can be seamlessly integrated into a single user-friendly platform, enabling the development of sensitive, rapid, cost-effective, and minimally or non-invasive diagnostic systems. These devices can continuously and accurately detect a wide range of biomarkers present in biological fluids such as urine, blood, and saliva [22,23]. Miniaturization of devices is getting more prevalent, especially in electrochemical and biosensing analyses [24]. Reducing electrode dimensions in electrochemical research significantly influences the detection limits and enhances the electrochemical methods' sensitivity, selectivity, repeatability, and accuracy. This miniaturization also supports cost-effective fabrication and

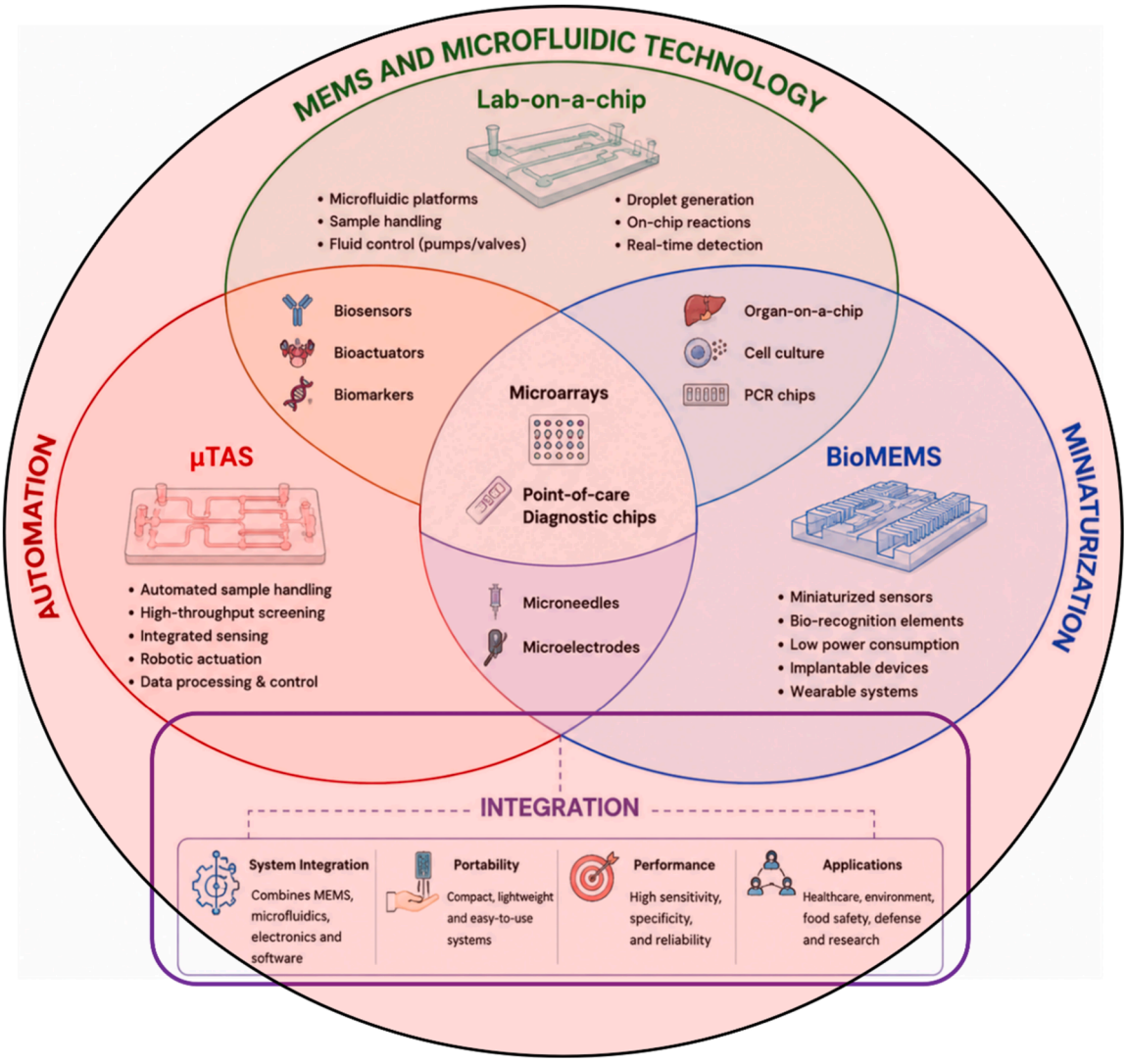


Fig. 1. Diverse applications of microfluidic technology within the Automation, Integration, and Miniaturization (AIM) framework.

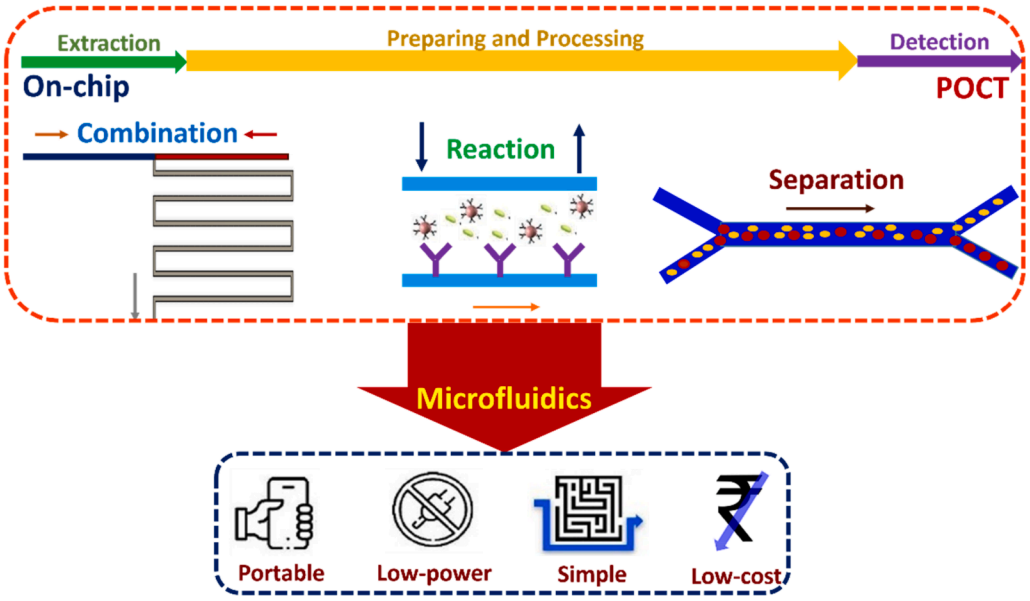


Fig. 2. A schematic representation of microfluidic technology with key beneficial parameters.

enables high-throughput analytical applications [25,26].

Biosensors have emerged as highly valuable tools in academic research, industry, and university settings. Their unique capability lies in detecting and quantifying biological or biochemical reactions by producing signals that correlate directly with the analyte concentration, thereby identifying physical events via a transducing system. The foundation of modern biosensor technology was established by Leland C. Clark Jr., who introduced the oxygen electrode in 1956, marking a significant milestone in this field. These devices function as biomarkers and are crucial in detecting diseases, environmental pollutants, and pathogenic microorganisms in various physiological fluids, including blood, saliva, urine, tears, and sweat [27–29]. In recent years, biosensors have significantly broadened their impact, offering novel opportunities in healthcare, food safety, environmental monitoring, and beyond. They have also become instrumental in pharmacology, high-throughput screening, agriculture, medicine, and homeland security. The first generation of biosensors utilized enzyme-catalyzed reactions and electrochemical transducers to convert biochemical changes into electrical signals [30]. Second-generation biosensors, affinity biosensors, introduced biological recognition elements such as antibodies, receptors, and nucleic acids. These biosensors rely on specific molecular interactions—such as DNA–DNA hybridization, antigen–antibody binding, and protein–nucleic acid recognition—between the target analyte and immobilized biomolecules on the sensor surface [31].

In recent years, numerous academics and scientists have given much thought to the biorecognition of the viral state at the receptor. Due to their close association with gene expression, nucleic acids rank among the most critical biomarkers and the most frequently researched macromolecules. Even slight nucleic acid sequence differences can have clinical and biological implications. Single-nucleotide polymorphisms (SNPs), for instance, reveal the innate knowledge of distinct virus predispositions and the mixed reactions to therapy. As a result, nucleic acid-based biosensors are crucial for integrating quantitative strategies to detect the presence of widespread common mutations [27,28]. The immobilized molecule offers sensitivity and specificity to the microfluidic-based biosensor system. Since then, the literature has reported on several biosensors used in medical diagnostics. 75% of the commercially available biosensor devices are used in this field. Typically, thermometric, optical, electrochemical, magnetic, and piezoelectric transducers are used with biosensors. In recent years, nucleic acid-based identification has started to be employed in clinical diagnostics for real-world applications, such as detecting actual mutations, genetic polymorphisms, and the presence of pathogenic strains that cause infections [30,31].

Detection of nucleic acids has become significantly essential in modern diagnostics, enabling the detection of infectious pathogens and target disease-specific biomarkers [32]. Microfluidics-based systems have become crucial in providing sensitivity, accuracy, rapidity, and portability for target detection [33]. They have been successfully identifying a varied array of pathogens [34]. PCR is a gold standard technique in diagnosing bacterial and viral infections, and genetic disorders [35]. The main focus of PCR is to amplify DNA in the sample through repeated cycles of a standard curve using thermostable Taq polymerase [36]. From conventional PCR to digital PCR, there has been a significant evolution in diagnostic methods [37]. Real-time PCR enables detection and amplification in real-time [38]. The detection signal of the integrated device has evolved to digital readouts. PCR offers several advantages in better quantification, higher precision, and improved reproducibility [37]. Traditionally, centralized laboratories with bulky, expensive equipment and trained personnel are required [39]. To address this, isothermal amplification methods have gained interest as quicker and easier options that can amplify DNA at a steady temperature using strand-displacing DNA polymerase [40]. RPA is a sensitive method that works at 37–42 °C in less time, increasing its application for decentralized point-of-care testing [41]. Microfluidic devices enable rapid analysis and easy transport, rendering immediate point-of-care applications [42].

This review focuses on the advancements of microfluidic-based biosensors to enable real-time, portable devices in nucleic acid detection. The discussion highlights innovations in fabrication techniques, system integration, automation, and miniaturized POCT platforms. These devices are particularly suited for detecting and diagnosing genital infections and microbial pathogens, offering rapid, sensitive, and accessible diagnostic solutions.

2. Methods for nucleic acid amplification and detection

Generally, two unique methods are used to detect nucleic acid in a microscale environment: PCR and isothermal detection.

