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Recent advancements in nucleic acid detection with microfluidic chip for molecular diagnostics

Zheng Li, Xiaojian Xu, Dou Wang**, Xingyu Jiang*

Shenzhen Key Laboratory of Smart Healthcare Engineering, Guangdong Provincial Key Laboratory of Advanced Biomaterials, Department of Biomedical Engineering, Southern University of Science and Technology, No. 1088, Xueyuan Rd., Xili, Nanshan District, Shenzhen, Guangdong, 518055, PR China

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ABSTRACT

The coronavirus disease 2019 (COVID-19) has extensively promoted the application of nucleic acid testing technology in the field of clinical testing. The most widely used polymerase chain reaction (PCR)-based nucleic acid testing technology has problems such as complex operation, high requirements of personnel and laboratories, and contamination. The highly miniaturized microfluidic chip provides an essential tool for integrating the complex nucleic acid detection process. Various microfluidic chips have been developed for the rapid detection of nucleic acid, such as amplification-free microfluidics in combination with clustered regularly interspaced short palindromic repeats (CRISPR). In this review, we first summarized the routine process of nucleic acid testing, including sample processing and nucleic acid detection. Then the typical microfluidic chip technologies and new research advances are summarized. We also discuss the main problems of nucleic acid detection and the future developing trend of the microfluidic chip.

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1. Introduction

Nucleic acid sequence carries rich genetic information and is the most specific marker for biological information identification. The research and development of nucleic acid detection methods for biochemical analysis are significant. Currently, the most mature nucleic acid detection technology is amplification based on PCR technology, which has been developed and applied in clinical practice for decades [1]. Since the outbreak of COVID-19 in 2019, it has been widely used and is regarded as the gold standard for COVID-19 detection [2,3].

Nucleic acid testing is systematic engineering, including sample processing, nucleic acid extraction and detection steps [4,5]. Conventional PCR-based nucleic acid detection technologies usually have critical drawbacks such as complex operation, high requirements for expensive equipment and long reaction time [6,7]. Therefore, developing rapid, accurate and straight-forward nucleic acid detection testing techniques has crucial practical application value. Many new nucleic acid detection technologies have been developed in recent years, such as isothermal amplification and amplification-free nucleic acid detection technology [8–12].

The highly miniaturized microfluidic technology can integrate complex nucleic acid detection processes on one chip [13,14], which lessens the complexity of the operation and helps build an automatic and efficient diagnosis system [15–18]. Various microfluidic chips have been developed to detect nucleic acid [19–22]. A series of new nucleic acid detection technologies have been developed in the past two years with the vast demand for the detection of COVID-19 [23–26]. Therefore, we write this review to summarize the recent progress of nucleic acid testing based on a microfluidic chip. We first summarized the routine process of nucleic acid testing, and then the advanced microfluidic chip technologies are summarized. Finally, we put forward the existing problems and development direction of the microfluidic chip for nucleic acid detection (Fig. 1).

2. Microfluidic chip and on-chip nucleic acid detection

2.1. Sample processing and nucleic acid extraction

The first step in the testing process is extracting nucleic acid from clinical samples [27]. The extraction and purification of nucleic acids will directly affect the assay results. The process

* Corresponding author.
** Corresponding author.
E-mail addresses: wangd9@sustech.edu.cn (D. Wang), jiang@sustech.edu.cn (X. Jiang).

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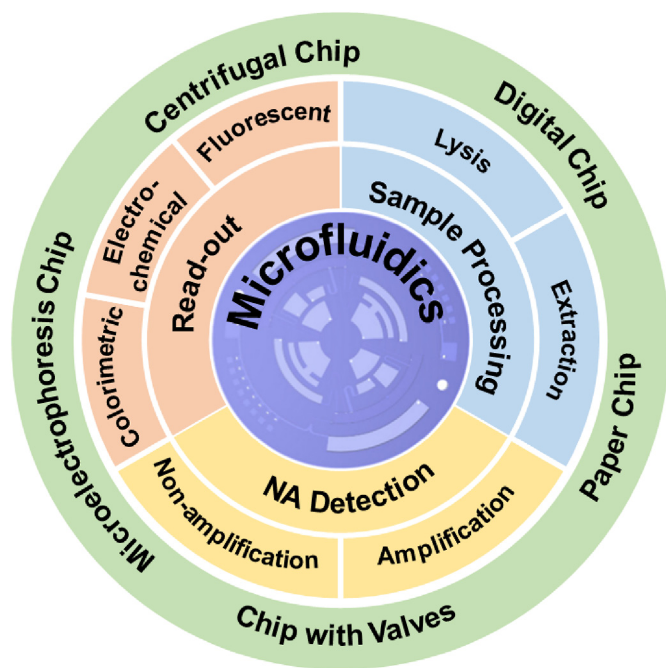

Fig. 1. Outline of microfluidics technology for the nucleic acid test.

usually requires three steps: sample lysis, nucleic acid extraction, and purification. Nucleic acid extraction first requires lysis of the cells to release the intracellular nucleic acid. Ideal lysis agents should not interfere with downstream assays and can be adapted to miniaturized devices such as microfluidic chips.

2.1.1. Lysis method

The lysis method falls into two major categories, chemical and mechanical lysis [28]. Chemical solutions, such as alkaline solutions, enzyme solutions, and decontaminants, can lyse cell membranes to release intracellular components [29]. The advantage of the chemical lysis method is that the process is simple and easy to handle, but the lysis reagent may affect the downstream nucleic acid amplification. Mechanical lysis is performed by physical means such as freeze-thawing, collision method, grinding, acoustic and electrolysis. For example, the grinding method uses shear stress to disrupt cell membranes from the outside, and the automated grinding microfluidic chip developed recently [30] can replace manual grinding with filtration and other steps after lysis, followed by elution using PCR buffer. The collision method is based on integrating nanostructures into the microfluidic chip to induce mutual collisions between cells to achieve lysis. In addition, introducing tiny beads (100 μm) into the microfluidic device can also induce cell fragmentation lysis.

