

Research on real-time nucleic acid detection device based on microfluidic technology

Shuo Wu^{1,2}, Jianxin Cheng²,
Xiaohua Cao¹, Jingdong Bo¹, Shilun
Feng³ and Chuanjin Cui^{1,*}

¹North China University of Science
and Technology, Tangshan, Hebei
Province, 063000, China

²Xiangfu Laboratory, Jiaying,
Zhejiang Province, Jiashan, 314100,
China

³State Key Laboratory of
Transducer Technology, Shanghai
Institute of Microsystem and
Information Technology, Chinese
Academy of Sciences, Shanghai
200050, China

*E-mails: 15933378516@163.com,
chuanjincui@126.com

Received for publication
October 08, 2024.

Abstract

In recent years, the point-of-care testing (POCT) device for nucleic acid detection has received extensive attention. POCT device provides rapid, high sensitivity and accuracy of on-site diagnosis and can quantify the nucleic acid in clinical samples in real time. The integration of microfluidics ensures accurate fluid control, minimizes sample volume, and improves detection efficiency. In addition, POCT devices can conduct multichannel analysis, facilitate continuous monitoring, and provide comprehensive quantitative data. Due to its portability, cost-effectiveness, and ease of use, POCT device represents a promising solution for extensive, reliable, and timely nucleic acid detection in various medical environments. This review summarizes the research and development of nucleic acid detection POCT devices based on microfluidic technology in recent years, and the development trend of the device has prospected.

Keywords

microfluidic technology, nucleic acid test, point-of-care testing

1. Introduction

Nucleic acid testing can directly identify the genetic material of pathogens and analyze it using molecular biology techniques to detect the presence or absence of specific pathogens or genes. This precision is crucial in quickly identifying infectious diseases such as viral infections. During the peak period of the epidemic, nucleic acid testing can provide results within a few hours, providing doctors with timely and accurate negative and positive judgments. Nucleic acid testing, through accurate and rapid detection methods, has been applied in various aspects such as primary healthcare, epidemic prevention and control, animal and food safety, infectious disease monitoring, etc. [1], providing indispensable support for us to build a safe and healthy living environment.

Point-of-care testing (POCT) refers to a real-time testing method conducted near or at the patient's location, also known as proximity testing or bedside testing. POCT can provide nucleic acid test

results in a short period of time, enabling doctors to quickly make diagnostic and treatment decisions. Additionally, due to its simple operation and lack of specialized laboratory facilities, it can be used in a wider range of scenarios. The application of POCT devices for nucleic acid testing is very important in harsh environments such as emergency departments, intensive care units, rural rescue stations, and even in the wild [2]. Compared with traditional nucleic acid detection methods, POCT devices may have incomplete extraction of nucleic acid from biological samples containing complex impurities, which may reduce the detection sensitivity and specificity. In the nucleic acid amplification process, due to the small size of microfluidic chip and the small reagent and sample system, there may be uneven mixing of samples and actual samples, resulting in incomplete amplification reaction. In the microfluidic POCT device, due to the limited available space and fluid control, primer efficiency and reaction conditions may be limited. There will also be surface adsorption in

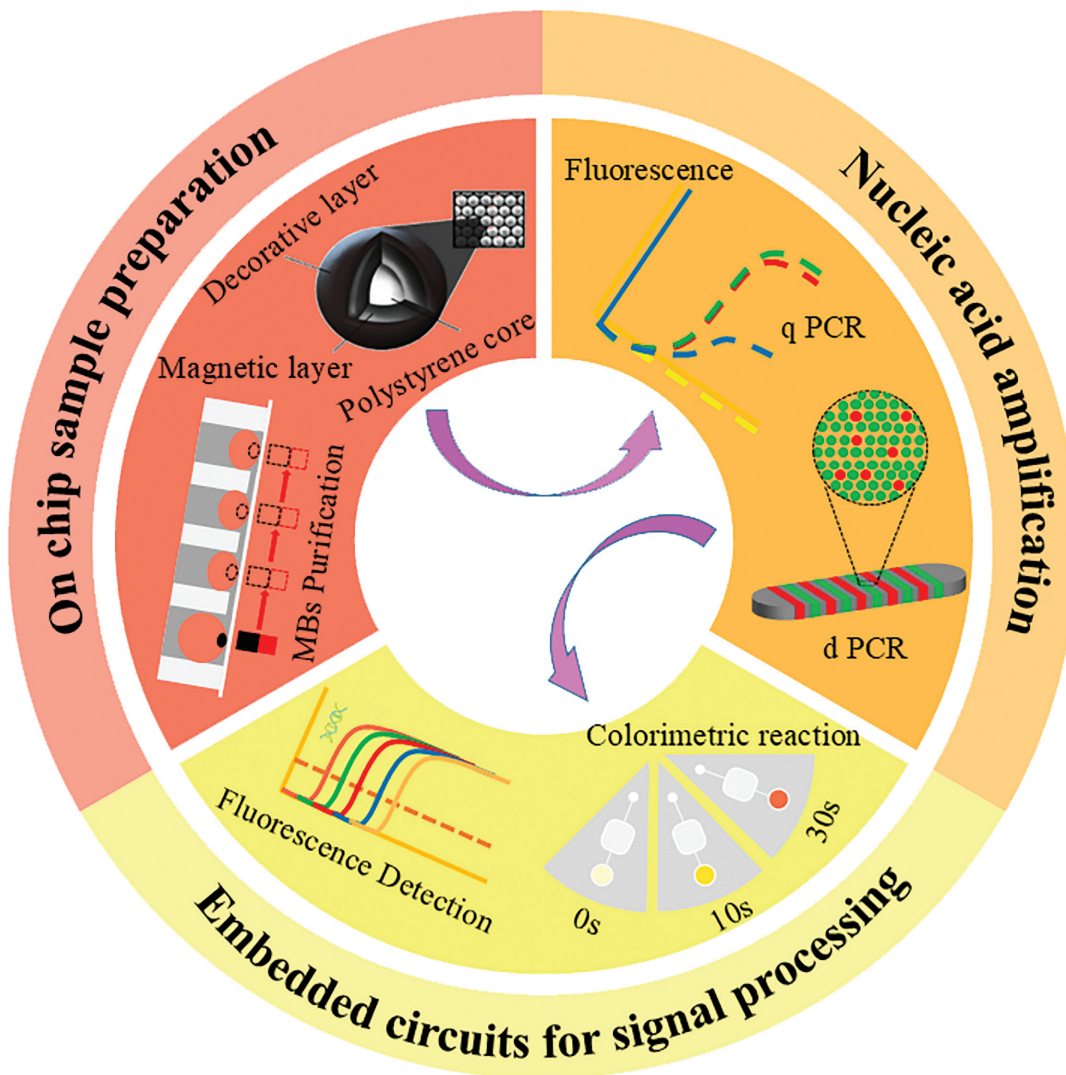


Figure 1: Steps and methods of nucleic acid testing using POCT device. dPCR, digital PCR; POCT, point-of-care testing.

the chip, which may cause some nucleic acid molecules to fail to participate in the amplification reaction effectively, thereby reducing the amount of amplification products or affecting their quality [3]. However, the POCT device is especially suitable for applications requiring rapid diagnosis and low resources, such as clinical emergencies, remote mountainous areas, field hospitals, etc. In these scenarios, traditional nucleic acid detection methods cannot provide rapid detection results because of the need for complex equipment, long-time reaction, sample transportation, and other factors. POCT device can detect on-site and provide nucleic acid detection results within a few minutes to an hour, enabling doctors and medical personnel to make treatment decisions in time, especially for the rapid screening of infectious

diseases. The commonly used nucleic acid testing steps and methods for POCT devices are shown in Figure 1. Through integrated and automated design, POCT device greatly reduces the manual intervention, reagent consumption, and operation complexity in traditional nucleic acid detection, thus reducing the detection cost [4]. Although microfluidic POCT devices still have some limitations compared with traditional nucleic acid detection methods in sensitivity and specificity, these devices have great application potential, especially in the field of field detection, resource limited environment, and rapid diagnosis. With the continuous progress of technology, POCT device will overcome the existing challenges and become an indispensable part of the modern medical and public health system.

Microfluidic technology integrates basic operational units such as sample separation, reaction, and detection in biological, chemical, and medical analysis processes into chips that are several centimeters square by controlling high-throughput and microvolume fluids. It has the advantages of requiring smaller volume of liquid sample for detection, rapid reaction, and the ability to process multiple samples in parallel in large quantities [5,6]. The nucleic acid detection POCT device based on microfluidic technology is not only convenient and fast to use, with a wide range of applicable scenarios, but also integrates the advantages of high specificity and parallel processing of large amounts of samples of microfluidic chip detection technology. With the continuous development of microfluidic technology, the microfluidic POCT device will be a necessary step to improve the quality of medical services and also the key to global health and disease control.

This article reviews the latest research progress of nucleic acid detection POCT devices based on microfluidic technology. The next three sections will review the three steps of nucleic acid detection using POCT devices: on-chip sample preparation, nucleic acid amplification, and signal output. The final section outlines the development trend of POCT devices for nucleic acid detection.

II. On-Chip Sample Preparation

Nucleic acid purification is the initial and critical step in nucleic acid testing. The purity and integrity of the purified nucleic acids significantly impact the accuracy of subsequent testing results [7]. Traditional liquid-phase purification methods require complex procedures, extensive reagents, and time, with the added risk of nucleic acid contamination from toxic agents [8–10]. In contrast, the solid-phase purification method showed strong affinity and adsorption capacity for nucleic acids in the purification process [11]. This results in greater efficiency and time savings, addressing inherent issues in liquid-phase methods. Consequently, solid-phase purification methods are extensively employed in automated nucleic acid purification equipment [12]. In nucleic acid detection POCT devices, nucleic acid purification commonly employs silicon-based solid-phase and magnetic bead-based methods. In nucleic acid POCT devices, various silica structures such as silicon microcolumns [13], monolithic silicas [14], sol-gel coated hybrid silica beads [15], and organic polymer monoliths have been utilized for nucleic acid purification. Additionally, silica-coated magnetic particles

are utilized in microfluidic chips for nucleic acid purification and detection [16]. However, due to the unique characteristics of magnetic beads such as large specific surface area, ability to modify various active groups, and ease of being guided by external magnetic fields, magnetic bead-based nucleic acid purification methods are more widely used in microfluidic nucleic acid real-time detection POCT devices [17–19]. However, in POCT devices, the simplicity and rapidity of on-site nucleic acid extraction are at the expense of the potential limitations of the detection mechanism, which may introduce errors affecting the sensitivity and specificity of the detection results. The subsequent review will focus solely on POCT devices that utilize magnetic bead-based nucleic acid purification methods for on-chip sample preparation.

The integration of magnetic bead-based solid-phase nucleic acid purification technology with microfluidic technology offers a highly promising approach for on-chip sample preparation in POCT devices. Wu et al. [20] developed a droplet-based magnetically controlled microfluidic chip that integrates nucleic acid extraction, purification, and amplification, using permanent magnets as the driving mechanism. The chip encapsulates reagents within oil to create droplets, which then direct the movement of magnetic beads between these droplets using permanent magnets, facilitating the nucleic acid purification steps. This chip is capable of automatically extracting nucleic acid from multiple samples in <20 min. The chip structure is shown in Figure 2A. However, in the process of nucleic acid purification, nucleic acid capture is often incomplete or lost, especially when using magnetic beads with poor optimized magnetic field strength or poor surface properties. Shen et al. [21] designed and developed a highly integrated microfluidic chip. The nucleic acid extraction efficiency reached 93%, comparable to that of commercially available kits. The chip features a microporous array structure at the bottom, which utilizes the inherent gravity of the magnetic beads and the magnetic force of a permanent magnet to capture the beads post-elution, thus ensuring that the beads do not interfere with subsequent nucleic acid amplification and detection. When used in conjunction with the developed POCT devices, it enables a complete sample-answer detection process. Li et al. [22] designed an integrated microfluidic chip that synergistically combines micropillar structure with a bubble capture array. This design allows for thorough mixing of reagents and magnetic beads within the chip under acoustic streaming. This not only ensures