2.1. Microfluidic PCR devices

PCR has become a unique method for sensitive nucleic acid amplification since its invention over 40 years ago by Kary Mullis in 1983 [43]. PCR is mainly used to amplify DNA for analysis and diagnosis, and further deals with the study of genes to detect bacteria, diseases, and pathogens. There are typically three temperature zones in a PCR cycle. The double-stranded DNA (dsDNA) is denatured (94 °C) and split into two distinct single strands. After that, the temperature is lowered for the annealing stage (56 °C), wherein single-stranded DNA (ssDNA) templates anneal using short primers complementary to the target genes. Additionally, primers bind to the single-stranded template, and the third region, with the extension stage, was heated to 72 °C. As the DNA polymerase extends the primers, the sample's nucleotides are used to synthesize a new complementary nucleic acid strand [44,45]. Fig. 3 shows the PCR process with different thermal zones required for the amplification of the nucleic acid: denaturation (94–95 °C), annealing (55–60 °C), and extension (72 °C), representing (A) the double helix structure of DNA. (B) PCR process for nucleic acid detection and (C) Temperature gradients in PCR.

Since then, much research has been devoted to microfluidics-based PCR. Continuous-flow microfluidic PCR systems are currently being developed because they may have several advantages over steady-flow approaches and have magnificent potential for device miniaturization, integration, and contraction [46]. Moreover, single-molecule and single-cell investigations can be performed using continuous-flow-based microfluidics devices. Despite this, just a few critical issues must be resolved to enable the creation of fully automated, tractable, and integrated continuous-flow microfluidic systems. Furthermore, PCR can be executed using two fundamental approaches: continuous flow-based and chamber-based scenarios [47,48]. Fig. 4 illustrates the classification of microfluidic-based PCR devices: chamber-based and continuous-flow (CF) based approaches.

2.1.1. Chamber-based devices

The reaction sample and all PCR reagents are added to the reservoir in the device. Then, using pre-established thermal loops connected to temperature zones, the entire chamber is heated from the bottom of the chamber and actively cooled with a fan [49]. The temperature varied over time while the sample stayed stationary. The temperature of the sample depends on time; hence, this method is a time-domain process. The temperature distribution is independent of the microchannel geometry.

Additionally, heating and cooling processes can be modified to accommodate any changes to PCR thermal loops. Other names for chamber-based PCR include microchamber PCR, static PCR chips, and time-domain PCR. The four types of chamber-based PCR chips include single-chamber, multi-chamber array, droplet Micro-PCR (μPCR) chip, and isothermal amplification process. They are the miniature version of a regular thermocycler [50]. Fig. 5 illustrates **(A)** the single-chamber-based approach and **(B)** the multi-chamber-based approach.

In 1993, Northrup et al. [51], developed a single-chamber PCR microdevice with a simple yet effective design, utilizing silicon and borosilicate glass as substrate materials. The PCR chamber was formed by anisotropic silicon wafer etching, and the chamber was sealed by anodic bonding to a borosilicate glass slide. This well-based PCR device amplified a 50 μL β-actin gene sample. It marked one of the earliest

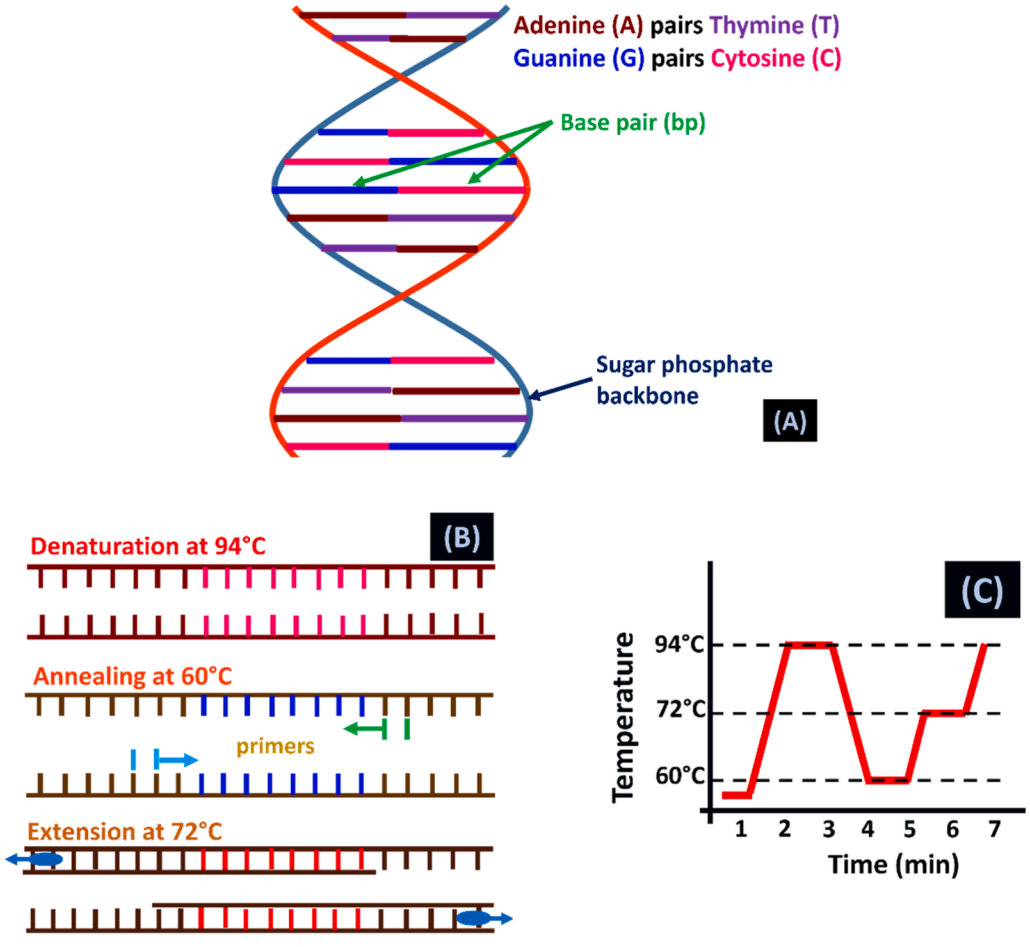


Fig. 3. **(A)** Representation of the double helix structure of DNA. **(B)** An illustration of the PCR process for nucleic acid detection. **(C)** Temperature gradients in PCR.

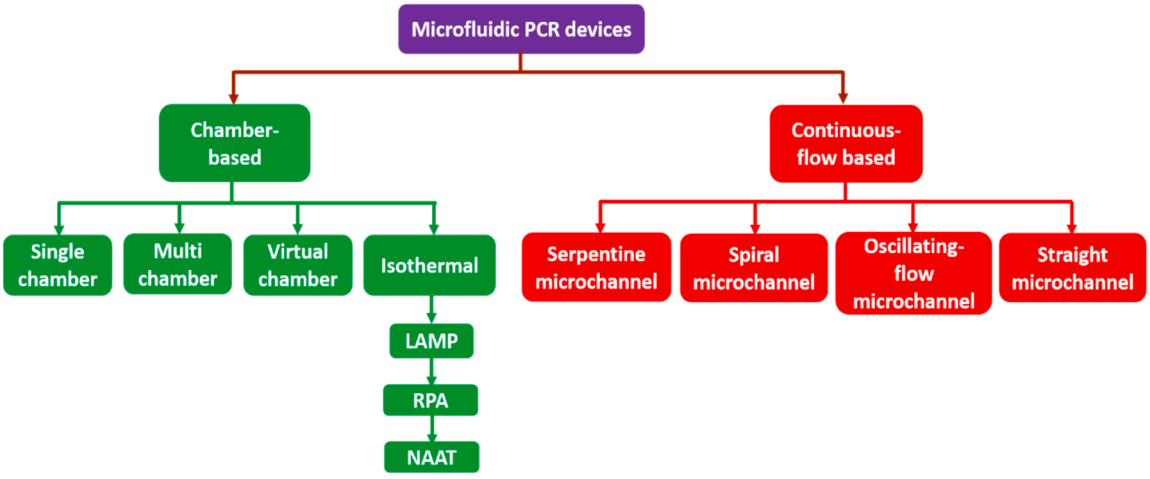


Fig. 4. Classification of microfluidic-based PCR devices.

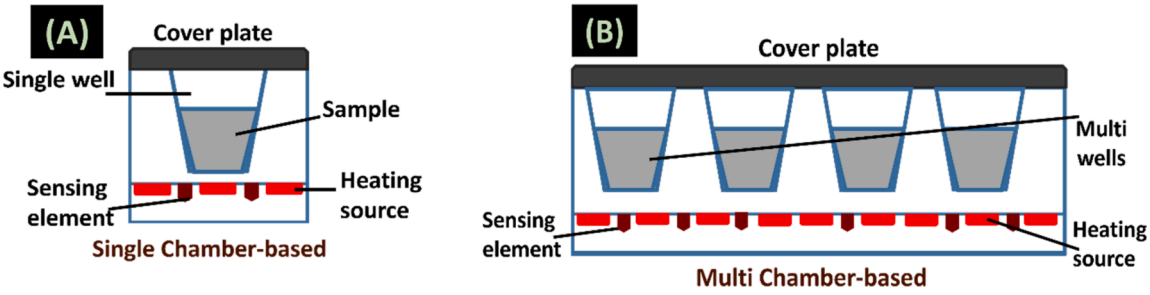


Fig. 5. A schematic representation of **(A)** single-chamber-based and **(B)** multi-chamber-based approaches.

efforts to miniaturize PCR technology, offering a compact platform with a reduced reaction volume and a streamlined experimental configuration. This innovation laid the groundwork for the development of chamber-based microfluidic PCR systems. However, expensive tools and processes that call for specialized microfabrication facilities were needed for the chip's fabrication and the bonding of the PCR [52].

2.1.2. *Continuous-flow-based devices*

The sample is continually moved through the microchannel in continuous-flow PCR equipment, also known as space domain or flow-through PCR devices, mounted on top of pertinent heating blocks. Here, the corresponding heating block maintains a static temperature during the reaction time [53]. Denaturation (94–95 °C), also called the melting stage, where high temperature is applied, and the dsDNA is split into a single strand, is one of three stages that the PCR process goes through to amplify the DNA samples. Small pieces of template DNA known as primers are added during annealing (60 °C), which increases the primers' ability to synthesize single-stranded DNA (ssDNA). The extension of a whole strand into two separate DNA strands occurs during the extension (72 °C) stage. This cycle must be repeated multiple times to amplify the DNA, with the amount doubling each round. Since then, research has been intensely concentrated on microfluidics-based PCR. Since they may have various advantages, such as device automation, integration, and miniaturization [54,55]. Fig. 6 shows the classification of continuous-flow-based microfluidic PCR devices.