Other physical lysis methods also basically use physical means to disrupt the cell membrane to allow the escape of intracellular components, acoustic methods use mechanical vibrations to disrupt the cell membrane, and electrolysis uses high voltage to perforate the cell membrane and to perform its effect [31]. The advantages of the physical lysis method are that it does not require chemical reagents and is suitable for highly automated microfluidic devices. The disadvantage is that the high shear stress and heat generated in the process may damage the DNA.

2.1.2. Extraction methods

There are two main extraction methods: liquid-liquid extraction and solid-phase extraction. Liquid-liquid extraction uses two immiscible solvents to extract and concentrate the analytes in one phase. The traditional phenol-chloroform system, for example, uses a large amount of volatile organic solvents and requires many sample processing steps. Nucleic acid extraction has been miniaturized to require only microliter or milliliter volumes to reduce the use of toxic organic solvents [32,33], but it is still difficult to be compatible with microfluidic devices for reasons such as corrosiveness. Instead, solid-phase nucleic acid extraction methods, which allow for the separation of substances with limited amounts of reagents, are more frequently used as assays paired with microfluidic chips [34–36]. Therefore, we focus on the latest advances in the solid phase extraction field.

2.1.2.1. Silicon-based material extraction method. Silica is a biocompatible substrate material that is stable, easy to modify, and compatible with nucleic acids. Nucleic acids can be adsorbed or eluted from silica by changing pH or salt concentration conditions [37]. Various silica-based materials, such as silica beads and membranes, have been widely used for nucleic acid extraction in microfluidics. Depending on the material properties, silica-based materials can be used in various ways on microchips [38]. For example, silica beads or nano-filters can simply be placed into the wells or microchannels of a microfluidic chip to extract nucleic acids from the sample. Researchers [39] utilized the microfluidic chip embedded with chitosan-modified silicon dioxide capillaries and a smartphone-based detection unit to construct a system for rapidly extracting and detecting ZIKV RNA. Using nanofiltration membranes made from low-stress silicon nitride, researchers [40] proposed a method to collect negatively charged nucleic acids directly from biological samples by adding an electric field between the sample and the collection buffer separated by the nanofiltration membrane.

2.1.2.2. Magnetic-based strategies. Magnetic nanoparticles generally contain magnetic nuclei and outer shell coated with silica or other derivatives with nucleic acid trapping ability. The excellent surface biocompatibility and large specific surface area of magnetic beads ensure their ability to adsorb nucleic acids effectively, and the superparamagnetic properties ensure that the beads can be uniformly dispersed throughout the medium in the absence of an external magnetic field [41]. Magnetic beads move and aggregate rapidly when placed in an external magnetic field for separation. Solid-phase extraction based on magnetic materials is the primary choice for high-throughput and automated nucleic acid extraction because it does not rely on centrifuges or other complicated equipment, significantly reduces separation times, and the implementation of automation eliminates the errors that tend to occur in manual operations. Commonly used magnetic particles include silica-coated, amino-coated, carboxyl-coated Fe_3O_4 or γ-Fe_2O_3 magnetic particles [42]. Compared to silica-based solid phase extraction techniques, magnetic particles are easier to manipulate and control with external magnets. The principle of extraction for nucleic acids is simple, nucleic acids are adsorbed on the surface of silica-coated magnetic particles at high salt and low pH conditions, while the molecules can be eluted again at low salt and high pH conditions [43]. The silica-coated magnetic beads allow the extraction of DNA from large volumes of samples with the help of magnetic field-guided enrichment.

To fully integrate nucleic acid extraction, amplification and detection on a microfluidic system and to enable the detection of five high-risk HPV indicators, researchers [44] used chitosan-modified magnetic microspheres for pH-induced nucleic acid extraction and integrated this approach into a centrifugal microfluidic chip (Fig. 2A). The microfluidic system includes cell lysis, nucleic acid capture and release, isothermal amplification, and real-