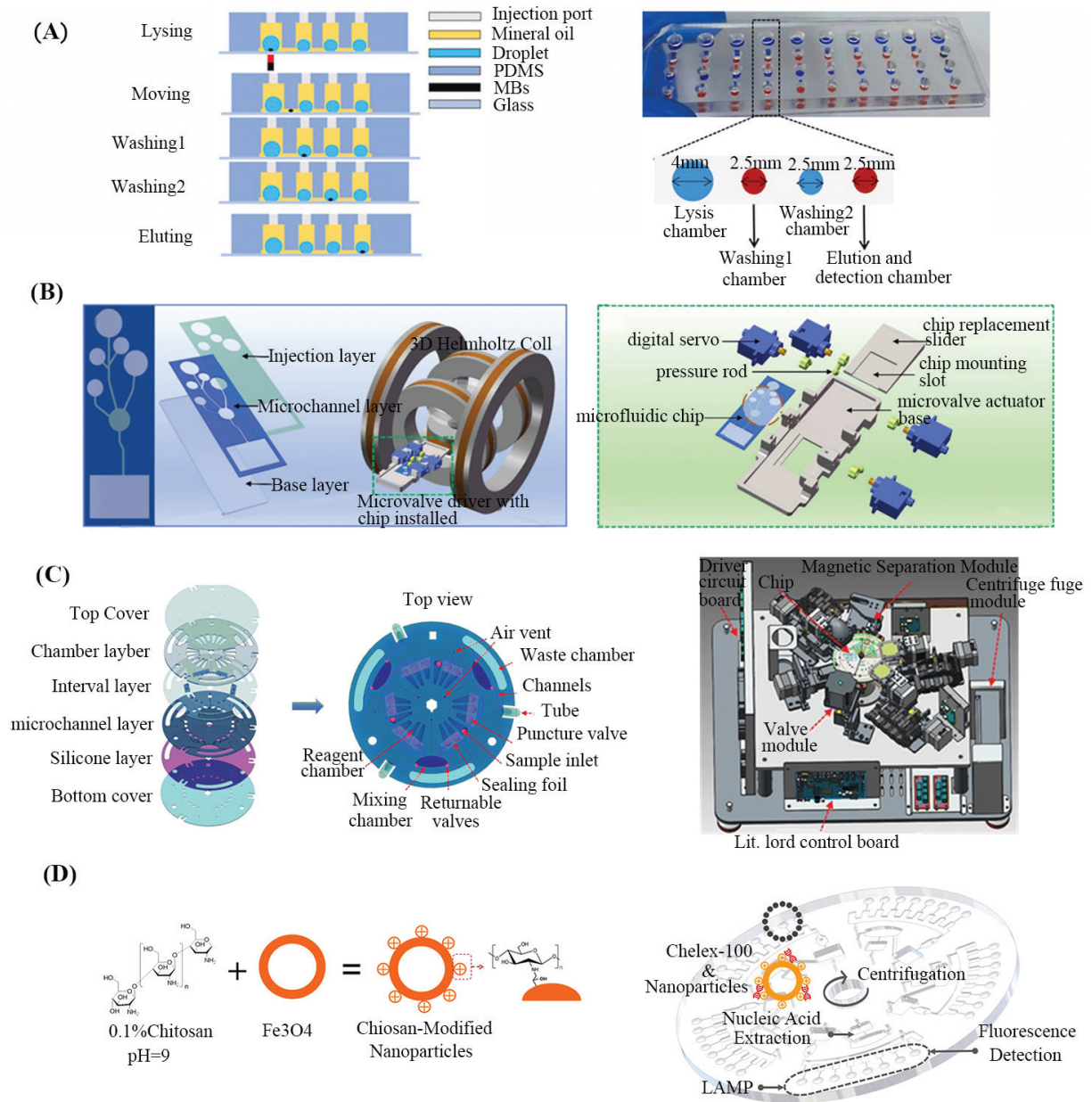


Figure 2: Sample preparation of nucleic acids in POCT. (A) Schematic diagram of the operation process of nucleic acid extraction and purification using MBs in one assay unit and the image of the chip [20]. (B) 3D Helmholtz coil active magnetic capture nucleic acid chip and device explosion diagram [24]. (C) A centrifugal microfluidic chip and its supporting automation platform [30]. (D) Chitosan and polydopamine modified magnetic beads and corresponding chip [31]. LAMP, Loop mediated adiabatic amplification; POCT, point-of-care testing.

effective mixing of the magnetic beads but also enhances their capture efficiency for nucleic acids. While these devices provide the benefits of enhanced automation, they also introduce the complexity associated with device miniaturization and reagent distribution uniformity. The variation of flow rate or fluid dynamics in the microfluidic channel will lead to

incomplete mixing of magnetic beads and reagents, which will lead to unsatisfactory nucleic acid capture and extraction [23]. In addition, external factors such as temperature fluctuations or electrostatic interference may affect the behavior of magnetic beads, thus affecting the efficiency and repeatability of the purification process.

Zhang et al. [24] introduced a novel biological detection strategy. They employed a three-dimensional Helmholtz coil to facilitate the active magnetic capture of nucleic acids by magnetic beads. In contrast to traditional hybrid capture methods, active magnetic capture significantly enhances capture efficiency via automated magnetic manipulation. The chip design concept and chip structure are shown in Figure 2B. Li et al. [25] developed a fully integrated POCT device employing magnetic rods for the manipulation of magnetic beads. Every step of nucleic acid purification was optimized, achieving a magnetic bead recovery efficiency of 94.98% and a nucleic acid extraction efficiency of 91.83%. The nucleic acid extraction efficiency reached 93%, comparable to that of commercially available test kits [26]. Although magnetic beads provide a large surface area for nucleic acid binding, the inconsistency of magnetic bead coating or the binding ratio between magnetic beads and nucleic acid may lead to incomplete recovery of nucleic acid, which directly affects the sensitivity of detection. If the magnetic field intensity is not ideal, whether it is too weak or too strong, it will lead to the reduction of capture efficiency and further affect the repeatability of detection and the specificity of nucleic acid detection [27]. Pearlman et al. [28] examined the high gradient magnetic separation (hgms) extraction technique. They investigated the impact of surface magnetic field, fluid flow rate, and viscosity on the nucleic acid extraction efficiency using magnetic beads. Before attaining the necessary magnetic field strength for external field saturation, the capture efficiency of magnetic beads rises with increasing field strength. However, once the requisite magnetic field strength for external field saturation is reached, further increases in field strength paradoxically leading to a decline in the capture efficiency of the magnetic beads. Besides employing permanent magnets to regulate the movement of magnetic beads within the chip, these beads also demonstrate effective performance in microfluidic chips powered by centrifugal force. Sciuto et al. [29] employed variable rotational speeds to regulate the release of preloaded reagents within the chips, utilizing the magnetic field from permanent magnets to extract and purify nucleic acids. Moreover, they designed a complementary POCT device to facilitate a seamless sample-answer detection process.

Li et al. [30] segmented the chip's functional areas and integrated the section dedicated to nucleic acid purification onto the centrifuge functional disk. The structure of the central functional disk is shown in Figure 2C. Additionally, the chip incorporates various

control valves for regulating the release of reagents and the activation or deactivation of microchannels. They even employ magnetic valves for swift control of magnetic bead aggregation and dispersion. The chip achieves automatic nucleic acid purification within 30 min after adding the sample, which boosts the efficiency of nucleic acid extraction by an order of magnitude compared to traditional manual methods. Additionally, the device can automatically perform subsequent nucleic acid testing steps. Zhao et al. [31] developed a microfluidic device for whole process nucleic acid detection. The chip structure is shown in Figure 2D. To prevent reagent contamination of the nucleic acids and its adverse impact on subsequent amplification and detection, they optimized the nucleic acid purification process on a centrifugal microfluidic chip. They decorated magnetic beads with chitosan and polydopamine, enabling the adsorption and desorption of nucleic acids via magnetic beads through electrostatic interactions and pH control of reagents. Additionally, Chelex-100 Sigma-Aldrich (St Louis, MO, USA) particles are incorporated to aid thermal cracking, which removes certain impurities. The efficiency of nucleic acid extraction is marginally higher than that of commercially available test kits. However, in practical applications, more complex environmental conditions are usually encountered, such as magnetic field interference and sample matrix interference, which will affect the performance of POCT devices. Or, under the condition of magnetic field, due to pollutants generated by environmental exposure, the integrity of nucleic acid capture may be affected, which may not be fully considered in the laboratory environment.

Although the nucleic acid purification method based on magnetic beads in POCT devices has significant advantages in efficiency and time saving, there are still some technical and operational challenges. In low viral load samples, nucleic acid purification may not be able to effectively concentrate or separate enough target nucleic acids, which may lead to false negative. In addition, cross contamination during sample processing may be a problem of special concern in manual or semiautomatic POCT devices, which may lead to false positive, thus affecting specificity. Magnetic beads are vulnerable to residual contamination from one sample to another, especially when the washing steps are not optimized, which may lead to false positive in subsequent tests [32,33]. In addition, the reuse and recycling of magnetic beads and microfluidic chips are still the key issues of environmentally sustainable POCT solutions. At present, many POCT devices use

disposable magnetic beads and disposable microfluidic chips, which will lead to a large amount of waste. After the purification of nucleic acid, the magnetic beads may retain nucleic acid or pollutants, making it unable to be reused in subsequent cycles [34]. Incomplete recovery of nucleic acid or accumulation of pollutants will reduce the performance of purification process, which may lead to false negative or false positive in future detection.

In the future development, efforts can be made to optimize the surface chemical composition of magnetic beads to enhance their ability to be cleaned and reused without loss of binding load. People can develop self-cleaning magnetic beads or modify anti-fouling coatings or develop automatic magnetic bead cleaning solutions and integrate them into POCT devices to reduce the detection cost [35,36]. The development of reusable microfluidic chips made of durable and easy to clean materials can significantly reduce waste. For example, the use of sterilized and reusable silicon-based chips or biodegradable polymers can help make these devices more sustainable. From the standpoint of POCT devices, the degree of integration and automation for the cell lysis to nucleic acid elution processes leaves much to be desired. The high integration of these systems provides a promising method for simplifying nucleic acid detection, but it must be recognized that hardware limitations and user errors are the potential factors of failure in POCT applications. Improper handling may lead to significant deviations from the expected results. In the future, we can focus on more stable magnetic bead formula, optimizing the miniaturization and modularization of sample preparation scheme and equipment. Ultrasonic module [37], oscillation module [31] or other equipment used for pathogen lysis can be integrated into POCT system. This will minimize these limitations and improve the performance of POCT devices in clinical diagnosis.

III. Nucleic Acid Amplification

Amplifying the target gene sequence is a crucial step in the nucleic acid testing process for POCT devices. Nucleic acid amplification technology can significantly replicate minute quantities of the target gene sequence, thereby enhancing the sensitivity of POCT devices. This enables the nucleic acid detection POCT device to detect a smaller number of target nucleic acid sequences in the sample. Common amplification techniques are categorized into isothermal and nonisothermal amplification technologies. With the advancements in nonisothermal and

isothermal amplification technologies, nucleic acid detection POCT device integrated with nucleic acid amplification module came into being.

a. Nonisothermal amplification

Nonisothermal amplification technology involves nucleic acid amplification steps at different temperatures during the reaction process. The most extensively applied nonisothermal amplification technique is the polymerase chain reaction (PCR) [38]. PCR technology mimics the natural process of DNA self-replication and amplification. The PCR process involves repeated cycles of DNA/RNA through three stages: denaturation (93–94°C), annealing (40–60°C), and extension (70–75°C). Each cycle of denaturation, annealing, and extension exponentially amplifies the target sequence. Thus, PCR is an efficient technology that amplifies nucleic acids exponentially. In genetic disease and infectious disease testing, PCR is widely considered the gold standard for detecting target DNA and RNA [39,40]. Performing PCR amplification on a microfluidic chip can enhance various aspects of PCR performance, including amplification efficiency, reducing reaction time, simplifying the procedure, and lowering costs [41]. Temperature control is critical for PCR amplification, as precise thermal cycling is required for the denaturation, annealing, and extension of target sequences. POCT devices, due to their compact nature, often face challenges in maintaining stable and uniform temperatures, leading to potential thermal fluctuations [42]. Inconsistent heating or cooling can result in incomplete denaturation, inefficient primer binding, or suboptimal extension, all of which could decrease amplification efficiency and compromise specificity, leading to false negatives or false positives. Additionally, several POCT devices have been developed and designed [43].

PCR modules incorporated into POCT devices for nucleic acid detection can be broadly classified into fixed microchamber and flow-through types [44]. The PCR module with fixed microchamber usually seals the samples and reagents in the specific chamber of the microfluidic chip and uses the temperature control module with rapid temperature rise and fall function to provide different temperatures required in the PCR reaction process. The PCR module with a fixed microchamber can be heated using various methods, including contact heating devices such as film resistive heaters [45], box heaters [46], hot plates [47], and printed circuit heaters [48], as well as noncontact methods such as infrared heating [49], microwavable [50], wireless induction heating [51]. The flow-through

PCR module achieves the desired temperature conditions by managing the reagents' passage through various constant temperature zones within the microchannel of the reaction chamber [52]. In addition, there is a thermal convection PCR technology [53] that creates a stable temperature gradient within the capillary by applying heat to one end. This temperature gradient will induce the reaction reagent to circulate spontaneously between the cold and hot regions in the capillary and accomplish nucleic acid amplification.