2.1.2.1. Serpentine microchannel-based PCR devices. The reagent is passed via a short, serpentine microchannel with distinct heat zones, and the length needed for nucleic acid quantification in continuous-flow PCR systems. In this case, the microchannel's location determined the thermal condition. Thus, the term space domain describes this kind of technology. The flow-through PCR or dynamic PCR systems are other names for continuous-flow PCR equipment [56]. Research applications last a long time thanks to the adaptability of microfluidic device design, ease of production, biocompatibility, and integration potential with other methods. In research requiring unique microfluidic design, microchannel-based modules play a crucial role. Research applications last a long time thanks to the adaptability of microfluidic device design, ease of production, biocompatibility, and integration potential with other methods. The DNA gyrase gene of Neisseria gonorrhoeae was studied using the serpentine microfluidic PCR apparatus by Kopp et al.

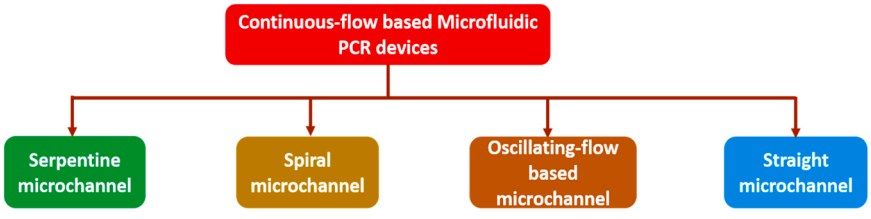


Fig. 6. Classification of continuous-flow-based microfluidic PCR devices.

[57], in 1995, using 176 bp fragments. This was accurately measured using a 10 μl reaction mixture in a micro-machined chemical process, driven under hydrostatic pressure, through a CF approach, and traveling through three different temperature zones. The device's performance depended on the sample moving through the constraints for 20 cycles. With an amplification factor, the time ratio for the three zones' melting, annealing, and lengthening was 4:9 for each. Gel electrophoresis was used for the validation process. The manufactured microchip allowed for many chip reuses and the potential for high-throughput multiplexed PCR. A heating platform consisting of three copper blocks kept at 94 °C, 72 °C, and 62 °C was used to position the microchip. The reaction mixture was forced through a serpentine microchannel etched on a glass slide, which caused the reaction mixture to be heated or cooled via predefined temperature zones [58,59].

2.1.2.2. Spiral microchannel-based PCR devices. Here, apparatuses can be classified as flow-driven or space-domain techniques. As a result, the reaction composition will proceed through the spiral microchannel, with the intake and exit passing through temperature zones that are high, low, and in the center, respectively. The spiral technique effectively and efficiently rearranges the temperature zones into a sectoral style [60]. The Spiral PCR platform offers an effective solution to challenges in maintaining three distinct temperature zones and optimizing channel geometry. In this design, the sample transitions directly from the denaturation zone to the annealing zone, thereby minimizing the risk of DNA renaturation, which can negatively affect amplification efficiency. Two main configurations of spiral microchannel PCR systems have been reported: the cylindrical and the planar spiral designs [61].

2.1.2.3. Oscillating flow-based PCR devices. Recent advancements in microfluidic technology have led to the development of innovative oscillating-flow-based PCR machines. The microchannel length does not affect the mathematical value of thermal cycles in oscillating flow PCR machines. Thus, the cycles in an oscillating flow are not constrained by the channel size [62]. These PCR systems typically consist of a heater and sensor unit, a pressure-driven system, and a microchip [63].

2.1.2.4. Straight channel-based PCR devices. Herein, the straight (single line/ electrode) channel can be designed and manufactured using a straightforward process. A linear channel also has a variety of uses in a microfluidic platform. Straight microchannels with an *IN* and *OUT* are typical [64]. The T, π, and Y channels and cross-junction-based microfluidic devices are a few of the straight channels employed in the microfluidic environment [65,66]. Fig. 7 illustrates **(A)** the serpentine-based approach, **(B)** the spiral-based approach, and **(C)** the oscillating flow-based approach for microfluidic PCR devices.

Table 1 summarize the differences between chamber-based PCR and continuous-flow-based PCR approaches.

2.2. Digital PCR (dPCR)

Digital PCR (dPCR) is a cutting-edge nucleic acid amplification assay allowing highly sensitive and absolute quantification of DNA or RNA due to partitioning of a reaction mixture into hundreds to millions of discrete microreactors. Different from conventional PCR and qPCR, where amplification takes place in one bulk reaction, dPCR divides nucleic acid target molecules into many partitions, ideally consisting of no, one, or a few target copies. After completion of the PCR amplification process at the endpoint, individual partitions are then sorted into positive and negative classes according to the fluorescence intensity, and the initial target concentration is computed using Poisson statistics without an external calibration curve. Microfluidics has greatly enhanced dPCR performance through precise high-density partitioning, low reaction volume, efficient heat transfer, low reagent usage, and throughput analysis. These features offer dPCR with remarkable sensitivity and accuracy for the detection of low-abundance nucleic acids, rare mutations, copy-number variations, pathogens, and cancer mutations. Moreover, dPCR shows higher resistance to some PCR inhibitors and can be highly beneficial when subtle differences in the target concentration should be quantified precisely. Despite this, difficulties such as non-uniform partitioning, sophisticated fluid manipulation, the need for thermal cycling, high instrument cost, sample preparation, and integrated fluorescence detection limit its use in decentralized and point-of-care diagnostics [26,52].

2.3. Droplet digital PCR (ddPCR)

It is known that droplet digital PCR (ddPCR) is a very sensitive technology of nucleic acid amplification that provides for absolute

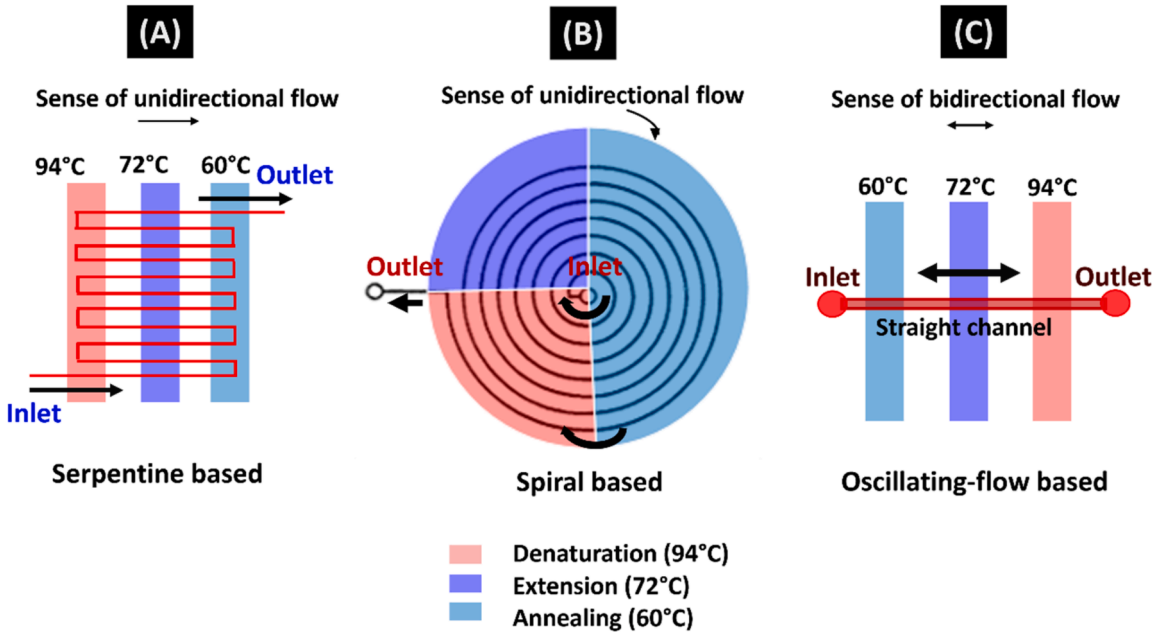


Fig. 7. Schematic representation of **(A)** Serpentine, **(B)** Spiral, and **(C)** Oscillating-flow-based microfluidic PCR devices.

Table 1
A comparison of chamber-based PCR and continuous-flow PCR approaches.

Parameter	Chamber-Based PCR	Continuous-Flow PCR	Advantages	Disadvantages	Applications	Ref.
Reaction Mode	Stationary sample with temporal thermal cycling	Moving sample with spatial thermal cycling	Chamber-based: simple operation; Continuous-flow: rapid cycling	Chamber-based: relatively slower; Continuous-flow requires precise fluid control	Micro-PCR and cartridge-based diagnostics	[67]
Sample Handling and Loss	Greater possibility of evaporation, particularly at elevated temperatures	Generally lower sample loss when channels are properly sealed	Continuous-flow systems can minimize evaporation and sample loss	Chamber-based systems may experience evaporation; continuous-flow systems may encounter leakage or unstable flow	Small-volume nucleic acid amplification	[68]
Thermal Control	Sample is repeatedly heated and cooled within a fixed chamber	Sample passes through predetermined spatial temperature zones	Chamber-based: straightforward thermal control; Continuous-flow: rapid thermal transitions	Chamber-based: possible thermal non-uniformity; Continuous-flow: requires accurate temperature-zone control	Rapid and ultrafast PCR	[69]
Contamination Risk	Relatively low in completely sealed chambers	Potential contamination at fluidic interfaces or channel boundaries	Closed chambers provide good contamination control	Continuous-flow configurations require careful channel isolation	Closed POCT cartridges and continuous PCR platforms	[67]
Heating Configuration	Generally requires fewer heating/ sensing elements	Requires multiple spatially separated temperature zones	Chamber-based systems have simpler heater architecture; continuous-flow systems avoid repeated heating and cooling of the entire device	Multiple heating zones increase fabrication and control complexity	Automated microfluidic PCR systems	[67, 68]
Multiplexing Capability	Relatively difficult, particularly within a single reaction chamber	More readily achieved through parallel channels or multiple reaction pathways	Continuous-flow systems offer better parallelization and throughput	Complex primer interactions, fluid routing, and cross-reactivity may affect multiplex performance	Multiplex pathogen detection and genetic analysis	[69]
Processing Mode	Primarily sequential or batch processing	Continuous or parallel processing	Chamber-based: simple batch operation; Continuous-flow: high-throughput capability	Sequential processing limits throughput; continuous processing requires accurate flow control	High-throughput molecular diagnostics	[68]
System Architecture	Time-domain thermal cycling	Space-domain thermal cycling	Chamber-based architecture resembles conventional PCR and facilitates miniaturization; continuous-flow architecture enables rapid amplification	Chamber systems may exhibit greater thermal inertia; continuous-flow architectures are more complex	Portable PCR and rapid-flow PCR	[67, 68]
Device Complexity	Relatively simple chamber, heater, and sensing configuration	Requires microchannels, multiple temperature zones, and controlled fluid transport	Chamber-based systems are comparatively simple to fabricate and operate	Continuous-flow systems require precise channel geometry and flow-rate control	Lab-on-Chip and integrated molecular diagnostic devices	[68, 69]
Throughput	Generally low to moderate	Generally moderate to high	Continuous-flow architecture facilitates parallel and continuous sample processing	Increasing throughput increases fluidic and instrumentation complexity	Pathogen screening and high-throughput molecular testing	[69]