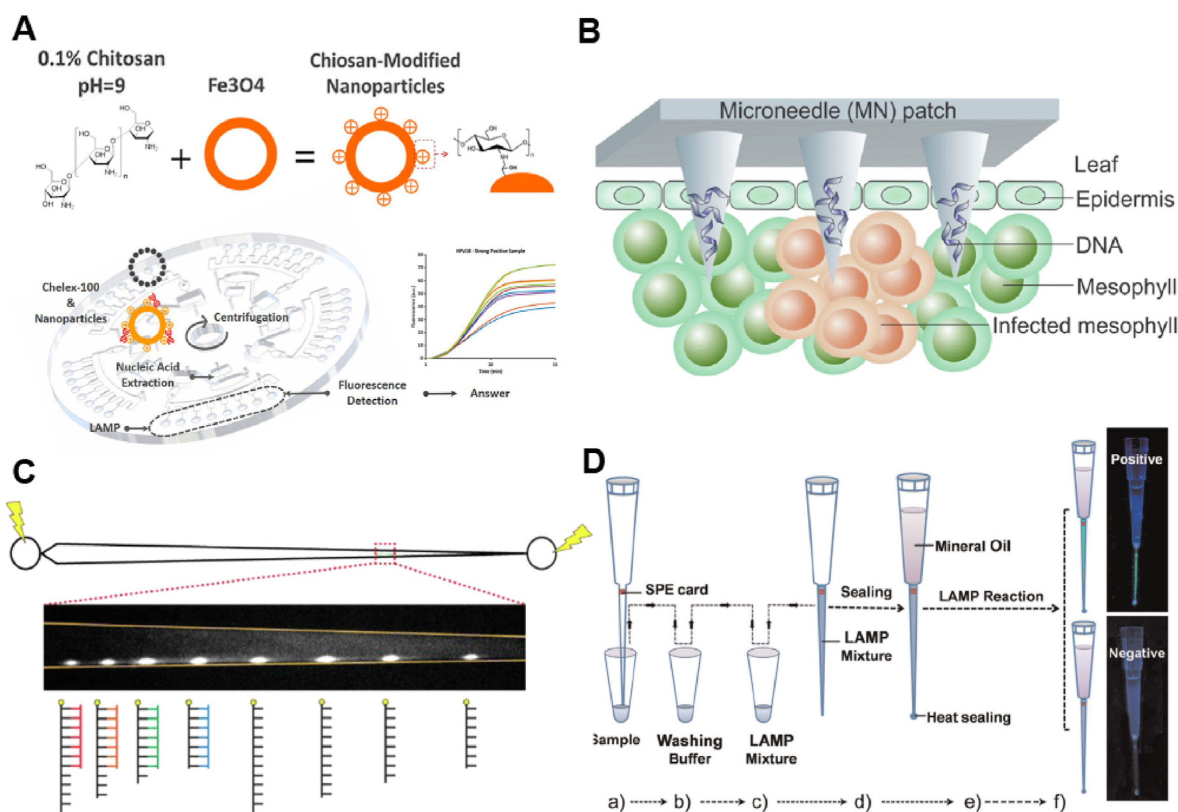

Fig. 2. Diagram of nucleic acid extraction method. (A) Chitosan-modified magnetic microspheres for pH-induced nucleic acid extraction and integrated into a centrifugal microfluidic chip. Reproduced with permission from Ref. [44]. (B) Rapid plant DNA extraction method using disposable polymeric microneedle patches. Reproduced with permission from Ref. [46]. (C) Using ME chip with thermal gel electrophoresis to directly separate and quantify multiple miRNAs. Reproduced with permission from Ref. [51]. (D) A micro-pipette tip-based nucleic acid test (MTNT) for high-throughput sample-to-answer detection of both DNA and RNA from crude samples including cells, bacteria, and solid plants. Reproduced with permission from Ref. [53].

time fluorescence detection, all the processes are controlled by centrifugal force and magnetic control. The detection system exhibits good specificity, stability, and high detection speed. The microfluidic system that introduced chitosan-modified magnetic microspheres as a solid-phase extraction material can successfully achieve pH-induced nucleic acid extraction and avoid the adverse effects of organic solvents on subsequent procedures.

For the detection of ultra-low-abundance exosomal nucleic acids, the researchers proposed a simple, efficient and "lab-in-tube" exosome nucleic acid detection system that fully integrates exosome enrichment using immunomagnetic beads (IMB) (10 min), rapid exosome-based lysis (5 min) and sensitive loop-mediated isothermal amplification (LAMP) in a tube [45]. The platform has demonstrated good performance in the direct analysis of exosomal HOTTIP RNA in human serum samples and has the potential to detect low abundance exosomal nucleic acid biomarkers in cancer.

As an effective nucleic acid extraction tool, magnetic microspheres have dominated the point of care testing (POCT) field with many advantages such as low sample consumption, high automation and excellent stability.

2.1.2.3. Microneedle-based nucleic acid extraction. Microneedle-based nucleic acid extraction methods, which allow rapid in situ extraction of nucleic acid molecules, have been applied in fields such as plant and food pathogen detection., etc.

Current conventional protocols for extracting DNA from plant tissues and performing in situ molecular diagnoses are cumbersome and time-consuming. Researchers [46] have developed a rapid plant DNA extraction method using disposable polymeric microneedle (MN) patches (Fig. 2B). The MN-extracted DNA is used for direct PCR without purification. This simple, cell lysis-free and purification-free DNA extraction method may be a transformative approach to facilitate rapid sample preparation for molecular diagnosis of various plant diseases directly in situ. In addition, nucleic acid-based assays are very promising for risk assessment in the food sector. However, cumbersome protocols are often required to isolate nucleic acid components due to the complexity of food matrices. To rapidly track allergens in food, researchers [47] developed an instant and multiplex DNA extraction method based on polyvinyl alcohol microneedle patches. By performing a simple press-and-peel operation in less than 1 min, samples suitable for DNA-based analysis can be collected, and by further combining this with a recombinase polymerase amplification (RPA) assay, rapid screening of complex samples such as shrimp balls and cheesecake for allergy risk can be achieved in less than 30 min.

2.1.2.4. Separation based on electrophoresis. Since electrophoresis-based separation methods do not require additional sample processing and nucleic acid extraction and can be directly combined with microchips for the separation and detection of nucleic acids, we summarized the latest developments in this field. Electrophoresis-based separation utilizes an electric field that allows the migration of negatively charged NA toward a positively charged cathode [48]. The principle of separation is that analytes have different migratory mobility when located in high applied electric fields (up to 800 V/cm) according to the size and charge-to-mass ratio. The use of electrophoresis has unique advantages over traditional DNA extraction, such as the isolation and enrichment of

small nucleic acid fragments can be completed in a few minutes. Cleaving large DNA sequences into fragments using restriction endonucleases also allows for high-speed separation and detection of large nucleic acid fragments.

Among the materials that can be used as matrices for capillary electrophoresis sieving, gels are indispensable materials similar to plate electrophoresis gels. Polyacrylamide and agarose are generally used as the matrices for conventional plate gel electrophoresis, which are prone to generate Joule heat resulting in poor reproducibility. As permanent polymers, they can only be used once [49]. Researchers [50] developed a DNA-modified polyacrylamide hydrogel that captures and releases 20–1000 bp ssDNA and dsDNA with high specificity and sensitivity. In this work, ssDNA and dsDNA from serum are electrophoresed into a modified hydrogel, and then thermos cycled to capture DNA by hybridization. After multiple electrophoreses, DNA is concentrated in the gel and retrieved, which can significantly improve the detection efficiency of free DNA in blood samples.