When the target sequence is short (<300 bp), the annealing and extension processes in PCR amplification can be conducted concurrently. For target sequences shorter than 100 bp, the extension time can be reduced to 0 s [54]. The PCR amplification process has been simplified, requiring only two temperatures for target sequence amplification, resulting in a significant reduction in PCR amplification time. Scientists have conducted research on and optimized the temperature differential between the temperature control module and the reaction reagents, the thermal resistance of materials, and the heat capacity of both the microfluidic chip and the reaction reagents. Consequently, some devices capable of performing ultrafast PCR have been developed. Nouwairi et al. [55] designed a nucleic acid amplification device that facilitates ultrafast thermal cycling. The traditional 50-min reverse transcription-polymerase chain reaction (RT-PCR) amplification process was compressed to under 10 min using two thermo-electric Peltier devices. An et al. [56] introduced a temperature control strategy that significantly enhances the temperature ramping rate of the solution by widening the temperature gap between the heating plate and the solution. Within a specific range, the temperature rise rate of the solution is linearly related to the temperature difference, the greater the temperature gap, the more rapidly the rise rate. The maximum heating and cooling ramp rates for a 25 μL solution reached $24.12^{\circ}\text{Cs}^{-1}$ and $25.28^{\circ}\text{Cs}^{-1}$, respectively, which is 6–8 times faster than that of conventional commercial PCR devices. The PCR amplifier designed using this method can complete 45 PCR cycles in just 9 min. Trauba et al. [57] employed a two-step PCR to amplify the target sequence, configuring the temperature control module with two distinct temperature zones at 90°C and 65°C . In total, 35 temperature cycles were executed within 52 s. Yeom et al. [58] developed an ultrafast PCR amplification device using inexpensive thin glass chips and thermal resistors. This device can heat the reaction reagents at a maximum rate of

$28.8^{\circ}\text{Cs}^{-1}$, achieving ultrafast PCR amplification while reducing the manufacturing cost of the device. These devices with fast thermal cycling show promise by significantly accelerating the temperature ramp, but the problem of temperature uniformity in the heating region is still a huge challenge, especially in small sample sizes [59]. Moreover, these ultrafast thermal cycling techniques may affect the accuracy of amplification, especially when dealing with highly complex or low abundance targets. Rapid cycling may also increase the possibility of nonspecific amplification or primer dimer formation, thereby reducing specificity. Salman et al. [60] introduced a comprehensive system comprising a PCR chip, thermal cycler, and fluorescence detector. Following nucleic acid amplification, the products can be fluorescently detected to yield results. The system has a heating rate of $1.8^{\circ}\text{Cs}^{-1}$ and a cooling rate of 2°Cs^{-1} . This represents a significant reduction in heating and cooling rates compared to the real-time PCR system developed by Petralia et al. [61], which boasts a heating rate of 15°Cs^{-1} and a cooling rate of 8°Cs^{-1} . Analysis of the two devices suggests that the primary cause of this difference may be the varying sizes of the heating elements and microfluidic chips. The thinner the microfluidic chip, the smaller the reaction reagent volume, resulting in faster temperature ramps. However, too little reagent volume may also lead to unsatisfactory reagent concentration or insufficient reagent mixing, thus reducing the sensitivity. In addition, reducing the volume of reagent may damage the efficiency of downstream detection steps [62]. Excessive miniaturization may also increase the risk of evaporation, especially in closed systems, which may affect the consistency and reproducibility of the reaction, resulting in changes in amplification yield. The thermal cycling required for PCR constrains the further miniaturization of microfluidic devices in POCT. Huang et al. [44] developed an ultrafast and highly sensitive real-time PCR system that integrates the best features of microchamber and flow-through amplification systems. This system includes a microfluidic chip optimized for rapid heat transfer and an amplification device with five temperature zones, complemented by a fluorescence detection module for quantitative nucleic acid assays. It addresses the previous dilemma of achieving both rapid temperature rates and flexibility in amplification modules [63]. The system minimizes mechanical complexity, enabling rapid temperature fluctuations while significantly controlling manufacturing costs. Additionally, the team compared the performance of their real-time PCR system with commercial PCR

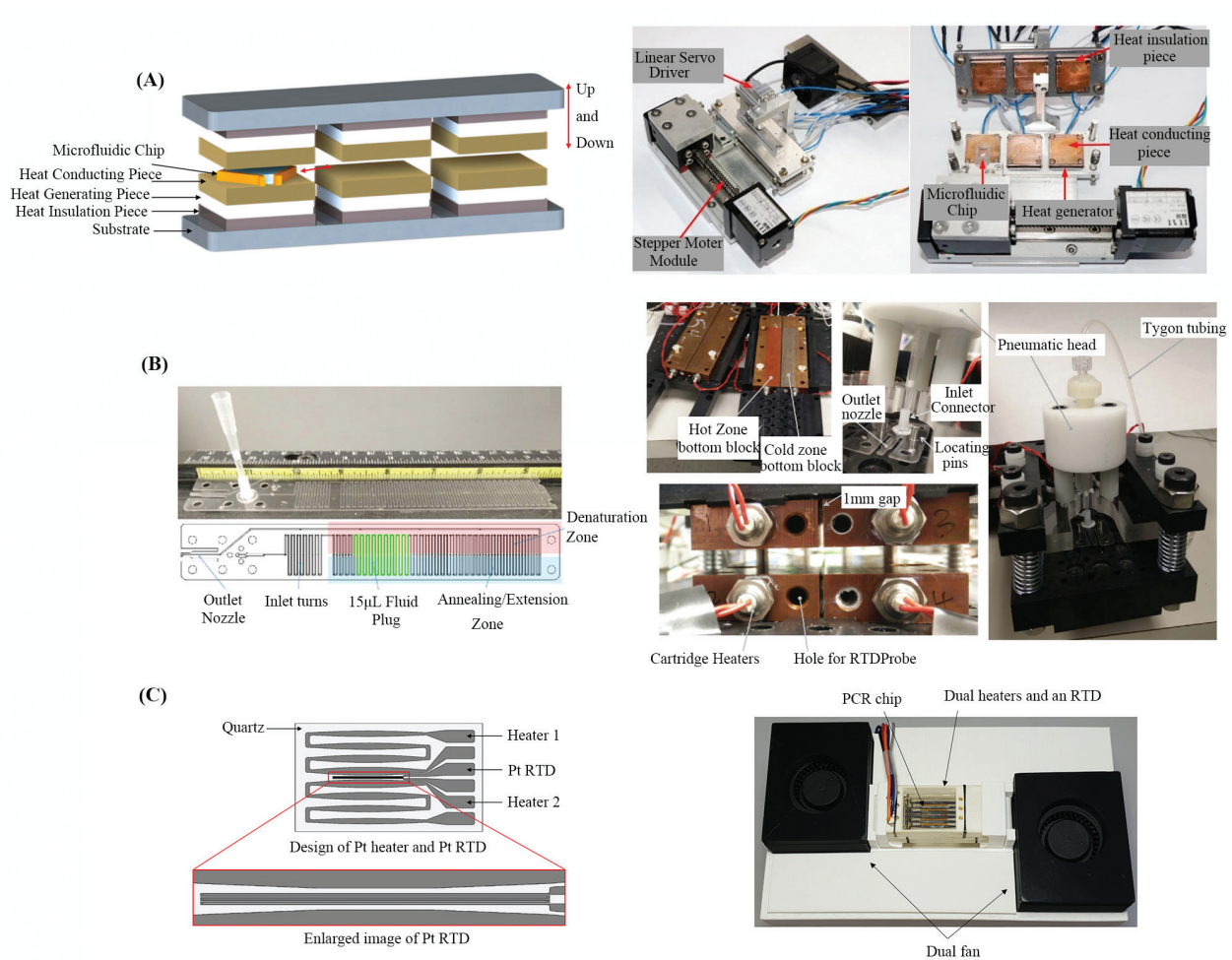


Figure 3: Microfluidic chips and temperature control devices for ultrafast PCR. (A) Ultrafast PCR design and hardware device [56]. (B) Ultrafast PCR chip with 35 cycles in 1 min and fast rising and cooling device [57]. (C) Microfluidic chip to improve RTDs performance and the temperature control device [62]. PCR, polymerase chain reaction; RTDs, resistance temperature detectors.

devices, finding that the results of biological sample tests were in agreement with those from commercial devices, despite reducing the total testing time from 1 h to 15 min. Utilizing the real-time PCR system for nucleic acid testing not only ensures sensitivity but also significantly reduces testing time, offering a promising solution for ultrafast real-time PCR. The preceding text introduced various schemes and structures, and Figure 3 illustrates several representative schemes among them.

To sum up, now there are many POCT devices that can achieve rapid PCR amplification, which greatly improves the efficiency of nucleic acid amplification. However, there are still challenges in detecting low copy target sequences. As shown in Table 1, compared with other PCR techniques, digital PCR (dPCR) technology has extremely high sensitivity and

absolute quantification advantages. In the future, as dPCR technology becomes more economical and compact, integrating it into POCT system can completely change the sensitivity by allowing absolute quantification of target sequences, even at very low concentrations [64]. Developing rapid heating and cooling devices with good temperature uniformity using new materials can help reduce thermal cycling errors [65]. Developing integrated temperature sensors and more powerful thermal control modules will improve sensitivity and specificity. Integrating and optimizing existing on-chip sample preparation technologies, such as automatic nucleic acid purification or improved inhibitor removal methods, will help to eliminate sample interference and reduce false negative effects caused by PCR inhibition. POCT devices shall be designed with built-in pollution prevention

Table 1: PCR techniques applied in POCT devices

Method	Advantage	Disadvantage
PCR	Low cost; complete standards; and the product is recyclable	The operation is cumbersome; low specificity and sensitivity; easy to be contaminated; and cannot perform quantitative analysis
qPCR	High specificity and sensitivity; quantitative analysis	High cost, nonrecyclable product; fluorescent probe or dye required
RT-PCR and RT-qPCR	Widely applicable and can be used for all types of RNA	RNA is easily degraded; additional reverse transcription steps are required
dPCR	Absolute quantification; extremely high sensitivity and specificity	Expensive cost; complex operation; and long-time consuming

dPCR, digital PCR; PCR, polymerase chain reaction; POCT, point-of-care testing; RT-PCR, reverse transcription-polymerase chain reaction.

function, such as closed disposable reaction chamber, which will reduce cross contamination and improve the specificity of results.

b. Isothermal amplification

Compared to PCR amplification technology, the advent of isothermal amplification technology provides a bright strategy to the challenge of further reducing the size of POCT devices, which was previously impossible due to the necessity of temperature control modules in nonisothermal amplification devices [66]. Common isothermal amplification techniques include Loop mediated adiabatic amplification (LAMP), recombinant polymerase amplification (RPA), and nucleic acid sequence-based amplification (NASBA). The subsequent review will solely concentrate on POCT devices for nucleic acid amplification employing LAMP technology.

Since its invention by Hase et al. in 2000, LAMP technology has been a primary focus in the development of POCT, owing to its simplicity, high sensitivity, and speed. LAMP is carried out at 60–65°C and can amplify DNA to 10^9 – 10^{10} times within 15–60 min. The LAMP reaction generates >50 times the number of amplicons compared to any other PCR-based amplification technique. LAMP also has the potential to amplify target DNA of various sizes ranging from 130 bp to 300 bp, which can be used for multiplex amplification of pathogens [67,68]. Compared to other isothermal amplification techniques, LAMP employs 4–6 primers that offer superior specificity and can amplify both DNA and RNA. But if the primer design is unsatisfactory, it will increase the possibility of non-specific binding. Improper primer selection or unsatisfactory primer concentration will lead to the formation of primer dimer, which competes with the target

amplification and reduces the reaction efficiency. In addition, if the primers are combined with unexpected sequences, nonspecific amplification may occur, resulting in false positive. LAMP is a one-step amplification technique that requires no additional sample processing, utilizes a single DNA polymerase, and demonstrates outstanding resistance to polymerase inhibitors [69]. Therefore, LAMP is currently the most prevalent isothermal amplification technology in the field of POCT. Jiang et al. [70] developed a microfluidic chip for rapid nucleic acid detection using LAMP, achieving a detection limit of 102 copies μL^{-1} . Xiao et al. [71] leveraged microfluidic technology to develop a handheld microfluidic lamp colorimetric chip. This chip enables direct DNA extraction from meat samples using microneedles and swiftly transfers the samples to the microfluidic chip for LAMP amplification. El Tholoth et al. [72] used LAMP to realize real-time detection of multichannel pathogens, which can detect multiple different virus samples at the same time. Loo et al. [73] developed a highly integrated nucleic acid detection platform based on LAMP, utilizing a disk platform to provide a sample-answer solution. A silicon membrane is employed for on-chip solid-phase extraction and purification of bacterial DNA; centrifugal force drives the movement of reagents within the chip, and SYTO-9 (New England Biolabs) fluorescent dye is used for real-time fluorescence detection. This microfluidic platform can complete the entire nucleic acid detection process within 2 hr, analyzing multiple samples simultaneously. Liu et al. [74] developed a fully automated centrifuge chip that can simultaneously detect five different bacteria and designed a corresponding automated supply platform. Nucleic acid testing can be completed within 70 min, with a detection limit of 10 copies μL^{-1} at the limit of detection. LAMP is qualitative in nature,

which means that although it has high sensitivity, it cannot provide absolute quantification of target molecules. For applications requiring accurate quantification, such as viral load measurement or therapeutic effect monitoring, the lack of quantitative accuracy may be a major limitation. Since the introduction of the digital LAMP (d-LAMP) method by Gansen et al. [75], it has been extensively applied for precise quantification of nucleic acids. Rane et al. [76] developed a microfluidic device leveraging d-LAMP technology to perform all the steps required for digital nucleic acid detection. The device can produce 1 million droplets every 110 min, with an average droplet size of 10 μL . Following amplification, fluorescence detection was conducted, achieving a detection limit of 600 copies μL^{-1} . Although d-LAMP improves the accuracy of quantification, it still faces challenges related to the consistency of droplet formation and potential cross contamination between droplets. The detection limit of d-LAMP also depends on the sensitivity of the system. Suboptimal droplet size distribution or incomplete droplet sealing may affect the sensitivity and specificity. In addition, the integration of d-LAMP into compact POCT devices will bring additional complexity, which may increase the cost and require more complex fluid control. Xie et al. [77] developed a simple and cost-effective nucleic acid detection platform using colorimetric LAMP technology. Simple on-site disease diagnosis can be directly performed by visually discriminating colors with the naked eye. The detection limit stands at 101 copies μL^{-1} . The preceding text introduced multiple cases, and Figure 4 presents several representative ones among them.