quantification of DNA/RNA by dividing a reaction mixture into thousands of water-in-oil droplets. Every droplet acts as a separate PCR microreactor containing zero, one, or a few copies of the nucleic acid being targeted. After PCR amplification, every droplet is analyzed based on its fluorescence intensity and classified as being either positive or negative. Further, initial target concentration is calculated via the use of Poisson statistical analysis without requiring any calibration curve. There are several roles played by microfluidic technologies in droplet digital PCR, such as fast and uniform droplet generation, accurate sample partitioning, low consumption of reagents, effective thermal exchange and high-throughput analysis. This method demonstrates excellent sensitivity, accuracy and repeatability in nucleic acid quantification, especially in the case of low-concentration nucleic acids, rare genetic mutations, copy number variations, pathogens, viral loads and cancer biomarkers. Moreover, ddPCR allows reducing competition between the molecules of the target and improving the detection of rare sequences through the use of a compartmentalized reaction format. At the same time, some problems related to droplet generation and stability, complicated equipment, PCR amplification, fluorescence-based droplet detection, sample preparation, complexity of operation, and relatively high price prevent ddPCR from being integrated into portable diagnostic systems [16,29].

2.3.1. CRISPR–Cas detection (Cas12a/Cas13a)

CRISPR-Cas diagnostics have been developed as extremely programmable nucleic acid detection methods that employ both specific target recognition and collateral activity of nucleases to obtain a signal. As the two most studied diagnostic systems, Cas12a targets mainly DNA and Cas13a targets RNA, providing them with the opportunity to detect numerous infectious diseases, genetic disorders, and any other markers containing nucleic acid. In Cas12a detection system, activation of Cas12a enzyme occurs through recognition of the complementary target DNA by the CRISPR RNA (crRNA). After that, trans-cleavage activity of Cas12a toward single-stranded DNA reporters is observed. Recognition of the target RNA leads to similar processes for Cas13a system. Cleavage of the reporter creates fluorescent, colorimetric, electrochemical, and/or lateral flow signals, which makes it possible to detect the target efficiently. Using microfluidics can reduce the quantity of sample and reagents used and provide automatic processing, compartmentalization, multiplexing, and portability. CRISPR-Cas detection is usually used in combination with isothermal amplification techniques like RPA and LAMP to enhance analytical sensitivity. However, there are still some problems to solve, including nucleic acid extraction, dependence on amplification, off-target effects, reagent stability, contamination control, multiplexing difficulties, and lack of clinical sample validation [45, 62].

2.4. Isothermal amplification-based microfluidic PCR devices

Isothermal amplification represents a paradigm shift in molecular

biology, offering an alternative to traditional PCR that replicates DNA or RNA sequences at a stable temperature. Unlike PCR, which requires repeated cycles of heating and cooling for denaturation, annealing, and extension, isothermal methods require only a constant heat source – achievable with a water bath or a resistive heater [40]. Specialized enzymes are utilized here, such as strand displacement polymerases, helicases, and recombinases. These enzymes help in unwinding the double-stranded DNA and allow amplification to occur continuously at a single temperature [70]. Temperature ranges typically from 37–65 °C, depending on the method [71]. These methods are well-suited for resource-limited environments where laboratory infrastructure and trained personnel may not be available. Fig. 8 illustrates the schematic representation of the generalized workflow of isothermal amplification methods.

2.4.1. *Recombinase polymerase amplification (RPA)*

RPA is distinguished from other isothermal methods due to its ability to amplify nucleic acids at the lowest temperature range of 37- 42 °C, within a short time of 5 to 20 min [72]. The RPA mechanism mirrors the recombination process; recombinase proteins form nucleoprotein filaments with the amplification primers. Once the primer–recombinase complex identifies its target, the displaced strand is stabilized by single-stranded DNA-binding proteins (SSBs) and a strand-displacing polymerase to synthesize new DNA [73]. The newly synthesized strand displaces the downstream sequence, which allows for further primer binding and establishes an amplification loop [73]. The operating temperature of RPA is profound for practical point-of-care diagnostics; the reaction can theoretically proceed using body heat, chemical heat sources, or a water bath[40]. RPA reagents offer excellent room temperature stability, ideal for point of use [74]. A distinctive kinetic feature of RPA is its characteristic sigmoidal amplification curve shape, which differs from PCR's stepwise cycle-dependent curve. RPA exhibits a variable lag phase during which recombinase filament assembly and initial strand invasions establish the reaction, followed by a rapid exponential phase of 5–10 min, and then a plateau [75]. For detection, RPA is routinely coupled to fluorescent exo probes, analogous to TaqMan probes, which are used [76]. Limitations are also associated with higher reagent cost compared to LAMP due to the complexity and cost of recombinase and SSB protein production. Variable lag phase introduces reproducibility challenges [77]. RPA has been successfully adapted for the detection of HIV, hepatitis B and C viruses, Ebola, Leishmania, Cryptosporidium, MRSA, Salmonella, and SARS-CoV-2 [72, 77].

2.4.2. *Loop-mediated isothermal amplification (LAMP)*

LAMP is the most widely used isothermal amplification method in research and clinical diagnostics. It was introduced in 2000 by Notomi, which employs strand-displacing DNA polymerase – Bacillus stearothermophilus (Bst) polymerase [78]. A set of four to six designed primers, which recognise six to eight distinct regions on the target sequence, are used. These primers include two outer primers (F3 and B3), two inner primers (FIP and BIP), and optionally two loop primers (LF and LB) [79]. A loop and stem structure called a dumbbell complex is generated when the reaction proceeds at 60–65 °C. These loops serve as self-priming templates, leading to an amplified product in 15 to 60 min [78].

A significant advantage of LAMP is its tolerance to biological inhibitors commonly found in clinical samples, which would suppress PCR [80]. Detection of LAMP products is highly versatile, as magnesium pyrophosphate is massively accumulated, which causes turbidity. Colourimetric dyes produce a naked-eye colour change upon amplification, such as hydroxynaphthol blue (HNB) or phenol red. Intercalating fluorescent dyes like SYBR Green, which enables real – time fluorescence monitoring [61,81]. LAMP has been validated for the detection of Mycobacterium tuberculosis, malaria (Plasmodium species), dengue, Zika, HPV, influenza, SARS-CoV-2, and numerous bacterial and parasitic pathogens [82,83]. Single and multi-chamber-based μPCR machines have some disadvantages, including a higher likelihood of sample evaporation, the production of bubbles, increased heat dissipation, and substantial thermal losses. Continuous-flow-based microfluidic PCR devices were created to address these issues. The procedure calls for the reaction mixture to be moved along the entire microchannel length in continuous-flow PCR machines. This method uses a microfluidic platform to easily incorporate sample preparation, amplification, and extraction into a response sample, resulting in quicker heat transfer. The microfluidic architecture predetermines the number of thermal cycles and duration for various thermal phases of the thermocycler [84].

Table 2 summarizes the difference between isothermal and PCR techniques used for nucleic acid detection.

2.5. *Sample preparation: lysis and nucleic acid extraction*

Sample preparation is an important stage of microfluidic nucleic acid diagnostics as the effectiveness of cell lysis and nucleic acid isolation

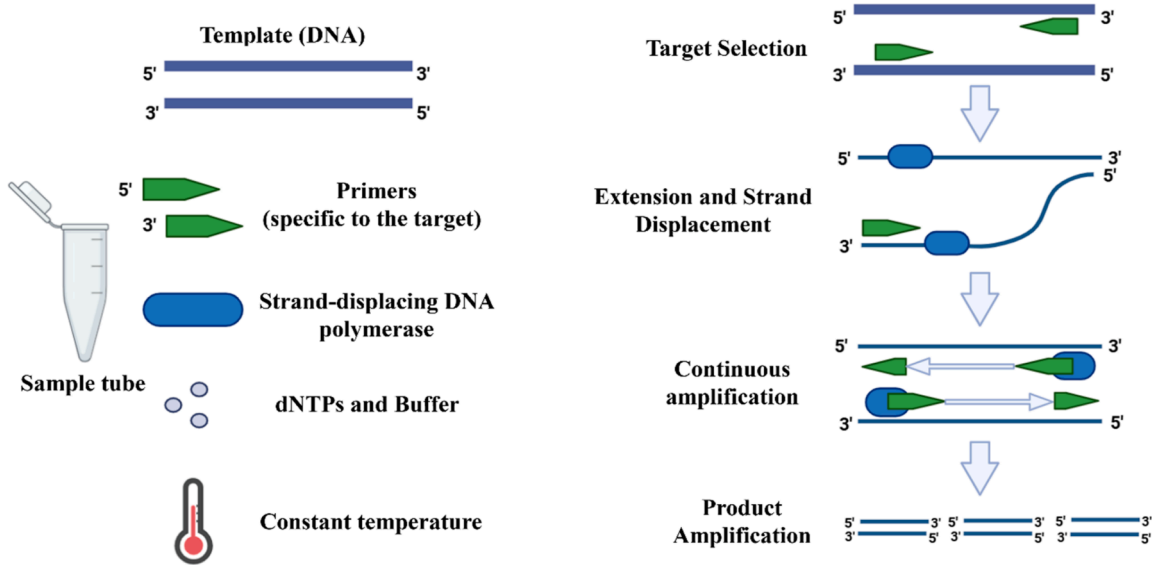


Fig. 8. Schematic representation of the generalized workflow of isothermal amplification methods.

Table 2
Comparison of Isothermal and PCR techniques used for nucleic acid detection.