In another example, researchers [51] developed a simple, rapid method to directly quantify multiple miRNAs using microfluidic thermal gel electrophoresis (TGE) (Fig. 2C). TGE is a thermally responsive polymer that changes viscosity based on temperature. The sample is poured directly into a liquid phase thermal gel that can be quickly loaded into the microfluidic channel. The gel is then cured by heating the device to lock the sample and electrolyte in the appropriate position and provide a sieving matrix. The method achieved a high resolution between four double-stranded miRNA-probe hybrids and four excess single-stranded probes. Wei et al. [52] developed a multiplex fluorescence signal amplification method based on an electrophoresis platform to separate and detect microRNAs. The method used two kinds of fluorescein-labelled DNA signal probes to hybridize with its target microRNAs and utilized T7 exonuclease to realize the fluorescence signal amplification. Two kinds of fluorescein-labelled DNA segments with different sizes were separated and detected on the Micro-electrophoresis (ME) laser-induced fluorescence detection platform. The method produces an excellent linear relationship between the fluorescence intensity and the amount of microRNA. The detection limit for microRNA can be as low as 15 pM.

2.1.3. Extraction-free and one-step nucleic acid detection

In some areas where resources are scarce extraction-free or one-step analysis methods for rapid detection are required.

In addition to solid phase extraction techniques, techniques such as FTA rapid nucleic acid extraction cards are also widely used. Our group have developed a micro-pipette tip-based nucleic acid test (MTNT) for high-throughput sample-to-answer detection of both DNA and RNA from crude samples without the need for sample pretreatment and complex operation [53]. MTNT consists of micro-pipette tips and embedded solid phase nucleic acid extraction membranes and fully integrates the functions of nucleic acid extraction from crude samples, LAMP of nucleic acids, and visual read-out of assays (Fig. 2D).

Some researchers developed a digitally enhanced one-pot method for nucleic acid detection using digital microwell arrays [54]. This method uses the combination of RT-RPA and CRISPR/Cas12. The target RNA is reverse-transcribed and amplified into DNA by RT-RPA, and the crRNA-Cas12a complex is activated to cut the single-stranded DNA fluorescent reporter gene to generate fluorescence. By confining this reaction to the digitized microwell, the local reaction concentration is increased, the signal is enhanced, and the sensitivity is improved. The method enables qualitative detection in <15 min and quantitative detection in 30 min with a high signal-to-background ratio, wide dynamic range, and high sensitivity. Other researchers have used CRISPR/Cas13 to detect COVID-19. Hsu et al. developed an integrated and rapid automated microfluidic detection system for nucleic acid detection [55]. The system consists of a single-use gravity-driven microfluidic cartridge and a compact instrument that automates the detection process from sample to result within 60 min, with a sensitivity of 40% for SARS-CoV-2 copies/μl. The combination with microfluidics makes one-step nucleic acid testing more feasible, and the simplicity and rapidity of these assays allows for on-site deployment, bedside diagnostics, and routine monitoring. The detailed comparison of the sample processing method is shown in Table 1.

2.2. Nucleic acid amplification techniques

For the detection of a low concentration of nucleic acid in samples, an amplification reaction is essential. Microfluidics combined with nucleic acid amplification can lead to a more sensitive test result and make it a powerful tool for clinical microbiology testing. In recent years, various microfluidic devices based on nucleic acid amplification have emerged for the rapid and accurate detection of DNA and RNA [56–58]. In general, nucleic acid amplification-based techniques are classified as PCR and isothermal nucleic acid amplification. This section will summarize those nucleic acid detection techniques based on microfluidic systems.

2.2.1. Polymerase chain reaction

Since being invented in the 1980s, PCR has become a powerful tool for detecting virtually all clinical microorganisms. PCR is an in vitro enzymatic DNA amplification performed at three discrete temperatures. PCR consists of a thermal cycle that includes denaturation of double-stranded DNA at high temperature, annealing of primers to genomic DNA complementary sequences, and extension with the aid of polymerase. The polymerase used in the reaction is Taq polymerase, which can tolerate high temperatures [59]. PCR can amplify a small amount of target DNA/RNA at an exponential rate, and the amplification process generates up to one billion copies of the original target gene, making PCR a powerful tool for rapidly detecting nucleic acids [60]. Due to advances in microfluidics, PCR has been widely integrated into microfluidic platforms used for diagnosis.

In recent decades, many portable microfluidic devices equipped with thermal cycling systems to perform PCR have been developed to meet the need for immediate diagnosis. Microfluidic PCR chips can be divided into two types of structures: chamber PCR and continuous flow PCR(CF-PCR) [2]. The chip is heated and cooled in a chamber PCR chip at a specific thermal cycling temperature after injecting the sample into the wells. CF-PCR chips transfer samples through fixed temperature zones for thermal cycling. CF-PCR chips can be further subdivided into serpentine, spiral, and straight-through channels [61,62]. These designs allow microfluidic PCR can be used in various molecular biology applications, such as gene detection and sequencing.

CF-PCR solves the problem of a discontinuous and time-consuming analysis process caused by traditional PCR's temperature rise and fall. CF-PCR can achieve rapid amplification by actively circulating the reagents into three preheating zones with a pump connected to the chip, and the design of long serpentine channels or short straight-through channels successfully avoids the transition phase among different reaction temperatures, thus significantly reducing the analysis time. Researchers [63] designed a CF-PCR microfluidic chip and fabricated a portable system for multiplex amplification of periodontal pathogens. They analyzed the thermal distribution of the heater used for PCR and then studied typical primers for *Porphyromonas gingivalis (Pg), Treponema denticola (T.d), and Tannerella forsythia (T.f)*. The CF-PCR showed an

Table 1
Comparation of the sample processing methods based on microfluidics.