The characteristic of LAMP constant temperature amplification is beneficial for making nucleic acid detection POCT devices more compact and convenient. As shown in Table 2, applying LAMP technology to nucleic acid detection POCT devices eliminates the need for cumbersome temperature control devices and has higher sensitivity and specificity compared to PCR, which helps to construct smaller POCT devices in resource limited environments. However, due to the high difficulty of primer design for LAMP and the expensive reagents used, the cost of POCT devices using LAMP technology is also higher, and their popularity in rural and remote areas is not as good as POCT devices using PCR technology.

Although these rapid amplification systems using PCR and lamp technology show great prospects, the current research lacks the actual performance verification in outdoor environment. GeneXper™ system is manufactured by Cepheid using PCR amplification and ID NOW™ platform is produced by Abbott

Laboratories using isothermal amplification have been tested and validated in remote areas such as Uganda and South Africa and have shown good performance during the pandemic [78,79]. The accuracy and efficiency of POCT devices that have not been tested in practical application scenarios under on-site conditions (such as rural clinics, disaster areas, and emergency response situations) have not been fully determined. It is very important to test these systems in the environment with changeable temperature and humidity to ensure their reliability and adaptability in practical applications. The ability to maintain high sensitivity and specificity outside the laboratory environment remains a major challenge. To improve the performance of POCT device in the actual scene, efforts should be made to develop devices that can handle rapid temperature changes while maintaining temperature uniformity. The integrated temperature sensor and powerful thermal control module will help to reduce the thermal cycle error and improve the sensitivity and specificity of the results.

IV. Embedded Circuits for Signal Processing

In nucleic acid detection POCT devices, nucleic acid detection results can be outputted through various methods [80,81], including colorimetry, fluorescence, electrochemistry, magnetism, and others. Nucleic acid information can be captured and analyzed by professional signal conversion devices, such as the naked eye, mobile phone cameras, optical microscopes, and industrial cameras. Colorimetric reaction involves the specific binding of colorimetric probes (such as fluorescent dyes [82], nanoparticles [83], or other pigment compounds) with amplification products. As the target nucleic acid increases, the colorimetric probe triggers a change in the solution's color, enabling the interpretation of test results. Compared to other methods, the cost of utilizing colorimetric reactions for nucleic acid detection is relatively lower. However, colorimetry is highly dependent on the precise design of the probe. Nonspecific binding or cross reactivity with similar nucleic acid sequences can lead to false positives. The presidential colored substance or other complex matrix in the sample may interfere with the signal, resulting in inaccurate results. This is particularly problematic in biological samples that may contain blood, food particles, or other compounds that may alter the colorimetric output [84]. The fluorescence method is employed to detect nucleic acid signals

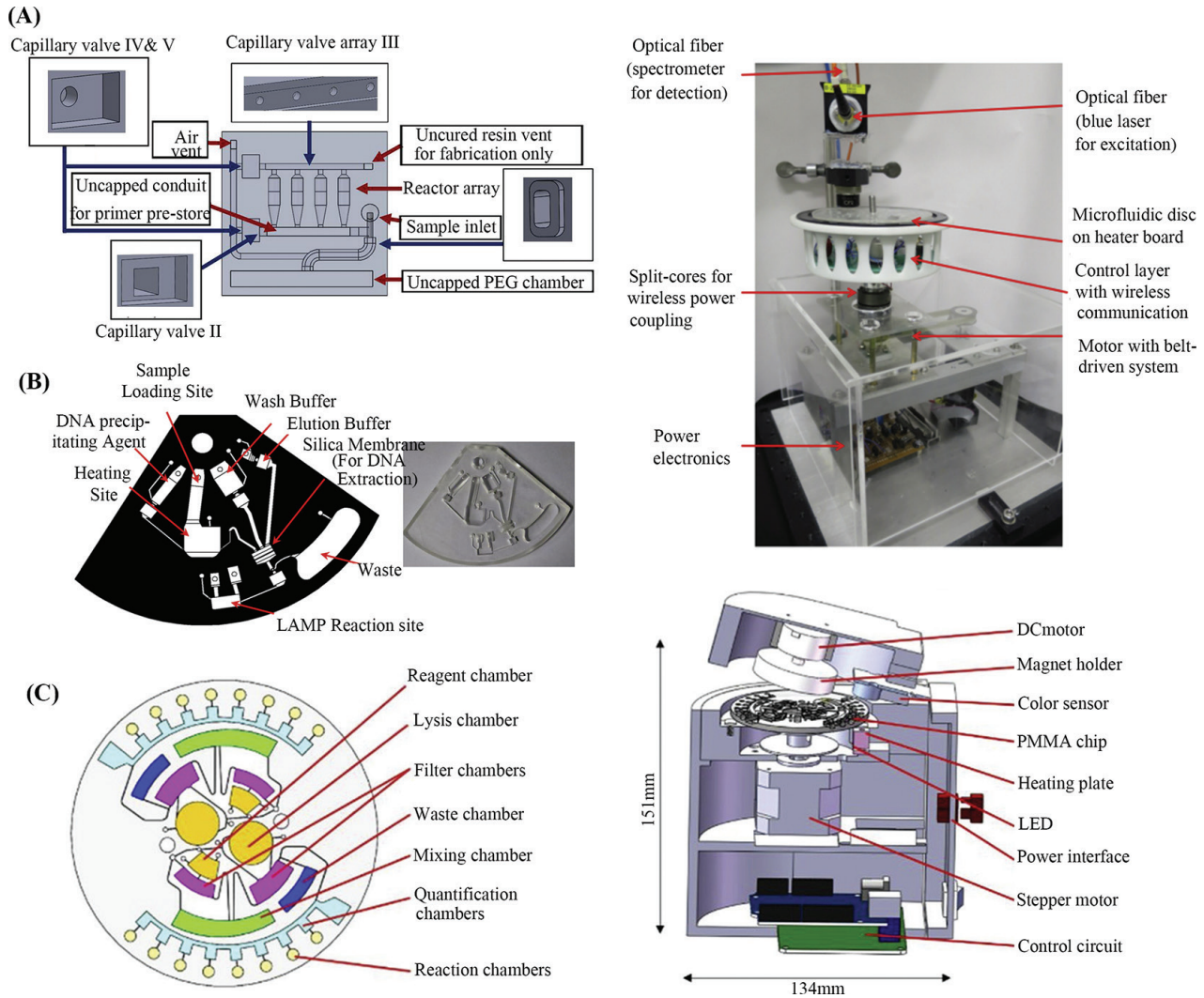


Figure 4: Microfluidic chips and temperature control devices for LAMP. (A) Portable device for multichannel real-time nucleic acid detection [72]. (B) Schematic diagram of the principle of the microfluidic chip (a quarter of the disk chip) for the whole process detection and schematic diagram of the matching automatic detection platform [73]. (C) Schematic diagram of disk microfluidic chip based on LAMP and its supporting automatic detection platform [74]. LAMP, loop-mediated adiabatic amplification; LED, light-emitting diode.

by utilizing fluorescent probes as reagents during the amplification process. These probes enable the amplified nucleic acid to exhibit a fluorescence reaction upon exposure to excitation light sources of specific wavelengths. Compared to other optical detection techniques, fluorescence detection excels in terms of sensitivity, specificity, and accuracy. Its high sensitivity enables the detection of nucleic acids at extremely low concentrations, facilitating precise quantitative analysis without being hindered by electromagnetic interference, pressure, or temperature fluctuations, thus ensuring high stability.

Furthermore, it is well-suited for the simultaneous detection of multiple samples [85]. On the contrary, electrochemical methods leverage the interaction between the nucleic acid molecules under investigation and the electrode surface. By closely monitoring changes in current, impedance, or potential associated with the electrochemical reaction involving the target nucleic acid, we can analyze the presence and concentration of the target nucleic acid within the sample. The electrochemical method offers simplicity in operating electrochemical sensors, facilitating the realization of portable detection [86].

Table 2: LAMP and PCR techniques in POCT devices

	LAMP	PCR
Equipment	Constant temperature equipment	PCR amplifier, thermal cycler
Reaction process	One-step amplification	Cyclic amplification
Amplification temperature	Constant temperature (60–65°C)	Denaturation (95°C), annealing (50–60°C), polymerization (72°C)
Reaction time	15–40 min	Depends on the temperature control rate of the thermal cycler
Primer design	4–6 primers, high design difficulty	2 primers, easy to design
Sensitivity	Higher sensitivity, but prone to false positives and more expensive	High sensitivity and strong specificity
Reagent prices	Higher sensitivity, but prone to false positives and more expensive	Cheap

LAMP, loop-mediated adiabatic amplification; PCR, polymerase chain reaction; POCT, point-of-care testing.

Nevertheless, overall technology remains immature and is still undergoing improvements. Factors such as ionic strength and pH of the sample can potentially interfere with detection outcomes. Additionally, certain electrochemical sensors exhibit sensitivity to environmental conditions, including temperature and humidity, thus limiting their applicability in various scenarios. The subsequent review will solely concentrate on POCT devices for nucleic acid detection via fluorescence detection approaches.

During the fluorescence detection process, the establishment of an optical system is paramount. Parameters such as wavelength selection, fluorescence emission efficiency, optical path efficiency, imaging quality, reproducibility, and stability have a direct impact on the accuracy and sensitivity of test results. Inaccurate optical alignment or inefficient optical elements will significantly reduce the sensitivity and reproducibility of the results, and the probe will degrade over time or be quenched by environmental factors (such as pH or ionic strength variants). These may lead to false negatives. When using multiple probes in multiple detection, signal crosstalk or spectral overlap may lead to interference, thus affecting the sensitivity and specificity of both. The use of probes with different spectra and advanced filters is essential for separating signals from different fluorophores. In addition, the dual emission proportional fluorescence strategy with two independent emission channels can minimize the spectral overlap and improve the detection accuracy [87]. Typically, the optical system utilized for fluorescence detection comprises a light source, focusing lens, filter, dichroic mirror, and photoelectric sensor [88]. Selecting the appropriate

excitation light based on the specific target is crucial. Commonly used excitation light sources include xenon lamps, mercury lamps, lasers, and high-power light-emitting diode (LED). Each type of light source possesses unique characteristics, thereby dictating their respective application scenarios. For instance, xenon lamps [89] and mercury lamps possess high luminous intensity, rendering them suitable for high-power analysis systems. However, their bulky size poses a challenge for integration. Lasers [90], on the contrary, exhibit high brightness and excellent monochromaticity but come at a hefty price. In contrast to these three light sources, LEDs are a more viable option for POCT devices, offering a wide color gamut, low cost, compact size, energy efficiency, and long service life [91]. In terms of photoelectric sensors, the mainstream photodetectors include avalanche photodiodes (APDs), charge-coupled devices (CCDs), photomultiplier tubes (PMTs), and photodiodes (PDs). APDs have the advantage of high gain and high quantum efficiency but narrow spectral response range; CCDs can scan all fluorescence signals simultaneously but with low sensitivity; and PMTs have the fast response and low noise regardless of the price advantages, and it is a good choice [92]. In contrast, PD with high sensitivity, high signal-to-noise, and low cost is more suitable for POCT devices.