Parameter	Isothermal	PCR	Advantages	Disadvantages	Applications	Ref.
Thermal Operation	Amplification occurs at a constant temperature	Requires repeated denaturation, annealing, and extension cycles	Isothermal methods simplify thermal control and reduce instrumentation requirements	PCR requires programmable thermal cycling; isothermal methods may show nonspecific amplification depending on chemistry	Rapid POCT and laboratory molecular testing	[85]
Operating Temperature	Typically, 37–65 °C, depending on the amplification method	Denaturation is typically near 95 °C, with lower temperatures for annealing and extension	Isothermal amplification can operate using simple heating systems	PCR requires precise control of multiple temperatures	Portable diagnostics and thermocycler-based molecular testing	[86]
Primer Design	Method-dependent; for example, LAMP commonly uses 4–6 primers	Conventional PCR generally uses two primers	Multiple primers can enhance target recognition and amplification efficiency	Complex primer design can increase primer–primer interactions and nonspecific amplification	Pathogen detection, genotyping, nucleic acid analysis	[87]
Readout Method	Compatible with colorimetric, fluorescence, electrochemical, turbidity, and lateral-flow detection	Commonly fluorescence or endpoint detection; other formats are also possible	Isothermal amplification enables simple visual and portable readout approaches	Some visual readouts may be less quantitative or more subjective	POCT, field diagnostics, laboratory analysis	[88]
Hardware	Usually requires a simple constant-temperature heater	Requires a programmable thermocycler	Lower instrumentation complexity supports miniaturization and portability	PCR instrumentation is comparatively more complex and power-intensive	Decentralized testing and centralized molecular diagnostics	[89]
Cost and Ease of Operation	Generally lower thermal-control complexity and potentially lower equipment cost	Relatively higher equipment and thermal-control requirements	Isothermal methods are attractive for resource-limited settings	Reagent and primer complexity can increase assay-development requirements	Resource-limited diagnostics and routine laboratory testing	[86]
Microfluidic Compatibility	Excellent; constant-temperature operation simplifies device integration	Excellent but requires accurate temporal or spatial thermal cycling	Both approaches benefit from reduced reaction volumes, rapid heat transfer, and automation	PCR requires more complex thermal architecture; isothermal systems may face amplification-control challenges	Lab-on-Chip and sample-to-answer diagnostics	[85, 88]
Multiplexing Capability	Possible, but primer interactions and nonspecific amplification may be challenging	Well established with appropriate primer/probe optimization	Enables simultaneous detection of multiple targets	Increasing multiplexing complexity can reduce specificity and increase assay optimization requirements	Multi-pathogen detection and genetic panels	[89, 88]

affects amplification sensitivity, specificity, and reproducibility. The process of lysis breaks cellular or viral structures and liberates DNA or RNA and can be performed chemically, enzymatically, thermally, mechanically, or electrically. While chemical and enzymatic lysis are comparatively easy to integrate into microfluidic systems, thermal and mechanical processes can enable fast lysis with minimal reagent usage. After lysis, nucleic acids must be separated from proteins, lipids, cellular waste, inhibitors of amplification, and other matrix elements. Silica membranes, functionalized magnetic beads, solid phase extraction, filtration, or electrokinetic approaches are the most common approaches used for extraction of nucleic acids in microfluidics. Magnetic-bead isolation is especially appealing for integration in microfluidic devices because magnetic beads can be manipulated inside the microchannels without the use of complicated mechanical elements. Integration of both lysis and extraction processes enables minimizing manual steps, reducing risks of contamination and loss of samples, and shortening the process of analysis. In addition, automated sample-to-answer analysis becomes possible. At the same time, variation in sample matrices, incomplete lysis, degradation of nucleic acids, inhibitor carryover, poor efficiency of isolation, and other fluidic manipulation problems should be taken into account. Therefore, development of effective microfluidic sample preparation is key to practical applications of nucleic acid amplification platforms [34,36,41].

2.6. Fabrication techniques

The technique used in the fabrication process has a significant impact on the cost, scalability, fluid handling, optical capabilities, and integrative capabilities of the microfluidic nucleic acid diagnostic systems. PDMS-based soft lithography is one of the widely used techniques for prototyping due to its optical clarity, biocompatibility, gas permeability, and precision replication of microscale channels. On the other hand, hydrophobicity, molecular adsorption, evaporation, and reliance on a cleanroom-based master fabrication process are some of the limiting factors that can hinder large-scale commercialization. 3D printing has emerged as one of the most promising methods in the fabrication of microfluidic devices since it enables rapid prototyping of microchannels, reaction chambers, valves, connectors, and sample handling structures without any lithographic process. Stereolithography and digital light processing could enable rapid changes in the design and customization of the device. It is worth noting that printing resolution, resin biocompatibility, surface roughness, optical clarity, and materials that are compatible with amplification reactions are very crucial. Paper-based microfluidic analytical devices (μPADs) form a very cheap and portable platform in which capillary action facilitates passive fluid transport without any pump. Hydrophilic channels and hydrophobic barriers facilitate sample handling, amplification, and visualization in a single disposable device. Evaporation, lack of quantification capabilities, storage of reagents, and nonspecific adsorption are some of the limitations. Fig. 9 illustrates a schematic of various microfluidic fabrication techniques highlighting the major processing steps from pattern design to final device. Our research group has already reported a detailed literature survey on various fabrication techniques used in the development of microfluidic devices for several diverse applications [12,47].

3. Recent advances in microfluidics-based nucleic acid detection

Kulkarni et al. [90], demonstrated an automated and fully integrated IoT-driven portable microfluidic CF-based PCR system, proficient with a photometric-based on-chip sensing platform. As proof of concept, the 594-bp housekeeping GAPDH gene was effectively quantified and detected on a solo platform (Fig. 10(A)). The disclosed mini-thermal platform has the potential to be used as a versatile microfluidic device for the identification of several infectious and non-infectious DNA

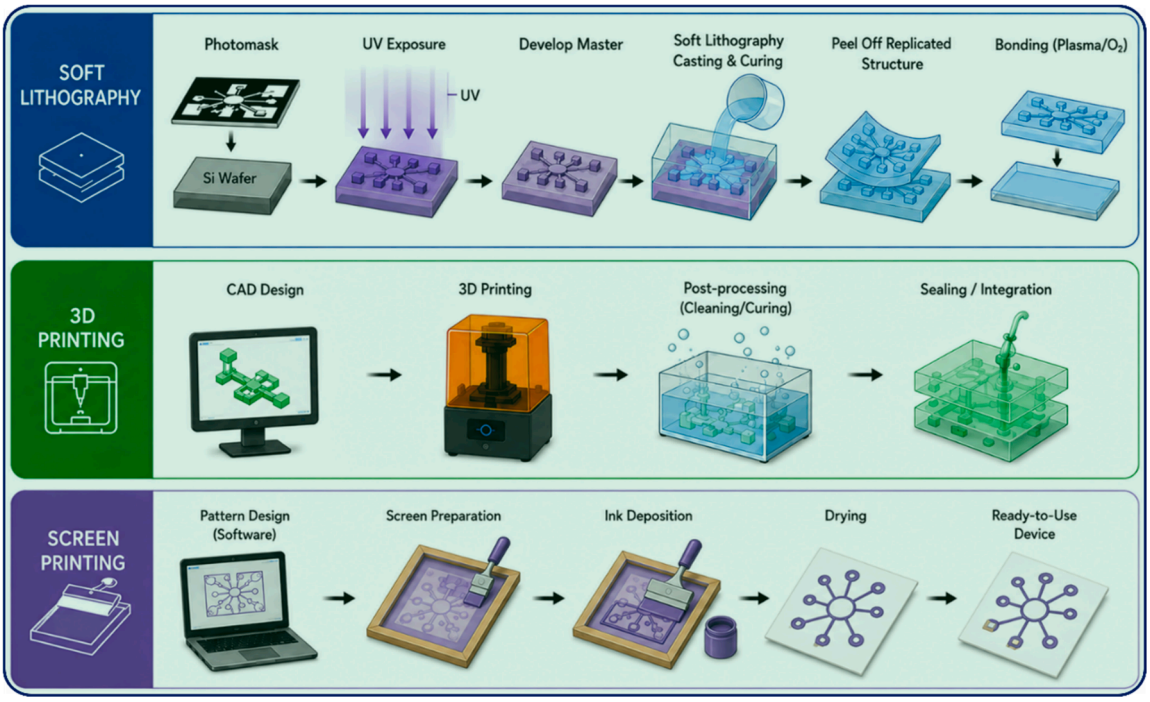


Fig. 9. Schematic representative of microfluidic fabrication techniques highlighting the major processing steps from pattern design to final device.

diseases.

Kulkarni et al. [91], proposed a microfluidic PCR device wherein a microfluidic device was made up on an acrylic substrate with 30 serpentine loops; the PCR microfluidic device also had an initial denaturation and final extension sections to improve the total quantification. Consequently, the assimilated results were numerically compared with the traditional PCR benchtop instrument (Fig. 10(B)). Furthermore, it was authenticated with an electrochemical workstation to detect nucleic acid using differential pulse voltammetry (DPV).

Zhiying et al. [92], described a novel Fd-MC-incorporated nucleic acid extraction using an RPA technique for quantification and conception formulated for quick tuberculosis detection. The Fd-MC's finger-actuated power system does not require additional pumps or specialized humans (Fig. 10(C)). The Fd-MC was created for multiplexed detection, distinguishing between MTB in human/bovis and TB/non-TB infection.

Hamed Tavakoli et al. [93], proposed a rapid, easy, and inexpensive technique highly desired for meningitis diagnosis. The primary meningitis-causing bacterium was detected in this investigation using a microfluidic Micro-Paper-based Analytical Device (μPAD) incorporated with LAMP and single-strand nucleic acid functionalized graphene oxide nanomaterials to enhance the sensing ability of the nucleic acid (Fig. 10 (D)). They offered flexible features, a qualitative diagnostic assay, assent testing, and quantitative scrutiny.

Xie et al. [94], developed a digital microfluidic platform integrating colorimetric LAMP for low-cost, on-chip diagnostics. This DMF chip features four parallel reaction units, enabling the simultaneous detection of multiple genes and samples. Post-amplification, the results were visually interpreted using endpoint detection with concentrated dry neutral red directly on the chip. The on-chip LAMP reaction time was reduced to just 20 min, and the entire diagnostic process was completed within 45 min (Fig. 11(A)). To validate its performance, the system was applied for the detection of four key pathogens affecting prawns: Enterocytozoon hepatopenaei, contagious hypodermal and hematopoietic necrosis virus (IHHNV), and white spot syndrome virus (WSSV), demonstrating its effectiveness for rapid, multiplexed aquatic pathogen detection.

Rivas-Macho et al. [95], produced a 3D-printed three-electrode electrochemical microfluidic-based biosensor using the LAMP technique to detect Listeria monocytogenes. To prevent the occurrence of this virus in the food supply chain, the food industry must have a rapid, responsive, and accurate detection tool for food quality management (Fig. 11(B)). One of the finest alternatives for quickly and on-site detecting bacteria is a POC biosensor based on the isothermal method and electrochemical detection.