Method	Material	Type of Extraction	microfluidic system	Analyte	Time	Advantages	Reference
Silicon-based material	Silica beads	SPE	Capillary chip	ZIKV RNA	25 min	Biocompatible material,	[39]
	Silicon nitride nanoporous membrane	SPE	Microelectrophoresis chip	miRNA	30 min	easy to perform, reproducible	[40]
Magnetic-based material	Magnetic Microspheres	SPE	Centrifugal chip	DNA	18 min	Rapid, automation, no need	[44]
	magnetic beads	SPE	N/A	RNA	15 min	centrifugation	[45]
Microneedle	Polyvinyl alcohol	SPE	N/A	DNA	1 min	In situ extraction, simple, cell-lysis-free, purification-free	[46]
electrophoresis-based separation methods	Polyacrylamide hydrogel	N/A	Microelectrophoresis chip	DNA	12 min	Simple, fast, low sample consumption, low cost	[50]
	Thermal gel	N/A	Microelectrophoresis chip	microRNA	5 min		[51]
	Agarose gel	N/A	Microelectrophoresis chip	microRNA	2 min		[52]
FTA card	Cellulose matrix	SPE	MTNT	DNA and RNA	30 min	Rapid, without pretreatment, easy operation	[53]

overwhelming advantage in reaction speed compared to conventional thermal cycler.

Digital PCR (dPCR), based on microdroplets and microfluidic chips, has gained widespread attention and applications. dPCR is a third-generation PCR technique that achieves accurate absolute quantification of nucleic acids for quantitative analysis with high sensitivity [64]. In the dPCR chip, the reaction mixture was divided into a large number of independent reaction units according to finite dilution and Poisson statistics [65]. Compared to conventional PCR, dPCR achieves absolute quantification of DNA templates, is not dependent on standard curves, and is more resistant to PCR inhibitors. The dilution of the sample and the amount and homogeneity of the dispersion can significantly affect the accuracy of the quantitative results. Researchers [66] reported a chamber-based dPCR microarray structure for high-throughput, high-sensitivity quantitative measurements of SARS-CoV-2 viral genes and mutant lung cancer genes (Fig. 3A). Samples will be assigned to each microchamber for independent reactions based on Poisson distribution. The prepared microarray chips successfully quantify a solution mixture containing both genes with a detection limit of 10 copies/μL at a throughput of 46200 microchambers. These chips are inexpensive and easy to industrialize. Researchers [67] proposed a simple method for rapid and low-cost dPCR assays. By subdividing a bulk sample volume into a large number of equal volume compartments using a self-digitizing chip, the method allows for low waste and large volume sample discretization in minutes.

2.2.2. Isothermal amplification

Compared to PCR, which requires a complex thermal cycler, isothermal nucleic acid amplification is an effective solution to replace conventional PCR because of the mild conditions and constant temperature requirements. Isothermal amplification methods have been widely used in microfluidic detection devices where nucleic acids are amplified at a constant temperature, thus facilitating low-cost and portable POCT devices for molecular diagnostics [68,69]. Here we focus on two primary isothermal nucleic acid amplification techniques: LAMP and RPA.

LAMP uses a set of four to six primers to identify the target gene sequence, including two outer primers (F3 and B3), a forward inner primer and a reverse inner primer. LAMP provides a highly sensitive molecular diagnostic tool with a reaction time of less than 1 h and can amplify billions of DNA copies [70–72].

Our group previously demonstrated a simple, robust, multiplexed and instant microcapillary LAMP (cLAMP) for the detection of nucleic acids [73]. The assay integrates capillaries (glass or plastic) for introducing and holding samples/reagents, a water droplet segment for preventing contamination, a pocket heater for providing heat, and a handheld flashlight for visual read-out of the fluorescent signal. cLAMP system allows simultaneous detection of two RNA targets of the human immunodeficiency virus (HIV) from multiple plasma samples and achieves two copies of the standard plasmid with high sensitivity. Our cLAMP holds great promise for immediate care applications in resource-poor settings (Fig. 3B).

In a recent study, researchers [74] used a LAMP method to amplify and detect specific nucleic acid (DNA/RNA) targets by introducing a microfluidic device that can sequentially distribute samples into multiple reaction microchambers in a single operation. This method provides a fast and straight-forward platform for multiplex gene diagnosis of multiple viral infectious diseases.

The determination of DNA methylation is still a challenge. Researchers have recently proposed a dual-mode LAMP incorporating magnetic bead separation to determine the methylated Septin9 gene in colorectal cancer [75]. A one-pot real-time fluorescent and colorimetric LAMP was used to detect the methylated Septin9 gene (60 min). The method was shown to detect methylated DNA in HCT 116 cells in the range of 2 to 0.02 ng/μL with a detection limit of 0.02 ± 0.002 ng/μL (RSD: 9.75%). The method also distinguished methylated Septin9 in 0.1% of HCT 116 cells (RSD: 6.60%), indicating its high specificity and sensitivity (Fig. 3C).