Fang et al. [93] and Wang et al. [94] designed a current-optical dual negative feedback LED driver circuit and developed two sets of highly integrated nucleic acid detection devices using one set and two sets of LEDs with different wavelengths, respectively, which integrated nucleic acid extraction, purification, amplification, and detection processes. The coefficient of

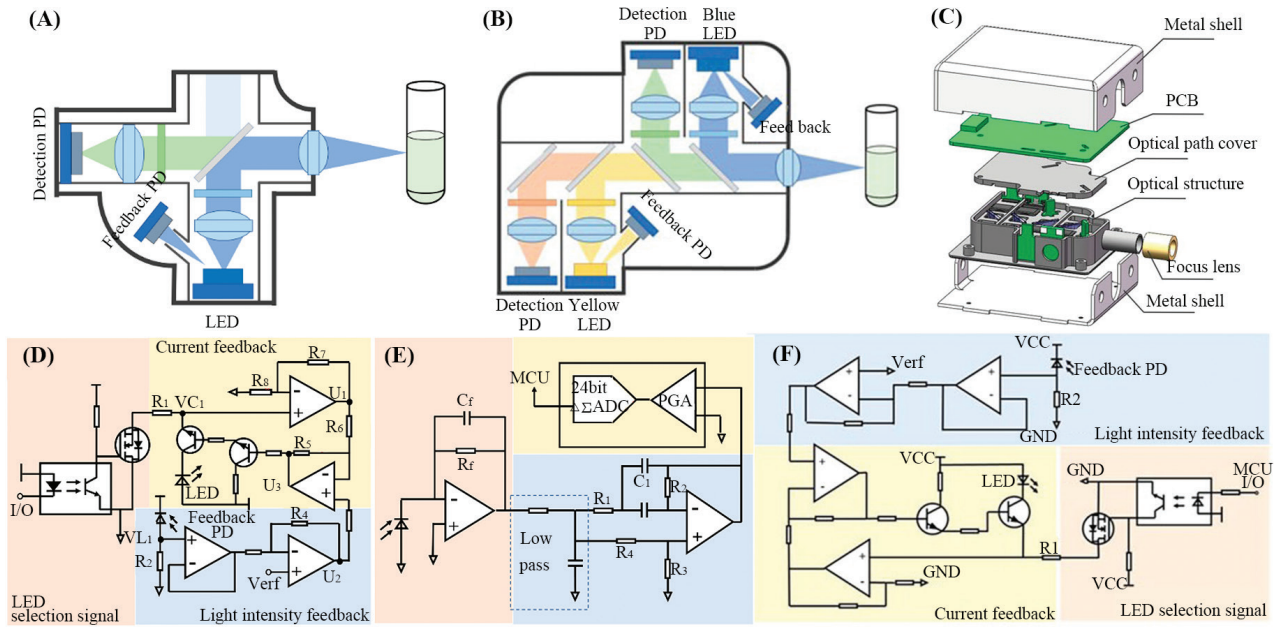


Figure 5: (A) Schematic diagram of optical path of single channel fluorescent module: schematic diagram of optical path blue indicates excitation, and green indicates the fluorescence emitted by the sample [94]. (B) Schematic diagram of optical path of dual channel fluorescent module: two excitation lights (blue and yellow) and two emission lights (green and orange) form a confocal optical path with the help of four dichroic mirrors [93]. (C) The three-dimensional (3D) illustration of the fluorescence module in an exploded view, which includes metal shell, PCB, optical path cover, optical structure, and focus lens [93]. (D) Schematic diagram of single channel current-optical double negative feedback led drive circuit, including three parts: current feedback, light intensity feedback, and LED selection signal [94]. (E) Schematic diagram of single channel photoelectric processing circuit, including: TIA, filter, and A/D conversion [94]. (F) The schematic diagram of dual channel circuit can be divided into three parts: current feedback, light intensity feedback, and led selection [93]. A/D, analog-to-digital; LED, light-emitting diode; PD, photodiode; TIA, transimpedance amplifier.

determination (R^2) of the two devices is >0.99 , and the sensitivity of the assay is comparable to that of a commercially available real-time PCR instrument (Step One Plus), even with better stability. The PNAD device uses two excitation light sources with different wavelengths and uses optical shielding principle and filter to build optical path to isolate excitation wavelength and emission wavelength. In addition, the use of time-resolved fluorescent or pulsed light sources helps to distinguish fluorescent signals from ambient light. Based on the principle of confocal optical path, two different wavelength excitation and emission optical paths are integrated into a single fluorescence detection module. Compared with the single wavelength emission, the dual emission proportional fluorescence strategy has a unique built-in self-calibration function, which can effectively avoid the interference of instrument parameters, fluctuation of light interference, uneven distribution of fluorescent substances,

and fluctuation of the microenvironment around the fluorescent source, improve the detection accuracy, expand the dynamic analysis range, and perform well in the field work outside the laboratory [95]. The two types of optical paths and circuits are shown in Figure 5. However, the optical path loss in the optical path will reduce the overall signal strength and affect the sensitivity and accuracy of detection. Using optical fiber can alleviate some of these problems by reducing path loss, but it will appropriately increase the complexity and cost of the system.

V. Conclusion and Prospect

Microfluidic technology has important application prospects in real-time nucleic acid detection devices. The rapid development of POCT devices for nucleic acid detection based on microfluidic technology has made it possible to perform nucleic acid detection in

resource limited environments. Microfluidic technology integrates basic operational units such as sample separation, reaction, and detection into a centimeter scale chip. On-chip nucleic acid amplification can increase the sensitivity of nucleic acid detection POCT devices. After amplification, the quantity information of nucleic acid can be converted into a series of physical signals such as optical signals and electrochemical signals. These signals are processed by POCT devices and output as results. In addition, microfluidic chips can perform multichannel and multisample monitoring [96], providing a feasible strategy for POCT devices to save more resources and time in resource limited environments. But there are still some challenges in its development and implementation.

a. Sensitivity and specificity

The microfluidic platforms can effectively extract and amplify nucleic acids, but it is still challenging to ensure that these POCT devices can detect low concentrations of nucleic acids in complex clinical samples (such as blood and sputum). The presence of inhibitors (such as proteins, salts, or metabolites) in the sample will interfere with nucleic acid extraction or PCR amplification, resulting in false negative or reduced detection accuracy [97]. It is essential to develop robust systems that can overcome these challenges while maintaining high robustness.

b. Integration

The POCT device for nucleic acid detection usually needs to integrate multiple steps of the detection process in a single, compact platform, including sample collection, nucleic acid extraction, amplification, and detection. This inevitably involves trade-offs between device size, function, and performance. As the size of the devices shrinks, the physical space required for reagent storage, thermal management (PCR or other amplification technologies), and necessary components such as sensors or detectors may be limited. Although miniaturization enhances portability, it also limits the ability to incorporate other functions or effectively execute certain programs [98]. Designing a system that is compact and capable of performing all necessary tasks without sacrificing performance is a major challenge. In addition, most POCT devices used for nucleic acid detection require external component power supply, data analysis, or connection (such as smart phones or computers). It is difficult to integrate these components into a single device without making the system too large or complex.

Powering devices in remote or resource limited settings can be challenging, especially for devices that need to work in an energy-scarce environment.

c. Cost in POCT device

The microfluidic system, micro electromechanical system (MEMS), and optical detection components need to be integrated into a compact portable system. These processes will significantly increase the production cost of POCT device. Moreover, the demand for high-quality components that can operate reliably in different environments and reagents that perform well in different environments further increases the total cost. General POCT devices are often customized for specific diseases, sample types, or certain specific environmental conditions. Customization of these POCT devices may require energy conversion components or reagents to obtain the best performance. In addition, the overall material sustainability of POCT devices should also be considered [99]. Adding environmentally friendly materials, such as biodegradable plastics or recycled polymers, to the construction of POCT devices will help reduce the impact on the environment. In the future, the waste management system can be integrated into the devices, such as the automatic treatment protocol of used components, which can help guide users to correctly and effectively dispose of materials. To sum up, manufacturers will face great challenges in developing and producing economical, efficient, and universal POCT devices that can be applied to a variety of scenarios.

d. User friendliness

For POCT device, ensuring user friendliness is a key factor for successful adoption, especially in clinics, remote areas, and even home-based nonlaboratory environments. POCT devices must be easy to use, require minimal training, and provide clear and reliable results. POCT devices for nucleic acid detection usually involve complex multistep processes, including sample processing, nucleic acid extraction, amplification (such as PCR), and detection. For users without technical background, it is a major challenge to have a simple and clear operation interface and as few operation steps as possible. Many devices require precise control of fluid flow, temperature, and reaction conditions, which may be difficult to manage manually. Ensuring that users can handle these tasks without advanced training while maintaining high performance and accuracy requires careful system

design and automation. Simplifying the interface and reducing the number of steps that users need to perform is critical to creating a user-friendly system. An intuitive user interface is essential to ensure that healthcare providers and even patients with minimal technical expertise can operate the device effectively. The human-computer interaction interface shall clearly guide the user to complete each step of the test process, from example to result interpretation. Designing a human-computer interaction interface that provides clear instructions, visual feedback, and easy to understand results is very important to improve the user experience [100]. In addition, the devices should include mechanisms such as visual or audible alarms to notify users of problems (e.g., insufficient sample size, incorrect reagent use, or device failure).

e. Field work performance

At present, POCT devices have made significant progress in the laboratory environment, but there are still many challenges to ensure that these devices can perform well in real application scenarios. In real application scenarios, especially in outdoor or remote areas, there will be many uncertain factors interfering with POCT performance. The collection of on-site samples may have different sources and have a high degree of complexity, so that the samples may contain impurities that affect the subsequent amplification and detection. There are huge temperature fluctuations and humidity changes, which will affect the stability of the devices and lead to inconsistent thermal cycle accuracy, thus affecting the amplification efficiency and sensitivity. In the detection process, interference signals generated by strong light, ultraviolet light, or other environmental light sources in the outdoor environment will affect the precision of the optical detection system of the devices, resulting in the decline of sensitivity [101]. There are still many challenges to improve the sample adaptability, amplification stability, and anti-interference ability of equipment in complex field environments.

In conclusion, although POCT nucleic acid detection device has broad development prospects, it still faces many challenges. This requires continuous research, innovation, and collaboration of multidisciplinary integration, and focus on improving the portability and intelligence of devices in combination with current new technologies.

Future POCT devices should be committed to making greater breakthroughs in ease of operation and automation. Many existing devices still require

operators to have certain professional knowledge, and the links of sample processing and data analysis are relatively complex. Future POCT devices must pay more attention to user friendliness, adopt more intuitive human-computer interaction interface, use less manual operation, and realize “fool type” operation. In addition, with the integration of artificial intelligence (AI) and machine learning technology, the devices should actively integrate with these technologies and strive to automate a series of processes such as sample collection, processing, and analysis, so as to further reduce the operation complexity and human error.

Real-time data monitoring and remote diagnosis: with the popularity of 5G and Internet of things (IoT) technology, future POCT devices will be able to transmit detection data in real time and connect with telemedicine platforms. This means that doctors or professionals can remotely monitor the detection process, timely obtain the detection results, and make rapid diagnosis and treatment decisions based on the data. This function is especially suitable for remote areas or emergency situations and can realize rapid disease screening and emergency response. However, this combination also has potential security and privacy issues, especially in clinical and sensitive environments involving patient data. Sensitive patient data may be intercepted or tampered with during transmission, which may result in personal health information being exposed to unauthorized third parties or altered diagnostic data, leading to incorrect diagnostic results [102]. These issues must be carefully addressed, and only through rigorous security testing and regular software updates to patch vulnerabilities can patient information be guaranteed. In addition, the device should be equipped with a secure boot process and authentication mechanism to ensure that the potential advantages of POCT-IoT integration are achieved without compromising patient safety, confidentiality, and trust.

These technologies have been applied to the rapid diagnosis of pathogens, gene mutation fragments, and cancer markers in POCT devices. To further improve the performance of nucleic acid detection POCT devices, the future development direction should focus on the complete automation of hardware control by the main control chip [103], the high integration of microfluidic modules in POCT devices [104], and the high intelligence of combining with smartphone software to perform nucleic acid analysis, provide results without the upper computer [105], and even try to combine with IoT technology to upload data to the cloud to form a data network [106].

By analyzing and processing uploaded data, disease monitoring and early warning can be achieved.