Chenda Wu et al. [96], introduced an integrated microfluidic detection platform based on loop-mediated isothermal amplification for the rapid and sensitive identification of Pyricularia grisea. The proposed system comprises three core components: a microfluidic chip, a temperature control module, and an OpenMV camera-based imaging unit. The microchip incorporates micromixing channels with shear-enhancing structures, achieving a mixing efficiency of approximately 98%. Additionally, flow-blocking valves are integrated to minimize reagent loss during the reaction process (Fig. 11(C)). The temperature module ensures a stable reaction temperature of 65 °C, essential for LAMP-based amplification. An OpenMV camera is used to monitor the reaction chamber within the microfluidic chip, allowing real-time image-based analysis. The system effectively detected P.grisea from 10 to 10^5 copies/μL, with a total assay time of <45 min. Specificity and interference experiments showed the platform's high reliability and selectivity.

Siqi Cui et al. [97], reported a fully automated, paper-based microfluidic system for simultaneous detection of multiple pathogenic bacteria. The system allows users to inject the sample; the entire diagnostic process runs autonomously, without external power sources, and is completed within 60 min or less. The innovative paper-based microfluidic chip is engineered to perform bacterial lysis and incorporate LAMP alongside the CRISPR/Cas12a detection system [98] on a single piece of paper, using minimal reagent volumes (Fig. 11(D)). All reaction reagents are pre-loaded in the form of freeze-dried powders, enabling long-term storage and on-demand activation.

Huan Li et al. [99], demonstrated a rapid and equipment-free method using a paper-based dipstick for nucleic acid extraction, capable of isolating nucleic acids within 2 min. Building on this, a

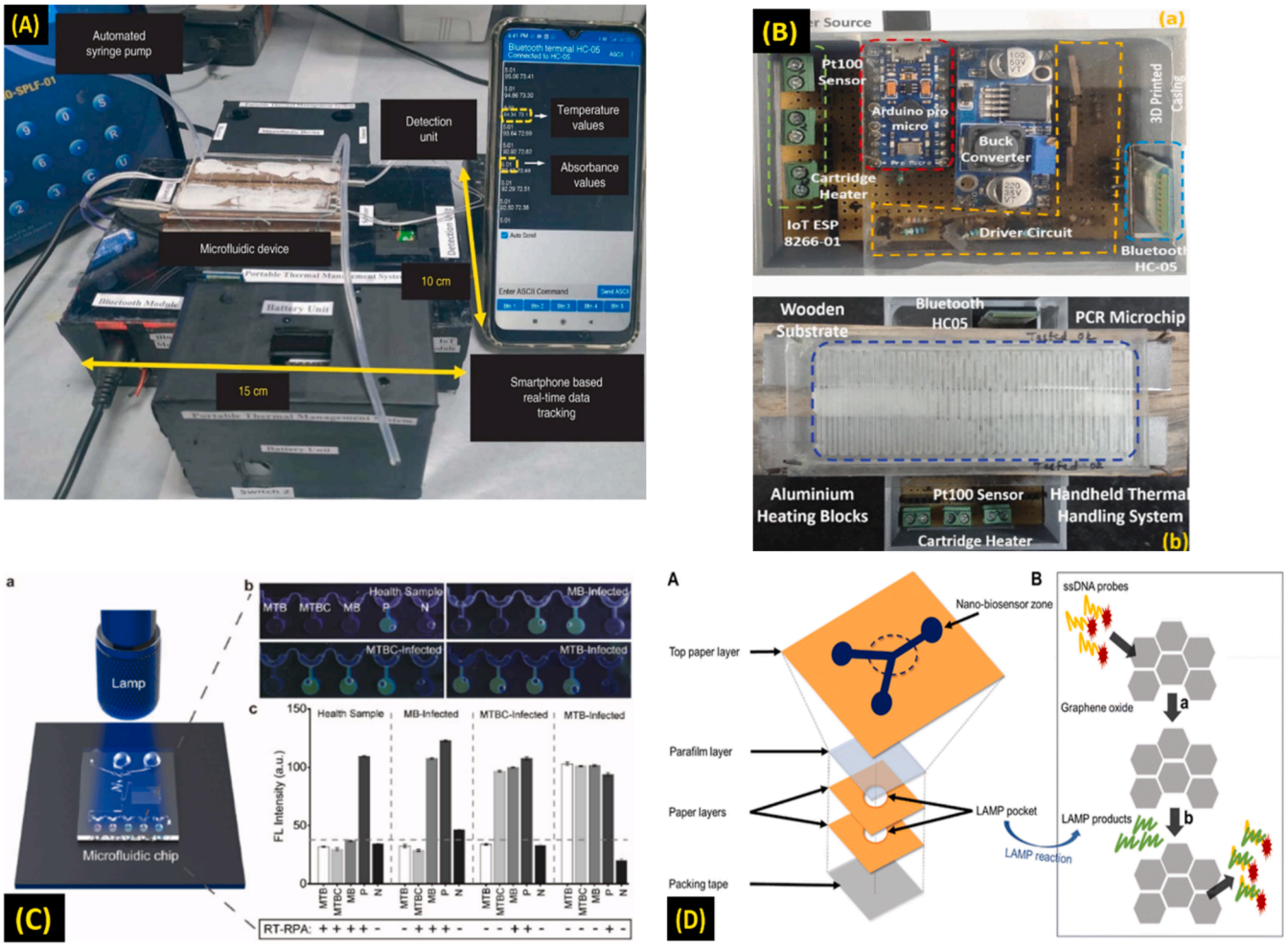


Fig. 10. **(A)** A fully integrated and portable CF-PCR for DNA quantification and detection on a solo platform [90]. **(B)** CF-PCR microdevice placed on the thermal management system [91]. **(C)** An illustration of a microfluidic chip for detection of Mycobacterium bovis [92]. **(D)** A schematic representation of the μPAD incorporated with the LAMP technique to detect Neisseria meningitides [93].
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microfluidic chip integrated with LAMP was developed to establish a portable, field-ready diagnostic system termed the microfluidic-LAMP assay. The platform enables the simultaneous detection of eight high-risk viruses with a total processing time of 70 min. The assay could detect between 7.02×10^2 and 8×10^4 copies/mL in simulated clinical samples. Sensitivity and specificity were observed compared to real-time PCR, in cross-reactivity tests against 21 different pathogens (Fig. 12(A)). These highlight the assay's potential as a low-cost, sensitive, and field-deployable diagnostic tool for viruses in outbreak scenarios.

Ji Lv et al. [100], reported the integration of LAMP with a microfluidic chip platform for simultaneous and rapid detection of four foodborne pathogenic bacteria in eight food matrices. Specifically, they developed and screened target-specific LAMP primers for Vibrio parahaemolyticus, *Escherichia coli* O157:H7, *Yersinia enterocolitica*, and Bacillus cereus. A 10-well microfluidic chip was used to improve detection throughput and avoid sample interference. The system was evaluated for its feasibility and accuracy in multiplex detection and was able to detect all four pathogens within 45 min, with detection from 1.78×10^4 to 1.59×10^5 copies/mL (Fig. 12(B)). The platform was performed in a variety of artificially contaminated food matrices, maintaining specificity and sensitivity. Detection results were visually detected by the naked eye and quantified using fluorescence analysis, demonstrating the potential of the platform's suitability for on-site, high-throughput food safety diagnostics.

Natish Kumar et al. [101], introduced an automated, quantitative, real-time diagnostic sensing platform for point-of-care nucleic acid testing. This compact and portable system integrates a polymer membrane filter-based nucleic acid extraction module, in situ isothermal amplification, and dual-mode optical detection. Precise, real-time detection of target gene sequences is achieved through analysis of the photovoltaic response, employing electrical polarity and photocurrent measurements for signal quantification (Fig. 12(C)). The platform's selective photovoltaic cut-offs allow quantitative discrimination of gene-specific variations without auxiliary equipment. The on-cartridge limit of detection (LoD) was 10 copies/μL, and the system demonstrated high clinical performance in the diagnosis of SARS-CoV-2 samples with 95% sensitivity and 100% specificity against the RT-PCR gold standard. This MILP-based device provides a promising platform for fast, accurate, and field-deployable molecular diagnostics.

Zhang et al. [102], developed a small and integrated Palm-Shaped One-Step System (P-SOS) with the full sample-to-result capacity for the rapid on-site detection of pathogens. The P-SOS platform consists of a microfluidic chip, a constant temperature heating module, a fluorescence detection unit, and genetic analysis software. To facilitate sample preparation, the sample release reagent lyses the pathogens directly off swabs, eliminating the need for nucleic acid extraction (Fig. 12(D)). The lyophilized RT-LAMP reagents are preloaded in the chambers of the chip, which allows storage at room temperature and easy use. The