To address the global threat of cryptococcal meningitis (CM), researchers [76] developed a multifunctional microfluidic module that integrates pathogen enrichment and on-chip nucleic acid extraction based on a portable one-pot, temperature-free LAMP lyophilization reagent bead. This module does not require additional instrumentation and is expected to develop a simple, rapid, and efficient method for "sample-in, result-out" testing of actual cerebrospinal fluid samples. To diagnose epidemic-transmitted infections caused by high-risk human papillomavirus (HPV), researchers [77] developed a microfluidic detection system consisting of a microfluidic chip and a corresponding detection device. The proposed method integrates nucleic acid purification, isothermal amplification and real-time fluorescence detection into a single device. Furthermore, it shows high specificity (100%) and excellent stability (coefficient of variation <6%) in five HPV genotypes. Compared to conventional qPCR, LAMP is more rapid. The integrated microfluidic assay system provides automated and rapid diagnosis in less than 40 min. However, the reaction temperature of LAMP reaches approximately 70 °C and inevitably generates air

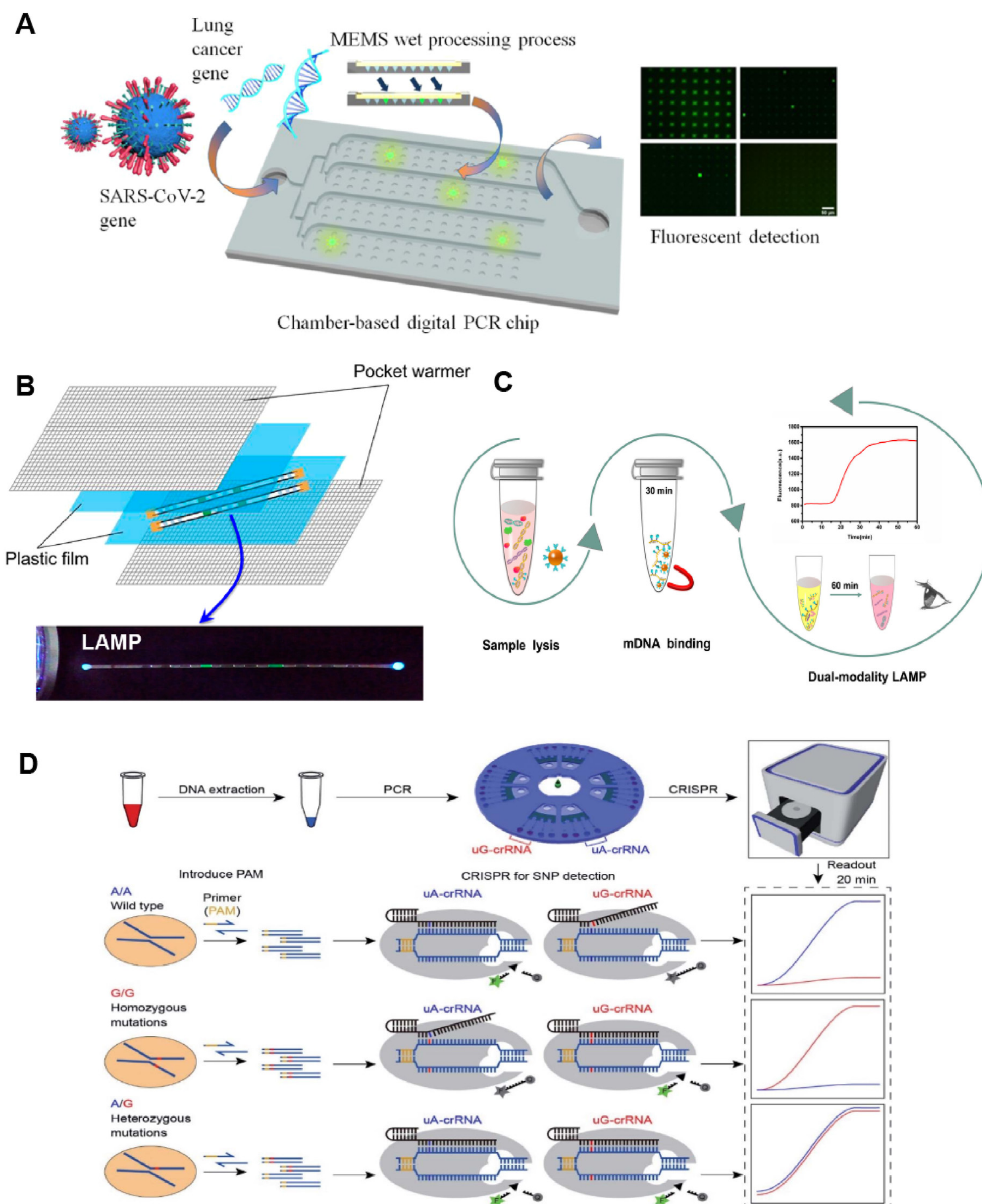

Fig. 3. Diagram of integrated microfluidic chips with nucleic acid amplification. (A) dPCR chip for high-throughput, high-sensitivity quantitative measurements of SARS-CoV-2 viral genes. Reproduced with permission from Ref. [66]. (B) Microcapillary LAMP for the detection of nucleic acids. Reproduced with permission from Ref. [73]. (C) Dual-mode LAMP incorporating magnetic bead separation to determine the methylated Septin9 gene in colorectal cancer. Reproduced with permission from Ref. [75]. (D) A CRISPR/Cas12a-based SNP detection genotyping method based on the centrifugal microfluidic device. Reproduced with permission from Ref. [16].

bubbles, which can lead to false positive results. The primer design of LAMP is more complicated than PCR.