POCT devices have enormous potential to revolutionize healthcare services, especially in low- and middle-income countries. For example, during the COVID-19 pandemic, the necessity of POCT device research has been highlighted. These devices provide fast, cost-effective, and user-friendly diagnostic solutions that can be deployed in resource limited environments. However, due to the significant cost investment required for the early development of POCT devices, it is necessary for local governments and international organizations to work together to provide subsidies or financial assistance for the research and development of POCT devices [107]. In the early stages of development, it is possible to simplify the design of the flow channel structure, reduce manufacturing complexity, and use inexpensive but functionally reliable materials to replace traditional expensive materials, thereby reducing manufacturing and research and development costs. The driving force for the development of POCT devices comes from the commercialization of POCT devices developed by relevant research and development devices. Commercial POCT devices should be simpler and more efficient to use and provide accurate and reliable detection results. Enable clinical doctors to easily access diagnostic information at a glance. In addition, before commercialization, the devices must comply with international regulatory standards for diagnostic tools, which is extremely important for ensuring the quality and performance of the devices and ensuring the confidentiality of patient privacy. This requires developers to comply with the ISO 13485 standard when researching and designing, ensuring that the device can meet user needs and regulatory requirements throughout its entire lifecycle. Develop clear devices specifications and implement risk management processes in accordance with ISO 14971 to identify, assess, and mitigate potential risks, particularly those related to patient safety, misdiagnosis, or devices failure. Devices related to IoT technology also need to ensure encryption of information transmission to ensure compliance with global data protection regulations [108]. Before commercialization, it is essential to conduct validation in actual application scenarios to ensure that the device can function properly in different scenarios. When quantifying production, relevant standards should be strictly followed to ensure consistent devices quality. Even after actual circulation in the market, it is necessary to actively collect adverse event reports and user feedback, regularly

reevaluate the performance of the devices to ensure that it can continue to comply.

Acknowledgments

This work was supported by grants from the equipment research and development projects of the Chinese Academy of Sciences (PTYQ2024YZ0010), the Science and Technology Commission of Shanghai Municipality Project (XTCX-KJ-2024-038), the Natural Science Foundation of Hebei Province (F2015209308), the Collaborative Education Project of the Ministry of Education (202174021, 220603632225737), and the Hebei Provincial Graduate Demonstration Course Construction Project (KCJSX2021061).

Conflict of Interest

There are no conflicts to declare.

Abbreviations

ADC, Analog-to-Digital Converter; COVID-19, Coronavirus Disease 2019; DNA, Deoxyribonucleic Acid; GND, Ground; MBs, Magnetic Beads; MCU, Micro Control Unit; PCB, Printed Circuit Board; PDMS, Polydimethylsiloxane; PEG, Polyethylene glycol; PGA, Polyglycolideacid; PMMA, Polymethyl Methacrylate; PNAD, portable nucleic acid detection (PNAD is the name of the device developed in the 93rd reference); qPCR, quantitative Polymerase Chain Reaction; RNA, Ribonucleic Acid; VCC, Volt Current Condenser (VCC means the supply voltage in the circuit).

References

- [1] Mangal, M.; Bansal, S.; Sharma, S.K.; Gupta, R.K. Molecular Detection of Foodborne Pathogens: A Rapid and Accurate Answer to Food Safety. *Crit Rev Food Sci Nutr* 2016, 56, 1568–1584, doi:10.1080/10408398.2013.782483.
- [2] Wang, Y.; Wang, C.; Zhou, Z.; Si, J.; Li, S.; Zeng, Y.; Deng, Y.; Chen, Z. Advances in Simple, Rapid, and Contamination-Free Instantaneous Nucleic Acid Devices for Pathogen Detection. *Biosensors (Basel)* 2023, 13, 732, doi:10.3390/bios13070732.
- [3] Phys. Rev. Lett. 120, 198001 (2018) - Elastohydrodynamic Lift at a Soft Wall Available online: <https://journals.aps.org/prl/abstract/10.1103/>

PhysRevLett.120.198001 (accessed on 6 December 2024).

[4] The Cost-Effectiveness of Point of Care Testing in a General Practice Setting: Results from a Randomised Controlled Trial | BMC Health Services Research | Full Text Available online: <https://bmchealthservres.biomedcentral.com/articles/10.1186/1472-6963-10-165> (accessed on 6 December 2024).

[5] Yeh, E.-C.; Fu, C.-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, doi:10.1126/sciadv.1501645.

[6] Kim, K.R.; Yeo, W.-H. Advances in Sensor Developments for Cell Culture Monitoring. *BMEMat* 2023, 1, e12047, doi:10.1002/bmm2.12047.

[7] Beltrán-Pavez, C.; Alonso-Palomares, L.A.; Valiente-Echeverría, F.; Gaggero, A.; Soto-Rifo, R.; Barriga, G.P. Accuracy of a RT-qPCR SARS-CoV-2 Detection Assay without Prior RNA Extraction. *J Virol Methods* 2021, 287, 113969, doi:10.1016/j.jviromet.2020.113969.

[8] Paul, R.; Ostermann, E.; Wei, Q. Advances in Point-of-Care Nucleic Acid Extraction Technologies for Rapid Diagnosis of Human and Plant Diseases. *Biosens Bioelectron* 2020, 169, 112592, doi:10.1016/j.bios.2020.112592.

[9] Abraham, G.R.; Chaderjian, A.S.; Nguyen, A.B.N.; Wilken, S.; Saleh, O.A. Nucleic Acid Liquids. *Rep. Prog. Phys.* 2024, 87, 066601, doi:10.1088/1361-6633/ad4662.

[10] Wiraswati, H.L.; Ma'ruf, I.F.; Ekawardhani, S.; Faridah, L.; Laelalugina, A.; Septanto, H.; Djati, I.D.; Gaffar, S.; Awaludin, A. Optimization of Nucleic Acid Extraction Methods for Rapid Detection in Pandemic Situations or Diseases with High Prevalence. *Journal of Pharmaceutical Analysis* 2023, 13, 1577–1579, doi:10.1016/j.jpha.2023.08.005.

[11] Li, P.; Li, M.; Yue, D.; Chen, H. Solid-phase Extraction Methods for Nucleic Acid Separation. A Review. *J of Separation Science* 2022, 45, 172–184, doi:10.1002/jssc.202100295.

[12] Fan, Y.; Dai, R.; Guan, X.; Lu, S.; Yang, C.; Lv, X.; Li, X. Rapid Automatic Nucleic Acid Purification System Based on Gas–Liquid Immiscible Phase. *Journal of Separation Science* 2023, 46, 2200801, doi:10.1002/jssc.202200801.

[13] Wu, Q.; Jin, W.; Zhou, C.; Han, S.; Yang, W.; Zhu, Q.; Jin, Q.; Mu, Y. Integrated Glass Microdevice for Nucleic Acid Purification, Loop-Mediated Isothermal Amplification, and Online Detection. *Anal. Chem.* 2011, 83, 3336–3342, doi:10.1021/ac103129e.

[14] Ji, H.M.; Samper, V.; Chen, Y.; Hui, W.C.; Lye, H.J.; Mustafa, F.B.; Lee, A.C.; Cong, L.; Heng, C.K.; Lim, T.M. DNA Purification Silicon Chip. *Sensors and Actuators A: Physical* 2007, 139, 139–144, doi:10.1016/j.sna.2007.05.033.

[15] Easley, C.J.; Karlinsey, J.M.; Bienvenue, J.M.; Legendre, L.A.; Roper, M.G.; Feldman, S.H.; Hughes, M.A.; Hewlett, E.L.; Merkel, T.J.; Ferrance, J.P.; et al. A Fully Integrated Microfluidic Genetic Analysis System with Sample-in-Answer-out Capability. *Proc Natl Acad Sci U S A* 2006, 103, 19272–19277, doi:10.1073/pnas.0604663103.

[16] Hu, F.; Li, J.; Peng, N.; Li, Z.; Zhang, Z.; Zhao, S.; Duan, M.; Tian, H.; Li, L.; Zhang, P. Rapid Isolation of cfDNA from Large-Volume Whole Blood on a Centrifugal Microfluidic Chip Based on Immiscible Phase Filtration. *Analyst* 2019, 144, 4162–4174, doi:10.1039/C9AN00493A.

[17] Jamshaid, T.; Neto, E.T.T.; Eissa, M.M.; Zine, N.; Kunita, M.H.; El-Salhi, A.E.; Elaissari, A. Magnetic Particles: From Preparation to Lab-on-a-Chip, Biosensors, Microsystems and Microfluidics Applications. *TrAC Trends in Analytical Chemistry* 2016, 79, 344–362, doi:10.1016/j.trac.2015.10.022.

[18] Song, H.; Khan, M.; Yu, L.; Wang, Y.; Lin, J.-M.; Hu, Q. Construction of Liquid Crystal-Based Sensors Using Enzyme-Linked Dual-Functional Nucleic Acid on Magnetic Beads. *Anal. Chem.* 2023, 95, 13385–13390, doi:10.1021/acs.analchem.3c03163.

[19] Tang, C.; He, Z.; Liu, H.; Xu, Y.; Huang, H.; Yang, G.; Xiao, Z.; Li, S.; Liu, H.; Deng, Y.; et al. Application of Magnetic Nanoparticles in Nucleic Acid Detection. *J Nanobiotechnology* 2020, 18, 62, doi:10.1186/s12951-020-00613-6.

[20] Wu, M.; Huang, Y.; Huang, Y.; Wang, H.; Li, M.; Zhou, Y.; Zhao, H.; Lan, Y.; Wu, Z.; Jia, C.; et al. Droplet Magnetic-Controlled Microfluidic Chip Integrated Nucleic Acid Extraction and Amplification for the Detection of Pathogens and Tumor Mutation Sites. *Analytica Chimica Acta* 2023, 1271, 341469, doi:10.1016/j.aca.2023.341469.

[21] Shen, H.; Dong, L.; Gao, Y.; Wang, X.; Dai, X. Integrated Microwell Array-Based Microfluidic Chip with a Hand-Held Smartphone-Controlled Device for Nucleic Acid Detection. *Analytical Chemistry* 2023, doi:10.1021/acs.analchem.3c03525.

[22] Li, J.; Gao, Z.; Jia, C.; Cai, G.; Feng, S.; Wu, M.; Zhao, H.; Yu, J.; Bao, F.; Cong, H.; et al. Simultaneous Detection of Multiple Respiratory Pathogens Using an Integrated Microfluidic Chip. *Anal Chem* 2024, doi:10.1021/acs.analchem.4c00990.

[23] Grönland, T.-A.; Rangsten, P.; Nese, M.; Lang, M. Miniaturization of Components and

Systems for Space Using MEMS-Technology. *Acta Astronautica* 2007, 61, 228–233, doi:10.1016/j.actaastro.2007.01.029.

[24] Zhang, Z.; Deng, X.; Zhang, W.; Chen, K.; Su, Y.; Gao, C.; Gong, D.; Zhu, L.; Cai, J. Manipulation of Magnetic Beads for Actively Capturing *Vibrio Parahaemolyticus* and Nucleic Acid Based on Microfluidic System. *Biomicrofluidics* 2024, 18, 034104, doi:10.1063/5.0193442.

[25] Li, Y.; Liu, S.; Wang, Y.; Wang, Y.; Li, S.; He, N.; Deng, Y.; Chen, Z. Research on a Magnetic Separation-Based Rapid Nucleic Acid Extraction System and Its Detection Applications. *Biosensors* 2023, 13, 903, doi:10.3390/bios13100903.

[26] Yang, K.; Zhou, J.; Zhao, J.; Liu, L.; Hua, C.; Hong, C.; Wang, M.; Hu, A.; Zhang, W.; Cui, J.; et al. Mobile Lab: A Novel Pathogen Assay Using the Nucleic Acid Automatic Assay System Assisted by a Self-Contained Microfluidic Cassette and Chitosan Decorating Magnetic Particles. *Sensors and Actuators B: Chemical* 2024, 419, 136413, doi:10.1016/j.snb.2024.136413.

[27] Seong, H.; Park, J.; Bae, M.; Shin, S. Rapid and Efficient Extraction of Cell-Free DNA Using Homobifunctional Crosslinkers. *Biomedicines* 2022, 10, 1883, doi:10.3390/biomedicines10081883.

[28] Pearlman, S.I.; Leelawong, M.; Richardson, K.A.; Adams, N.M.; Russ, P.K.; Pask, M.E.; Wolfe, A.E.; Wessely, C.; Haselton, F.R. Low-Resource Nucleic Acid Extraction Method Enabled by High-Gradient Magnetic Separation. *ACS Appl. Mater. Interfaces* 2020, 12, 12457–12467, doi:10.1021/acsami.9b21564.

[29] Sciuto, E.L.; Petralia, S.; Calabrese, G.; Conoci, S. An Integrated Biosensor Platform for Extraction and Detection of Nucleic Acids., doi:10.1002/bit.27290.

[30] Li, S.; Wan, C.; Xiao, Y.; Liu, C.; Zhao, X.; Zhang, Y.; Yuan, H.; Wu, L.; Qian, C.; Li, Y.; et al. Multiple On-Line Active Valves Based Centrifugal Microfluidics for Dynamic Solid-Phase Enrichment and Purification of Viral Nucleic Acid. *Lab Chip* 2024, 24, 3158–3168, doi:10.1039/D4LC00074A.

[31] Zhao, X.; Huang, Y.; Li, X.; Yang, W.; Lv, Y.; Sun, W.; Huang, J.; Mi, S. Full Integration of Nucleic Acid Extraction and Detection into a Centrifugal Microfluidic Chip Employing Chitosan-Modified Microspheres. *Talanta* 2022, 250, 123711, doi:10.1016/j.talanta.2022.123711.