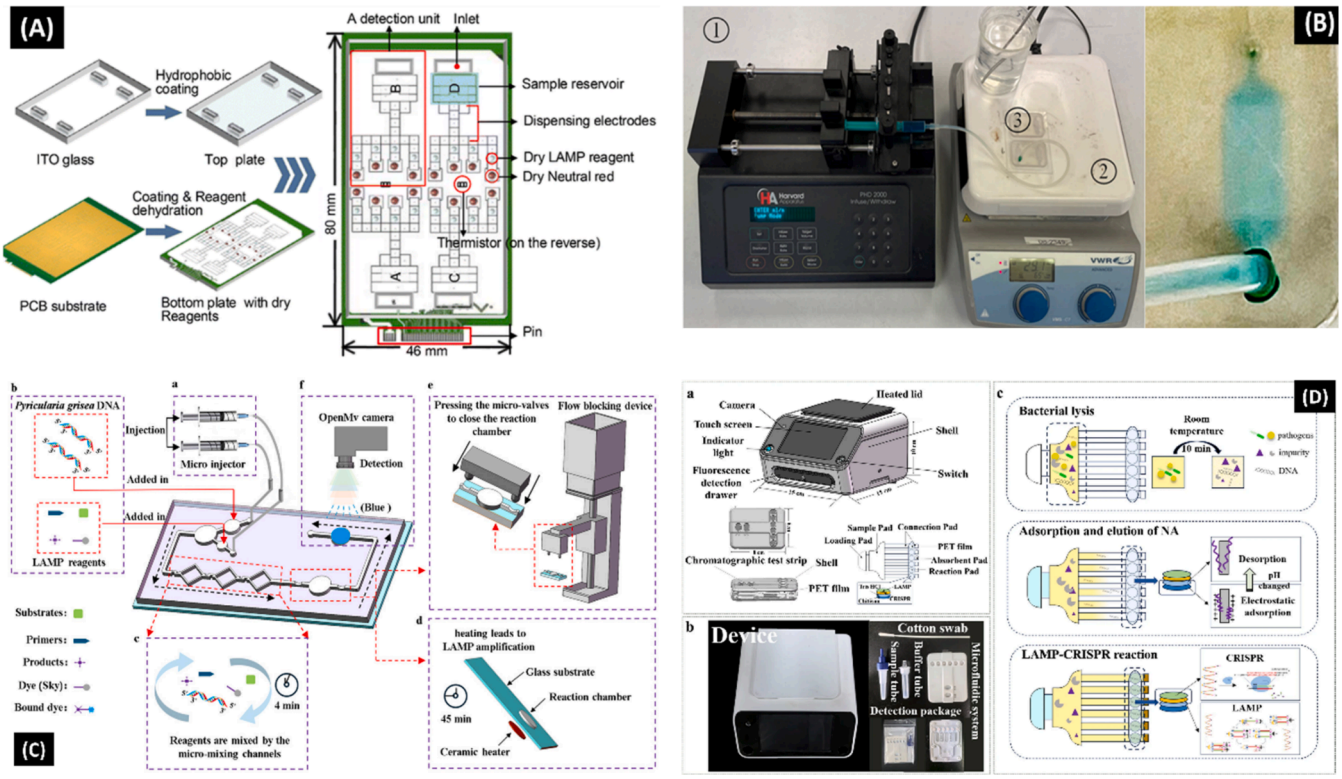


Fig. 11. **(A)** Digital microfluidic chip design and fabrication for detecting infectious hematopoietic necrosis and hypodermal virus using LAMP [94]. **(B)** Experimental setup of a microfluidic device for nucleic acid detection [95]. **(C)** Detection process of the microfluidic detection system for Pyricularia grisea [96]. **(D)** Representation and a photograph of a fully integrated microfluidic platform for on-chip bacterial detection [97]. Reprinted images with permission of copyright.

system has a detection limit of 25 copies/μL for the N gene of SARS-CoV-2 and can detect pseudovirus samples in 50 min. The simplicity, rapid turnaround time, and high sensitivity make the P-SOS platform a promising tool for point-of-care diagnosis of acute respiratory diseases.

Yuanyuan Zhu et al. [103], developed a micro-volume LAMP-Lateral Flow Assay platform (μLAMP-LFA) for on-site detection of aquatic animal pathogens. It is a system that combines a microfluidic chip with loop-mediated isothermal amplification (LAMP) and lateral flow assay (LFA) technologies, and a compact dedicated device called mini-LAMP. The chip is designed for automated fluid transfer: after amplification, a simple rotational mechanism directs the solution into the LFA chamber. Its hermetic construction prevents well aerosol contamination and thus reduces the risk of false-positive results. The chip also has a dedicated compartment for holding the dilution reagents separately to permit micro-volume operations and preserve the integrity of the assay (Fig. 12 (E)). The μLAMP-LFA chip shows good fluid control, efficient mixing, and excellent fluid dynamics. The mini-LAMP heats up quickly and keeps a stable isothermal environment, which is perfect for LAMP reactions. The system could detect white spot syndrome virus (WSSV) and Salmonella at concentrations as low as 28 copies/μL and 15 copies/μL, respectively. Furthermore, the simultaneous detection of two pathogens was validated in real samples of shrimp, indicating the suitability of the method for diagnostic applications in aquaculture under field conditions.

D Gao et al. [104] demonstrated an octopus-like multiplex microfluidic loop-mediated isothermal amplification (mμLAMP) chip for rapid point-of-care genetic testing. The device features a central fluidic inlet that distributes a single sample injection uniformly over ten individual reaction microchannels. The “dimension gradient bridge” is a key design feature of this platform, enabling seamless sample loading while preventing cross-contamination (cross-talk) between chambers with different pre-loaded primer sets. This radial design permits simultaneous detection and differentiation of multiple targets, e.g., different types of Influenza A virus subtypes and inherent positive and negative controls on a single miniaturised glass slide. Positive amplification can be easily observed on-site after isothermal reaction with end-point fluorescence under UV light, which allows for a robust and user-friendly platform for high-throughput, multiplexed diagnostics without sophisticated laboratory instrumentation. Fig. 13 illustrates a microfluidic chip featuring the device layout, fluid distribution network, reaction loading, and fluorescence-based detection of amplification products.

Table 3 Comparative analysis of recently reported microfluidic nucleic-acid detection platforms.

4. Discussion and conclusion

Herein, Fig. 14 states the SWOT analysis on the potential of microfluidic nucleic acid amplification platforms. Its major benefits are miniaturization, portability, reduced reagent volume, and rapid amplification without the need for thermal cycling. LAMP and RPA broaden the possibilities for decentralized testing, allowing detection of nucleic acids of interest with minimal instrumentation.

Microfluidic nucleic acid detection has progressed from miniaturized PCR systems toward increasingly integrated platforms combining sample preparation, nucleic acid extraction, amplification, detection, and digital readout. Chamber-based and continuous-flow PCR, isothermal amplification, digital PCR, and emerging CRISPR-assisted approaches offer important advantages in terms of reduced reagent consumption, shorter reaction times, portability, and potential sample-to-answer operation. Nevertheless, miniaturization alone does not guarantee analytical robustness or point-of-care readiness. Evaporation, bubble formation, thermal nonuniformity, nonspecific amplification, surface adsorption, amplification inhibition, contamination, and variability in

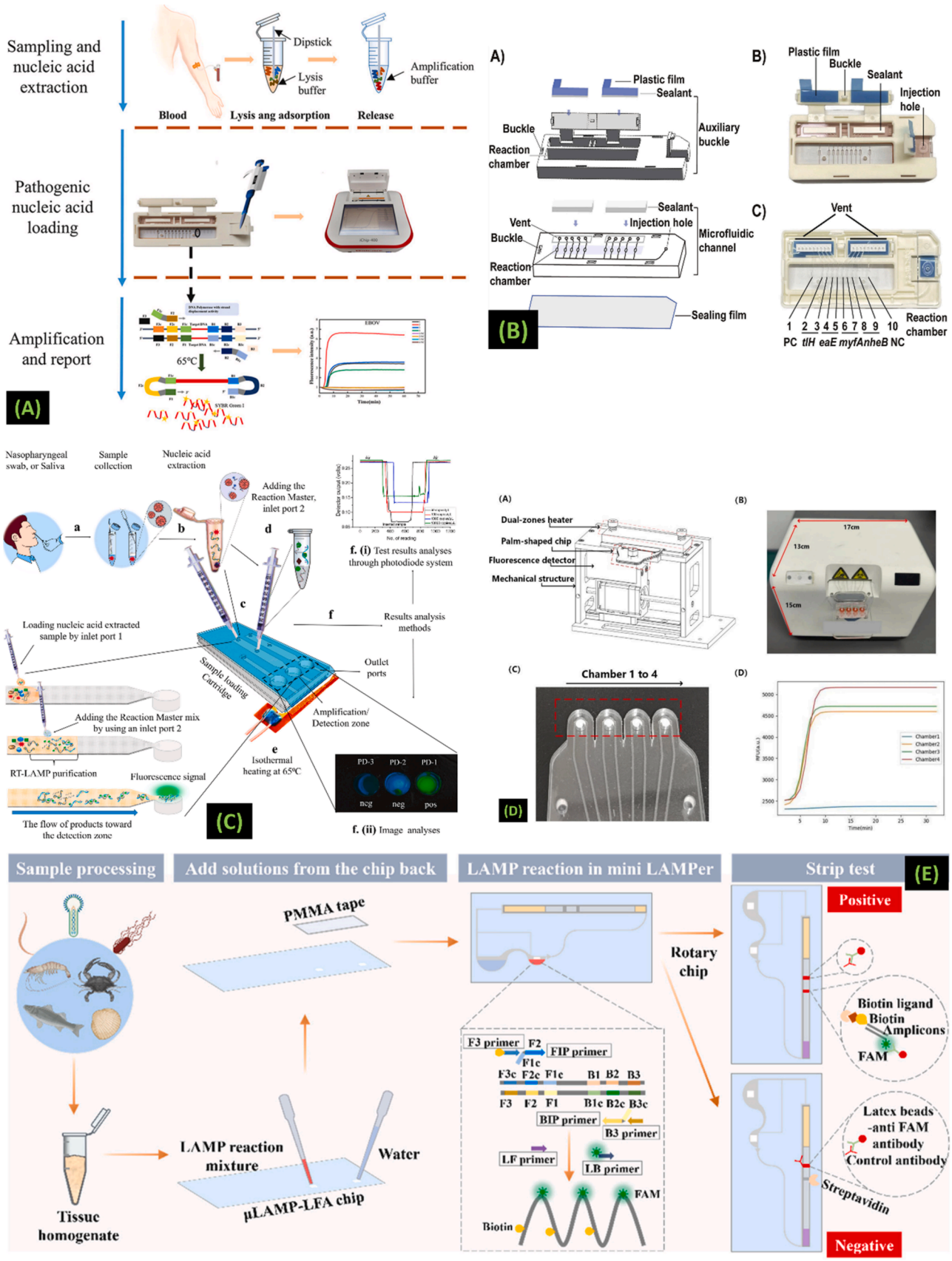


Fig. 12. **(A)** Schematic diagram showing the detection process of multiple pathogens [99]. **(B)** Structure and specificity detection of microfluidic chips [100]. **(C)** Illustrates the design and key steps of a MILP Point-of-care device for isoPCR testing using the RT-LAMP assay [101]. **(D)** Optimization design of the P-SOS [102]. **(E)** Overview of the detection process using μLAMP-LFA chip [103].
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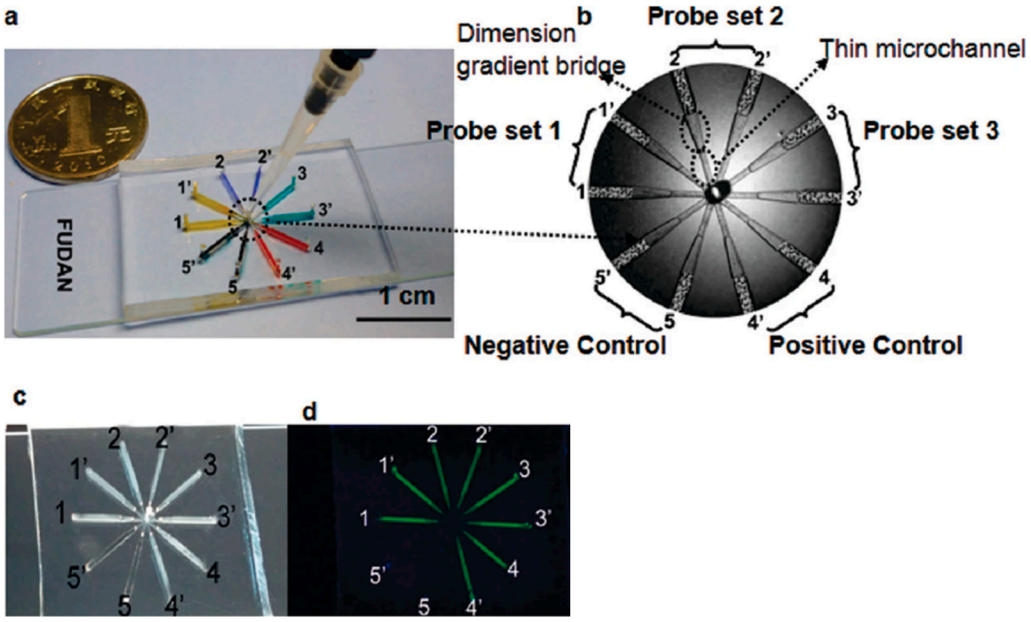