RPA can partially reduce LAMP's high false positive rate, as the relatively low reaction temperature (~37 °C) leads to relatively minor evaporation problems. In the RPA system, two opposite primers initiate DNA synthesis by binding to recombinase and can complete amplification in less than 10 min. Thus, the whole process of RPA is much faster than PCR or LAMP [78]. In recent years, microfluidic technology has been shown to increase the speed and accuracy of RPA. In response to the global outbreak caused by SARS-CoV-2, researchers [79] developed a CRISPR/CRISPR-associated (Cas) 13a-based biosensor combined with RPA to detect the S and Orf1ab genes of SARS-CoV-2 within 30 min, with detection limits as low as 0.68 fM and 4.16 fM for the two genes, respectively. In addition, they used lateral flow strips to visualize SARS-CoV-2 detection, which became a promising tool in the field of SARS-CoV-2 detection. In

another example, researchers [80] presented an integrated multiplex-digital recombinase polymerization amplification microfluidic chip. The chip combined DNA extraction, multiplex digital RPA, and fluorescence detection into a "sample-multiplex-digital-answer-output" system and was successfully demonstrated to detect three pathogenic bacteria simultaneously and give digital quantitative results in 45 min. However, the cost of individual RPA reaction is high. In addition, RPA may lead to non-specific amplification products under less rigorous reaction conditions. These limitations may affect the application of RPA in microfluidic systems and deserve further optimization. Well-designed primers and probes for different targets are also needed to improve the feasibility of RPA-based microfluidic strategies in POCT.

2.2.3. CRISPR-based nucleic acid detection methods

Beyond the widespread application as genome-editing and regulatory tools, CRISPR-Cas systems also play a critical role in nucleic acid detection due to their high sensitivity and specificity [81]. The recently developed Cas family have opened the door to developing new strategies for detecting different types of nucleic acids for various purposes [82]. Precise and efficient nucleic acid detection using CRISPR-Cas systems has the potential to advance the application and development of POCT.

Cas9, Cas13, and Cas12a have been combined with microfluidic technologies and are widely developed as diagnostic tools. They are activated upon binding to target DNA or RNA with the help of guide RNAs targeting pathogen-specific nucleic acids, respectively. Once activated, the Cas proteins begin cleaving, cutting off quenched fluorescent probes and releasing fluorescence [83].

We have developed a series of CRISPR-based nucleic acid assays in recent years and combined them with centrifugal microfluidic chips to develop a powerful tool suitable for POCT. To accurately and efficiently detect single nucleotide polymorphisms (SNPs) associated with multiple human diseases, we proposed a universal and high-fidelity genotyping method based on the microfluidic device of the CRISPR system [16]. Briefly, the universality of CRISPR/Cas12a-based SNP detection is improved by the systematic insertion of prototype spacer adjacent motif (PAM) sequences; sensitivity and specificity are improved by removing complementary ssDNA and introducing additional nucleotide mismatches. Preloading CRISPR/Cas12a reagents into the bedside biochip allows for process automation, improved stability and long-term storage. The biochip enables fast and easy genotype detection in less than 20 min (Fig. 3D).

For rapid, accurate and early detection of SARS-CoV-2, we combined CRISPR with recombinant enzyme-assisted amplification (RAA) to develop a dual CRISPR/Cas12a-assisted RT-RAA assay and "sample-to-answer" centrifugation microfluidic platform that can automatically detect 1 copy/μL of SARS-CoV-2 in 30 min [15]. The chip separates amplification (RAA) from detection (CRISPR), maximizing sensitivity and reducing time consumption by up to three times. For 26 positive and 8 negative clinical SARS-CoV-2 samples, this automated centrifugation microfluidic achieves 100% accuracy compared to the gold standard RT-PCR technique.

2.3. Signal read-out method

Signal acquisition is the final step in nucleic acid detection. Fluorescence-based, electrochemical, and colorimetric signal read-out strategies have been widely used. In addition, many signal amplification methods have been developed [84]. In this section, we will describe each method's principle, combination with microfluidics, and applications in molecular diagnostics.

2.3.1. Fluorescent read-out method

Fluorescence-based assays are widely used in molecular detection and other fields because of their high sensitivity, low cost, and immediate analysis. The principle is to use labelled fluorophores (e.g., fluorescent dyes and nanomaterials) to generate a detectable signal (fluorescence enhancement or quenching). Two standard methods are used to quantify DNA samples by fluorescence detection. The first is the use of fluorescent dyes embedded in dsDNA. Dyes are added to the PCR mix to bind the amplified dsDNA, and the complex can emit fluorescence. As the amount of DNA product increases with each thermal cycle, the measured fluorescence intensity also increases, thus allowing simultaneous quantification of DNA concentration. An alternative approach is using a modified DNA oligonucleotide probe that fluoresces when target DNA exist. This real-time fluorescence detection method is highly sensitive compared to conventional gel electrophoresis detection of PCR products and has attracted much attention.

Researchers [85] described a highly sensitive and versatile fluorescent biosensor for rapid and sensitive detection of pathogenic nucleic acids. The platform successfully detected four human-associated pathogens in clinical samples. The results were consistent with qPCR. The assay system can be designed to target different pathogen nucleic acids by simply changing the protector for different targets and the portion of the catalytic hairpin that binds to the protector. Researchers [26] demonstrated an integrated device for in situ fluorescence detection following reverse transcription, rapid thermal cycling (plasma heating via magnetic plasma nanoparticles) and magnetic removal of nanoparticles. SARS-CoV-2 RNA could be detected in 17 min using this portable device, which correctly classified all nasopharyngeal, oropharyngeal, and sputum samples from 75 COVID-19 patients and 75 healthy controls with fluorescence intensity in good agreement with standard RT-qPCR (Fig. 4A).

Fluorescence-based CRISPR technologies, such as SHERLOCK, DETECTER, and HOLMES, have also been proposed with high sensitivity and specificity for very convenient and rapid nucleic acid detection [86,87]. Researchers [88] reported a digital warm-start CRISPR (dWS-CRISPR) assay for sensitive, quantitative detection of SARS-CoV-2 in clinical samples (Fig. 4B). The dWS-CRISPR assay is initiated above 50 °C, overcoming premature target amplification at room temperature, allowing accurate and reliable digital quantification of SARS-CoV-2. By targeting the nucleoprotein gene of SARS-CoV-2, the dWS-CRISPR assay is able to detect SARS-CoV-2 RNA as low as 5 copies/μl in the chip, making it a sensitive and reliable CRISPR assay that facilitates accurate detection of SARS-CoV-2 for digital quantification.