[32] Schneider, L.; Cui, F.; Tripathi, A. Isolation of Target DNA Using Synergistic Magnetic Bead Transport and Electrokinetic Flow. *Biomicrofluidics* 2021, 15, 024104, doi:10.1063/5.0045307.

[33] Yamaguchi, A.; Matsuda, K.; Uehara, M.; Honda, T.; Saito, Y. A Novel Automated Device for

Rapid Nucleic Acid Extraction Utilizing a Zigzag Motion of Magnetic Silica Beads. *Analytica Chimica Acta* 2016, 906, 1–6, doi:10.1016/j.aca.2015.10.011.

[34] Huang, J.; Xia, L.; Xiao, X.; Li, G. A Recyclable PDMS Microfluidic Surface-Enhanced Raman Scattering Cu/AgNP Chip for the Analysis of Sulfadiazine in Aquatic Products. *New J. Chem.* 2024, 48, 11457–11464, doi:10.1039/D4NJ01825G.

[35] Lyu, C.; Jiang, Y.; Dai, Z.; Xu, X.; Cai, Y.; Liang, B.; Zhou, C.; Ye, X.; Wang, J. Optimizing Magnetic Separation and Cleaning Module in Fully Automated Chemiluminescence Immunoassay Analyzer Using a Special Arrangement of Spliced Magnets and a Three-Stage Magnetic Bead Collection Method. *Magnetochemistry* 2024, 10, 75, doi:10.3390/magnetochemistry10100075.

[36] Nisar, N.; Shah, R.; Zada, F.; Khan, B.; Aziz, S.; Rehman, N.; Soonmin, H.; Ahmad, N.; Khan, M.; Hanzala Civil Engineering Journal Novel Ni/ZnO Nanocomposites for the Effective Photocatalytic Degradation of Malachite Green Dye. 2024, 10, doi:10.28991/CEJ-2024-010-08-011.

[37] Zhang, J.; Su, X.; Xu, J.; Wang, J.; Zeng, J.; Li, C.; Chen, W.; Li, T.; Min, X.; Zhang, D.; et al. A Point of Care Platform Based on Microfluidic Chip for Nucleic Acid Extraction in Less than 1 Minute. *Biomicrofluidics* 2019, 13, 034102, doi:10.1063/1.5088552.

[38] Ji, T.; Liu, Z.; Wang, G.; Guo, X.; Akbar Khan, S.; Lai, C.; Chen, H.; Huang, S.; Xia, S.; Chen, B.; et al. Detection of COVID-19: A Review of the Current Literature and Future Perspectives. *Biosens Bioelectron* 2020, 166, 112455, doi:10.1016/j.bios.2020.112455.

[39] Saiki, R.K.; Gelfand, D.H.; Stoffel, S.; Scharf, S.J.; Higuchi, R.; Horn, G.T.; Mullis, K.B.; Erlich, H.A. Primer-Directed Enzymatic Amplification of DNA with a Thermostable DNA Polymerase. *Science* 1988, 239, 487–491, doi:10.1126/science.2448875.

[40] Boehme, C.C.; Nabeta, P.; Hillemann, D.; Nicol, M.P.; Shenai, S.; Krapp, F.; Allen, J.; Tahirli, R.; Blakemore, R.; Rustomjee, R.; et al. Rapid Molecular Detection of Tuberculosis and Rifampin Resistance. *N Engl J Med* 2010, 363, 1005–1015, doi:10.1056/NEJMoa0907847.

[41] Chen, S.; Sun, Y.; Fan, F.; Chen, S.; Zhang, Y.; Zhang, Y.; Meng, X.; Lin, J.-M. Present Status of Microfluidic PCR Chip in Nucleic Acid Detection and Future Perspective. *TrAC Trends in Analytical Chemistry* 2022, 157, 116737, doi:10.1016/j.trac.2022.116737.

[42] Ling, W.; Zhou, W.; Cui, J.; Shen, Z.; Wei, Q.; Chu, X. Experimental Study on the Heating/Cooling and Temperature Uniformity Performance of the Microchannel Temperature Control Device for Nucleic Acid PCR Amplification Reaction of COVID-19. *Applied*

Thermal Engineering 2023, 226, 120342, doi:10.1016/j.applthermaleng.2023.120342.

[43] İnce, G.T.; Yükksekaya, M.; Haberal, O.E. Micro-Polymerase Chain Reaction for Point-of-Care Detection and beyond: A Review Microfluidics and Nanofluidics. *Microfluid Nanofluid* 2023, 27, 68, doi:10.1007/s10404-023-02677-w.

[44] Huang, S.; An, Y.; Xi, B.; Gong, X.; Chen, Z.; Shao, S.; Ge, S.; Zhang, J.; Zhang, D.; Xia, N. Ultra-Fast, Sensitive and Low-Cost Real-Time PCR System for Nucleic Acid Detection. *Lab Chip* 2023, 23, 2611–2622, doi:10.1039/D3LC00174A.

[45] Qiu, X.; Ge, S.; Gao, P.; Li, K.; Yang, S.; Zhang, S.; Ye, X.; Xia, N.; Qian, S. A Smartphone-Based Point-of-Care Diagnosis of H1N1 with Microfluidic Convection PCR. *Microsyst Technol* 2017, 23, 2951–2956, doi:10.1007/s00542-016-2979-z.

[46] Li, Y.; Zhang, C.; Xing, D. Integrated Microfluidic Reverse Transcription-Polymerase Chain Reaction for Rapid Detection of Food- or Waterborne Pathogenic Rotavirus. *Analytical Biochemistry* 2011, 415, 87–96, doi:10.1016/j.ab.2011.04.026.

[47] Pham, Q.N.; Trinh, K.T.L.; Tran, N.K.S.; Park, T.-S.; Lee, N.Y. Fabrication of 3D Continuous-Flow Reverse-Transcription Polymerase Chain Reaction Microdevice Integrated with on-Chip Fluorescence Detection for Semi-Quantitative Assessment of Gene Expression. *Analyst* 2018, 143, 5692–5701, doi:10.1039/c8an01739e.

[48] You, D.J.; Tran, P.L.; Kwon, H.-J.; Patel, D.; Yoon, J.-Y. Very Quick Reverse Transcription Polymerase Chain Reaction for Detecting 2009 H1N1 Influenza A Using Wire-Guide Droplet Manipulationst. *Faraday Discuss* 2011, 149, 159–170; discussion 227–245, doi:10.1039/c005326k.

[49] Ouyang, Y.; Duarte, G.R.M.; Poe, B.L.; Riehl, P.S.; dos Santos, F.M.; Martin-Didonet, C.C.G.; Carrilho, E.; Landers, J.P. A Disposable Laser Print-Cut-Laminate Polyester Microchip for Multiplexed PCR via Infra-Red-Mediated Thermal Control. *Analytica Chimica Acta* 2015, 901, 59–67, doi:10.1016/j.aca.2015.09.042.

[50] Shaw, K.J.; Docker, P.T.; Yelland, J.V.; Dyer, C.E.; Greenman, J.; Greenway, G.M.; Haswell, S.J. Rapid PCR Amplification Using a Microfluidic Device with Integrated Microwave Heating and Air Impingement Cooling. *Lab Chip* 2010, 10, 1725–1728, doi:10.1039/C000357N.

[51] Chen, X.; Song, L.; Assadsangabi, B.; Fang, J.; Mohamed Ali, M.S.; Takahata, K. Wirelessly Addressable Heater Array for Centrifugal Microfluidics and Escherichia Coli Sterilization. In *Proceedings of the 2013 35th Annual International Conference of the IEEE*

Engineering in Medicine and Biology Society (EMBC); July 2013; pp. 5505–5508.

[52] Fernández-Carballo, B.L.; McBeth, C.; McGuinness, 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, doi:10.1007/s00216-017-0689-8.

[53] Qiu, X.; Shu, J.L.; Baysal, O.; Wu, J.; Qian, S.; Ge, S.; Li, K.; Ye, X.; Xia, N.; Yu, D. Real-Time Capillary Convective PCR Based on Horizontal Thermal Convection. *Microfluid Nanofluid* 2019, 23, 39, doi:10.1007/s10404-019-2207-0.

[54] Trinh, K.T.L.; Lee, N.Y. A Portable Microreactor with Minimal Accessories for Polymerase Chain Reaction: Application to the Determination of Foodborne Pathogens. *Microchim Acta* 2017, 184, 4225–4233, doi:10.1007/s00604-017-2451-5.

[55] Nouwairi, R.L.; Cunha, L.L.; Turiello, R.; Scott, O.; Hickey, J.; Thomson, S.; Knowles, S.; Chapman, J.D.; Landers, J.P. Ultra-Rapid Real-Time Microfluidic RT-PCR Instrument for Nucleic Acid Analysis. *Lab Chip* 2022, 22, 3424–3435, doi:10.1039/d2lc00495j.

[56] An, Y.-Q.; Huang, S.-L.; Xi, B.-C.; Gong, X.-L.; Ji, J.-H.; Hu, Y.; Ding, Y.-J.; Zhang, D.-X.; Ge, S.-X.; Zhang, J.; et al. Ultrafast Microfluidic PCR Thermocycler for Nucleic Acid Amplification. *Micromachines (Basel)* 2023, 14, 658, doi:10.3390/mi14030658.

[57] Trauba, J.M.; Wittwer, C.T. Microfluidic Extreme PCR: <1 Minute DNA Amplification in a Thin Film Disposable. *Journal of Biomedical Science and Engineering* 2017, 10, 219–231, doi:10.4236/jbise.2017.105017.

[58] Yeom, D.; Kim, J.; Kim, S.; Ahn, S.; Choi, J.; Kim, Y.; Koo, C. A Thermocycler Using a Chip Resistor Heater and a Glass Microchip for a Portable and Rapid Microchip-Based PCR Device. *Micromachines (Basel)* 2022, 13, 339, doi:10.3390/mi13020339.

[59] Oyewola, O.M.; Awonusi, A.A.; Ismail, O.S. Performance Optimization of Step-Like Divergence Plenum Air-Cooled Li-Ion Battery Thermal Management System Using Variable-Step-Height Configuration. *Emerging Science Journal* 2024, 8, 795–814, doi:10.28991/ESJ-2024-08-03-01.

[60] Salman, A.; Carney, H.; Bateson, S.; Ali, Z. Shunting Microfluidic PCR Device for Rapid Bacterial Detection. *Talanta* 2020, 207, 120303, doi:10.1016/j.talanta.2019.120303.

[61] Petralia, Salvatore & Castagna, Maria Eloisa & Spata, Massimo & Amore, Maria & Conoci, Sabrina. (2016). A Point of Care Real Time PCR Platform Based on Silicon Technology. *Biosensors Journal*. 5. doi:10.4172/2090-4967.1000136.