Fig. 13. (a) Image of the microfluidic device fabricated on a glass slide showing the central sample inlet and radial distribution into ten different reaction microchannels (colored with food dyes). A coin is provided for scale. (b) Schematic of the central fluidic distribution network, showing the dimension gradient bridge, thin microchannels, and the structural layout separating multiple probe sets (1–3), a positive control, and a negative control. (c) Bright-field image of the chip loaded with amplification reaction mix. (d) Detection of amplification products by end-point fluorescence under UV light with intense positive signals (green fluorescence) in the target probe channels (1–4′) and no signal in the negative control channels (5, 5′) [104].
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complex clinical and environmental matrices remain important limitations [105–109].

The next stage of development should therefore shift from demonstrating individual proof-of-concept devices toward quantitatively validated and reproducible integrated systems. In particular, the following research gaps require attention:

i) **Field validation of microfluidic–CRISPR platforms**: Emerging CRISPR–Cas12a/Cas13a microfluidic systems should be evaluated using authentic clinical or environmental specimens, with future studies targeting clinically relevant sensitivity and specificity (preferably >90%) and demonstrating performance against established reference methods rather than synthetic targets alone.
ii) **True sample-to-answer integration**: Future platforms should integrate lysis, nucleic acid extraction/purification, amplification, detection, and result interpretation within a closed device. For practical POCT, a desirable target is a total sample-to-result time of <30–60 min, with minimal manual reagent handling.
iii) **Reproducibility and inter-device robustness**: Analytical validation should extend beyond LoD and include intra-device and inter-device precision, batch-to-batch variability, invalid-test rates, and operator-dependent variation. A coefficient of variation of approximately <10–15% across independently fabricated devices would provide a useful development target where technically and clinically appropriate.
iv) **Multiplexing and clinically relevant analytical performance**: Future systems should progress beyond single-target demonstrations toward simultaneous detection of multiple pathogens, genetic variants, or resistance markers, while maintaining minimal cross-reactivity and clinically appropriate LoD. Multiplex performance should be evaluated using mixed-target samples rather than individual targets tested independently.
v) **Independent and field-based validation**: Many reported platforms remain validated using buffer, synthetic nucleic acids, or spiked samples. Future studies should include larger independent sample cohorts, blinded testing, comparison with PCR/qPCR or other accepted reference methods, and preferably multicentre or field evaluation to establish generalizability.

Beyond analytical performance, successful translation will depend on scalable fabrication, reagent stability, contamination control, power requirements, cost per test, user-friendly operation, and compatibility with portable readers. Manufacturing approaches such as thermoplastic replication, 3D printing, and paper-based microfluidics may help reduce dependence on specialized laboratory fabrication, but their reproducibility and large-scale manufacturing performance require systematic evaluation.

Integration with smartphone-based readout, wireless communication, and AI-assisted signal interpretation may further enable decentralized testing, automated quality control, and remote data transmission. However, computational integration should be validated independently and should complement rather than compensate for inadequate analytical performance.

Ultimately, progress toward clinically deployable microfluidic nucleic acid diagnostics requires a transition from "can the device detect the target?" to "can the complete system reliably detect the target under intended-use conditions?" Future platforms should therefore be benchmarked across analytical performance, sample-to-answer integration, reproducibility, multiplexing, manufacturability, cost, and real-world validation. Addressing these measurable gaps will be essential for translating microfluidic nucleic acid technologies from laboratory prototypes into reliable platforms for clinical diagnostics, infectious-disease surveillance, food safety, environmental monitoring, and decentralized point-of-care testing.

Declarations

Ethics statement

No human or animal samples were used during this study.

Table 3
Comparative analysis of recently reported microfluidic nucleic-acid detection platforms.

Ref.	Microfluidic Platform / Approach	Amplification / Detection Strategy	Target / Application	LoD	Assay Time	Multiplexing / Integration	Key Advantages	Major Limitation / Consideration*
[90]	Integrated portable continuous-flow PCR	CF-PCR with photometric on-chip sensing	594-bp GAPDH gene; potential molecular diagnostics	-	-	Automated, IoT-driven integrated platform	Portable, automated, integrated amplification and photometric sensing	Quantitative LoD and total assay time not reported in the present text
[91]	Acrylic continuous-flow PCR chip with 30 serpentine loops	CF-PCR + DPV electrochemical detection	Nucleic-acid amplification/ detection	-	-	Initial denaturation and final-extension regions integrated	Comparison with conventional benchtop PCR; electrochemical validation	Requires thermal-management and electrochemical instrumentation
[92]	Finger-driven microfluidic cartridge (Fd-MC)	Nucleic-acid extraction + RPA	Tuberculosis; MTB/ human–bovis and TB/ non-TB discrimination	-	Described as rapid;	Multiplex detection; extraction integrated	Finger-actuated operation without external pumps	Quantitative analytical performance not provided in the present text
[93]	Micro-paper-based analytical device (μPAD)	LAMP + ssDNA-functionalized graphene oxide	*Neisseria meningitidis* / meningitis diagnosis	-	-	Paper-based amplification and sensing	Low-cost, flexible and suitable for qualitative/ quantitative analysis	Quantitative LoD and assay duration not specified
[96]	Digital microfluidic platform	Colorimetric LAMP with neutral-red endpoint detection	*E. hepatopenaei*, IHHNV, WSSV and other prawn pathogens	-	20 min LAMP; 45 min total	Four parallel reaction units; multi-gene/sample detection	Low-cost visual detection and rapid multiplex analysis	LoD not stated in the supplied text
[95]	3D-printed three-electrode microfluidic biosensor	LAMP + electrochemical detection	*Listeria monocytogenes*; food-safety monitoring	-	-	3D-printed microfluidic/ electrochemical integration	Potential rapid on-site food pathogen detection	Quantitative performance not specified in the present text
[97]	Integrated microfluidic detection platform	LAMP + OpenMV image-based monitoring	*Pyricularia grisea*	10–10^5 copies/μL detection range	<45 min	Chip + temperature controller + camera; ~98% mixing efficiency	Real-time image analysis, controlled mixing, reduced reagent loss	Requires temperature-control and imaging modules
[98]	Fully automated paper-based microfluidic system	LAMP + CRISPR/ Cas12a	Multiple pathogenic bacteria	-	≤60 min	Lysis, LAMP and CRISPR/Cas12a integrated on paper	Equipment-free operation; preloaded freeze-dried reagents; automated processing	Quantitative LoD not provided in the present text
[99]	Microfluidic-LAMP platform with paper dipstick extraction	Rapid extraction + LAMP	Simultaneous detection of eight high-risk viruses	7.02×10^2–8×10^4 copies/ mL	70 min total; extraction 2 min	Eight-virus simultaneous detection	Low-cost, field-oriented and rapid extraction	Performance reported using simulated clinical samples in the supplied discussion
[100]	10-well microfluidic chip	Multiplex LAMP + visual/fluorescence detection	*V. parahaemolyticus, E. coli* O157:H7, *Y. enterocolitica, B. cereus*	1.78×10^4–1.59×10^5 copies/ mL	45 min	Four pathogens; eight food matrices	Multiplex, high-throughput and visually readable	Evaluated using artificially contaminated food matrices
[101]	MILP point-of-care platform	Membrane extraction + isothermal amplification + dual-mode photovoltaic/ optical detection	SARS-CoV-2	10 copies/μL	-	Extraction, amplification and detection integrated	95% sensitivity and 100% specificity versus RT-PCR; quantitative operation	Total assay time not specified in the present text
[102]	Palm-Shaped One-Step System (P-SOS)	Direct sample release + RT-LAMP + fluorescence	SARS-CoV-2 N gene / respiratory disease diagnosis	25 copies/μL	50 min	Sample preparation, amplification, heating, fluorescence and software integrated	Sample-to-result operation; lyophilized reagents; no separate extraction	Requires heating and fluorescence-detection modules
[103]	μLAMP-LFA microfluidic chip	LAMP + lateral-flow assay	WSSV and *Salmonella*; aquaculture diagnostics	28 copies/μL (WSSV); 15 copies/μL (*Salmonella*)	-	Dual-pathogen detection; automated fluid transfer	Hermetic construction, low contamination risk, micro-volume operation	Total assay time not stated in the supplied text
[104]	Octopus-like multiplex μLAMP chip	Multiplex LAMP + endpoint fluorescence	Influenza A subtypes / multiplex genetic testing	-	Rapid	Ten reaction microchannels with multiple primer sets and controls	Single-injection distribution, reduced cross-talk and high multiplexing capability	Quantitative LoD and reaction time not specified in the present text



Fig. 14. SWOT analysis of microfluidic nucleic acid amplification platforms.

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CRediT authorship contribution statement

Madhusudan B. Kulkarni: Writing – original draft, Validation, Supervision, Formal analysis, Data curation, Conceptualization. **Vinela:** Writing – review & editing, Formal analysis, Data curation, Conceptualization. **Naresh Mandal:** Writing – review & editing, Project administration, Investigation, Formal analysis. **Yashwanth N:** Writing – review & editing, Investigation, Funding acquisition, Data curation. **Panwala Fenil Chetankumar:** Writing – review & editing, Resources, Investigation, Formal analysis. **Sergio O. Martínez-Chapa:** Writing – review & editing, Resources, Methodology, Formal analysis. **Ricardo Izquierdo:** Writing – review & editing, Software, Project administration, Investigation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data is included within the manuscript.

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