- [62] Lim, J.; Jeong, S.; Kim, M.; Lee, J.-H. Battery-Operated Portable PCR System with Enhanced Stability of Pt RTD. *PLOS ONE* 2019, 14, e0218571, doi:10.1371/journal.pone.0218571.
- [63] Yang, Y.; Chen, Y.; Tang, H.; Zong, N.; Jiang, X. Microfluidics for Biomedical Analysis. *Small Methods* 2020, 4, 1900451, doi:10.1002/smt.201900451.
- [64] Si, H.; Xu, G.; Jing, F.; Sun, P.; Zhao, D.; Wu, D. A Multi-Volume Microfluidic Device with No Reagent Loss for Low-Cost Digital PCR Application. *Sensors and Actuators B: Chemical* 2020, 318, 128197, doi:10.1016/j.snb.2020.128197.
- [65] Effect of Gadolinium Doping on the Structure of $Ce_{1-x}Gd_xO_{2-x/2}$ Solid Solutions Prepared by Ionic Gelation Approach | Ilcheva | *Emerging Science Journal* Available online: <https://www.ijournalse.org/index.php/ESJ/article/view/2504> (accessed on 6 December 2024).
- [66] 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, doi:10.1016/j.bios.2020.112674.
- [67] Notomi, T.; Okayama, H.; Masubuchi, H.; Yonekawa, T.; Watanabe, K.; Amino, N.; & Hase, T. (2000). Loop-mediated isothermal amplification of DNA. *Nucleic acids research*, 28(12), E63. <https://doi.org/10.1093/nar/28.12.e63>.
- [68] Rabe, B. A., & Cepko, C. (2020). SARS-CoV-2 detection using isothermal amplification and a rapid, inexpensive protocol for sample inactivation and purification. *Proceedings of the National Academy of Sciences of the United States of America*, 117(39), 24450–24458. <https://doi.org/10.1073/pnas.2011221117>.
- [69] Suea-Ngam, A.; Bezing, L.; Mateescu, B.; Howes, P.D.; deMello, A.J.; Richards, D.A. Enzyme-Assisted Nucleic Acid Detection for Infectious Disease Diagnostics: Moving toward the Point-of-Care. *ACS Sens* 2020, 5, 2701–2723, doi:10.1021/acssensors.0c01488.
- [70] Jiang, L.; Lan, X.; Ren, L.; Yang, M.; Wei, B.; Wang, Y. Design of a Digital LAMP Detection Platform Based on Droplet Microfluidic Technology. *Micromachines (Basel)* 2023, 14, 1077, doi:10.3390/mi14051077.
- [71] Xiao, B.; Zhao, R.; Wang, N.; Zhang, J.; Sun, X.; Huang, F.; Chen, A. Integrating Microneedle DNA Extraction to Hand-Held Microfluidic Colorimetric LAMP Chip System for Meat Adulteration Detection. *Food Chem* 2023, 411, 135508, doi:10.1016/j.foodchem.2023.135508.
- [72] El-Tholoth, M.; Bai, H.; Mauk, M.G.; Saif, L.; Bau, H.H. A Portable, 3D Printed, Microfluidic Device for Multiplexed, Real Time, Molecular Detection of the Porcine Epidemic Diarrhea Virus, Transmissible Gastroenteritis Virus, and Porcine Deltacoronavirus at the Point of Need. *Lab Chip* 2021, 21, 1118–1130, doi:10.1039/d0lc01229g.
- [73] Loo, J.; Kwok, H.C.; Leung, C.C.H.; Wu, S.Y.; Law, I.L.G.; Cheung, Y.K.; Cheung, Y.Y.; Chin, M.L.; Kwan, P.; Hui, M.; et al. Sample-to-Answer on Molecular Diagnosis of Bacterial Infection Using Integrated Lab-on-a-Disc. *Biosens Bioelectron* 2017, 93, 212–219, doi:10.1016/j.bios.2016.09.001.
- [74] Liu, D.; Zhu, Y.; Li, N.; Lu, Y.; Cheng, J.; Xu, Y. A Portable Microfluidic Analyzer for Integrated Bacterial Detection Using Visible Loop-Mediated Amplification. *Sensors and Actuators B: Chemical* 2020, 310, 127834, doi:10.1016/j.snb.2020.127834.
- [75] Gansen, A.; Herrick, A.M.; Dimov, I.K.; Lee, L.P.; Chiu, D.T. Digital LAMP in a Sample Self-Digitization (SD) Chip. *Lab Chip* 2012, 12, 2247–2254, doi:10.1039/C2LC21247A.
- [76] Rane, T.D.; Chen, L.; Zec, H.C.; Wang, T.-H. Microfluidic Continuous Flow Digital Loop-Mediated Isothermal Amplification (LAMP). *Lab Chip* 2015, 15, 776–782, doi:10.1039/C4LC01158A.
- [77] Xie, M.; Chen, T.; Cai, Z.; Lei, B.; Dong, C. A Digital Microfluidic Platform Coupled with Colorimetric Loop-Mediated Isothermal Amplification for on-Site Visual Diagnosis of Multiple Diseases. *Lab Chip* 2023, 23, 2778–2788, doi:10.1039/d2lc01156e.
- [78] Daposang, E.S.; Hasanah, F.; Silaban, D. PROFILE OF PULMONARY AND EXTRA PULMONARY TUBERCULOSIS USE GENEXPER AT THE PIRNGADI HOSPITAL MEDAN. *BIOLINK (Jurnal Biologi Lingkungan Industri Kesehatan)* 2021, 8, 44–52, doi:10.31289/biolink.v8i1.4638.
- [79] Smithgall, M.C.; Scherberkova, I.; Whittier, S.; Green, D.A. Comparison of Cepheid Xpert Xpress and Abbott ID Now to Roche Cobas for the Rapid Detection of SARS-CoV-2. *Journal of Clinical Virology* 2020, 128, 104428, doi:10.1016/j.jcv.2020.104428.
- [80] Wei, Y.-J.; Zhao, Y.-N.; Zhang, X.; Wei, X.; Chen, M.-L.; Chen, X.-W. Biochemical Analysis Based on Optical Detection Integrated Microfluidic Chip. *TrAC Trends in Analytical Chemistry* 2023, 158, 116865, doi:10.1016/j.trac.2022.116865.
- [81] Zhang, L.; Huang, B.; Jin, J.; Li, Y.; Gu, N. Advances in Nanoprobes-Based Immunoassays. *BMEMat* 2024, 2, e12057, doi:10.1002/bmm2.12057.
- [82] Xu, J.; Tang, Q.; Zhang, R.; Chen, H.; Khoo, B.L.; Zhang, X.; Chen, Y.; Yan, H.; Li, J.; Shao, H.; et al. Sensitive Detection of microRNAs

Using Polyadenine-Mediated Fluorescent Spherical Nucleic Acids and a Microfluidic Electrokinetic Signal Amplification Chip. *J Pharm Anal* 2022, 12, 808–813, doi:10.1016/j.jpha.2022.05.009.

[83] Yao, Y.; Zhao, N.; Jing, W.; Liu, Q.; Lu, H.; Zhao, W.; Zhao, W.; Yuan, Z.; Xia, H.; Sui, G. A Self-Powered Rapid Loading Microfluidic Chip for Vector-Borne Viruses Detection Using RT-LAMP. *Sensors and Actuators B: Chemical* 2021, 333, 129521, doi:10.1016/j.snb.2021.129521.

[84] Jiang, K.; Wu, J.; Kim, J.-E.; An, S.; Nam, J.-M.; Peng, Y.-K.; Lee, J.-H. Plasmonic Cross-Linking Colorimetric PCR for Simple and Sensitive Nucleic Acid Detection. *Nano Lett.* 2023, 23, 3897–3903, doi:10.1021/acs.nanolett.3c00533.

[85] Fu, L.; Qian, Y.; Zhou, J.; Zheng, L.; Wang, Y. Fluorescence-Based Quantitative Platform for Ultrasensitive Food Allergen Detection: From Immunoassays to DNA Sensors. *Comprehensive Reviews in Food Science and Food Safety* 2020, 19, 3343–3364, doi:10.1111/1541-4337.12641.

[86] Surucu, O.; Öztürk, E.; Kuralay, F. Nucleic Acid Integrated Technologies for Electrochemical Point-of-Care Diagnostics: A Comprehensive Review. *Electroanalysis* 2022, 34, 148–160, doi:10.1002/elan.202100309.

[87] Zhou, P.; He, H.; Ma, H.; Wang, S.; Hu, S. A Review of Optical Imaging Technologies for Microfluidics. *Micromachines* 2022, 13, 274, doi:10.3390/mi13020274.

[88] Katzmeier, F.; Aufinger, L.; Dupin, A.; Quintero, J.; Lenz, M.; Bauer, L.; Klumpe, S.; Sherpa, D.; Dürr, B.; Honemann, M.; et al. A Low-Cost Fluorescence Reader for in Vitro Transcription and Nucleic Acid Detection with Cas13a. *PLoS One* 2019, 14, e0220091, doi:10.1371/journal.pone.0220091.

[89] Spibey, C.A.; Jackson, P.; Herick, K. A Unique Charge-Coupled Device/Xenon Arc Lamp Based Imaging System for the Accurate Detection and Quantitation of Multicolour Fluorescence. *ELECTROPHORESIS* 2001, 22, 829–836, doi:10.1002/1522-2683(200105)22:5<829::AID-ELPS829>3.0.CO;2-U.

[90] Chen, P.; Pan, D.; Mao, Z. Fluorescence Measured Using a Field-Portable Laser Fluorometer as a Proxy for CDOM Absorption. *Estuarine, Coastal and Shelf Science* 2014, 146, 33–41, doi:10.1016/j.ecss.2014.05.010.

[91] Velpula, R.T.; Jain, B.; Philip, M.R.; Nguyen, H.D.; Wang, R.; Nguyen, H.P.T. Epitaxial Growth and Characterization of AlInN-Based Core-Shell Nanowire Light Emitting Diodes Operating in the Ultraviolet

Spectrum. *Sci Rep* 2020, 10, 2547, doi:10.1038/s41598-020-59442-0.

[92] Baeg, K.-J.; Binda, M.; Natali, D.; Caironi, M.; Noh, Y.-Y. Organic Light Detectors: Photodiodes and Phototransistors. *Adv Mater* 2013, 25, 4267–4295, doi:10.1002/adma.201204979.

[93] Fang, Y.; Wang, Y.; Su, X.; Liu, H.; Chen, H.; Chen, Z.; Jin, L.; He, N. A Miniaturized and Integrated Dual-Channel Fluorescence Module for Multiplex Real-Time PCR in the Portable Nucleic Acid Detection System. *Front. Bioeng. Biotechnol.* 2022, 10, doi:10.3389/fbioe.2022.996456.

[94] Wang, Y.; Fang, Y.; Liu, H.; Su, X.; Chen, Z.; Li, S.; He, N. A Highly Integrated and Diminutive Fluorescence Detector for Point-of-Care Testing: Dual Negative Feedback Light-Emitting Diode (LED) Drive and Photoelectric Processing Circuits Design and Implementation. *Biosensors (Basel)* 2022, 12, 764, doi:10.3390/bios12090764.

[95] Zhu, Y.; Tong, X.; Wei, Q.; Cai, G.; Cao, Y.; Tong, C.; Shi, S.; Wang, F. 3D Origami Paper-Based Ratiometric Fluorescent Microfluidic Device for Visual Point-of-Care Detection of Alkaline Phosphatase and Butyrylcholinesterase. *Biosensors and Bioelectronics* 2022, 196, 113691, doi:10.1016/j.bios.2021.113691.

[96] Mumtaz, Z.; Rashid, Z.; Ali, A.; Arif, A.; Ameen, F.; Altami, M.S.; Yousaf, M.Z. Prospects of Microfluidic Technology in Nucleic Acid Detection Approaches. *Biosensors* 2023, 13, 584, doi:10.3390/bios13060584.

[97] Walker, F.M.; Hsieh, K. Advances in Directly Amplifying Nucleic Acids from Complex Samples. *Biosensors* 2019, 9, 117, doi:10.3390/bios9040117.

[98] Li, Z.; Bai, Y.; You, M.; Hu, J.; Yao, C.; Cao, L.; Xu, F. Fully Integrated Microfluidic Devices for Qualitative, Quantitative and Digital Nucleic Acids Testing at Point of Care. *Biosensors and Bioelectronics* 2021, 177, 112952, doi:10.1016/j.bios.2020.112952.

[99] Taylor, C.D.; Gully, B.; Sánchez, A.N.; Rode, E.; Agarwal, A.S. Towards Materials Sustainability through Materials Stewardship. *Sustainability* 2016, 8, 1001, doi:10.3390/su8101001.

[100] Human – Computer Interface Design Can Reduce Misperceptions of Feedback - Howie - 2000 - System Dynamics Review - Wiley Online Library Available online: [https://onlinelibrary.wiley.com/doi/10.1002/1099-1727\(200023\)16:3%3C151::AID-SDR191%3E3.0.CO;2-0](https://onlinelibrary.wiley.com/doi/10.1002/1099-1727(200023)16:3%3C151::AID-SDR191%3E3.0.CO;2-0) (accessed on 6 December 2024).

[101] POCT Analysts' Perspective: Practices and Wants for Improvement | The Journal of

Applied Laboratory Medicine | Oxford Academic
Available online: <https://academic.oup.com/jalm/article/5/3/480/5827435?login=false> (accessed on 6 December 2024).

[102] Point-of-Care Testing (POCT) and IT Security Concepts Available online: <https://www.degruyter.com/document/doi/10.1515/labmed-2019-0199/html> (accessed on 6 December 2024).

[103] Jinjin L.U.; Yongxin S.; Yuan T.; Tong H.O.U. The Flow Pump Control System Design Applied to Microfluidic Experimental Platform. *sykxyjs* 2023, 21, 50–56, doi:10.12179/1672-4550.20220641.

[104] Liu, D.; Wang, Y.; Li, X.; Li, M.; Wu, Q.; Song, Y.; Zhu, Z.; Yang, C. Integrated Microfluidic Devices for in Vitro Diagnostics at Point of Care., doi:10.1002/agt2.184.

[105] Xing, G.; Ai, J.; Wang, N.; Pu, Q. Recent Progress of Smartphone-Assisted Microfluidic Sensors for Point of Care Testing. *TrAC Trends in Analytical Chemistry* 2022, 157, 116792, doi:10.1016/j.trac.2022.116792.

[106] Zhou, D.; Zhang, Z.; Li, Y.; Ma, T.; He, H.; Li, H. Intelligent Textiles Make Life Wirelessly Energetic by Coupling Radiation Energy and Human. *BMEMat* 2024, 2, e12090, doi:10.1002/bmm2.12090.

[107] Sachdeva, S.; Davis, R.W.; Saha, A.K. Microfluidic Point-of-Care Testing: Commercial Landscape and Future Directions. *Front. Bioeng. Biotechnol.* 2021, 8, doi:10.3389/fbioe.2020.602659.

[108] Wiencek, J.; Nichols, J. Issues in the Practical Implementation of POCT: Overcoming Challenges. *Expert Review of Molecular Diagnostics* 2016.