2.3.2. Electrochemical read-out method

Electrochemical detection is a method to detect the measured components by converting the chemical signal generated by the analyte in solution into an electrical signal. Electrochemical detection is a powerful analytical method for POCT applications because it is fast, simple to manufacture, low cost, portable, and easy to control. The specific electrical signal can be generated by functionalized modifications of the electrode, such as immobilized probes, enzymes, and aptamers, which bind specifically to the amplification product [89–91]. Electrochemical detection is well suited to miniaturization and integration with microfluidics. To rapidly and accurately detect SARS-CoV-2 RNA to determine the immune status of individuals infected by the virus or vaccinated against the disease. The researchers [22] describe the development and application of a 3D-printed lab-on-a-chip that simultaneously detects SARS-CoV-2 RNA in saliva and *anti*-SARS-CoV-2 immunoglobulin in saliva with plasma added to the electrode as a function of SARS-CoV-2 Spike S1, nucleocapsid, and receptor-binding

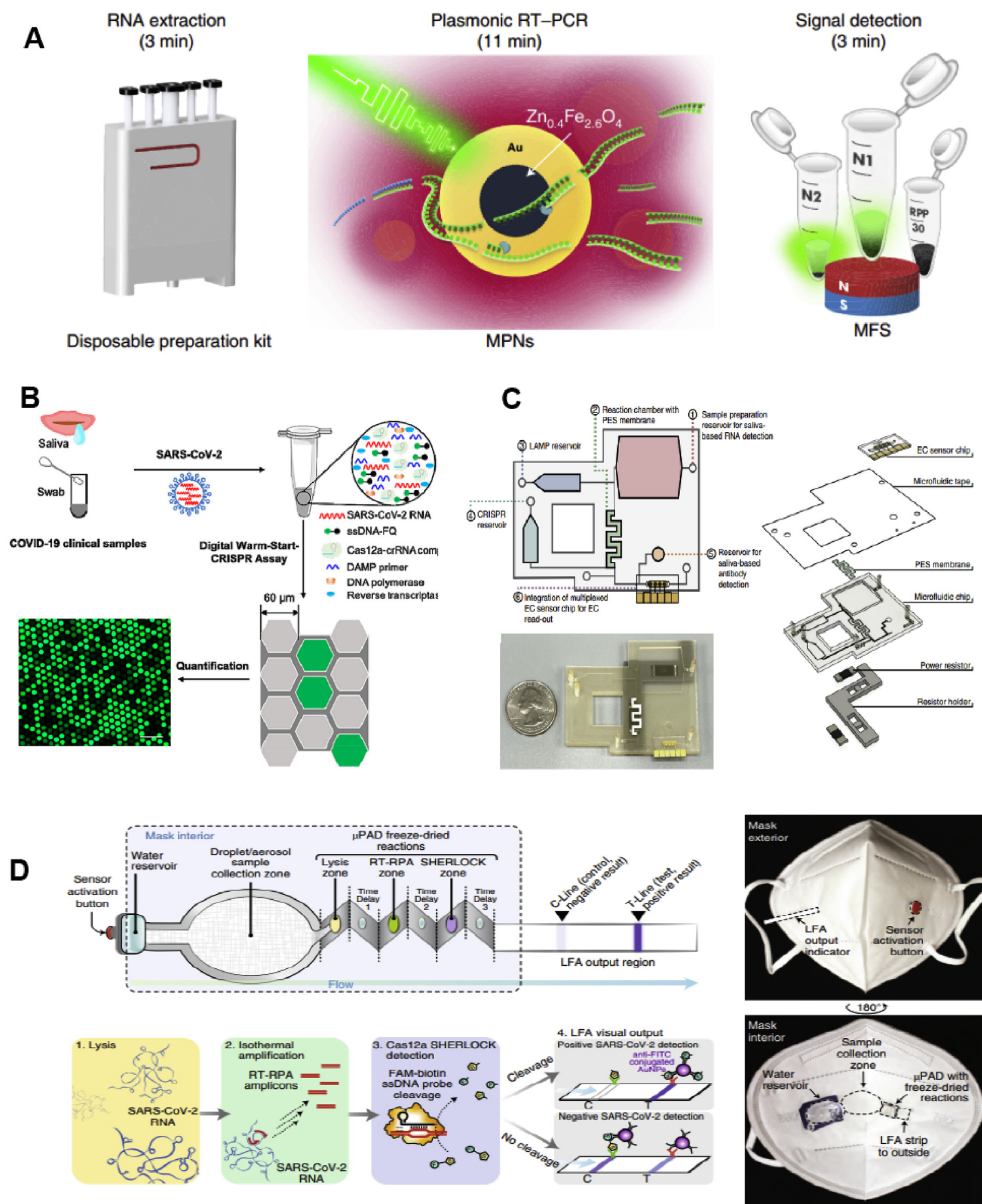

Fig. 4. Diagram of signal read-out method. (A) An integrated device for in situ fluorescence detection for SARS-CoV-2 RNA detecting. Reproduced with permission from Ref. [26].(B) Fluorescence-based digital warm-start CRISPR assay for sensitive, quantitative detection of SARS-CoV-2 Reproduced with permission from Ref. [88]. (C) A 3D-printed lab-on-a-chip that simultaneously detects SARS-CoV-2 RNA in saliva and *anti*-SARS-CoV-2 immunoglobulin within 2 h via multiplexed electrochemical output. Reproduced with permission from Ref. [22]. (D) A face mask with a lyophilized CRISPR sensor and a colorimetric sensing platform for wearable, noninvasive detection of SARS-CoV-2. Reproduced with permission from Ref. [98].

domain antigens within 2 h via multiplexed electrochemical output. The inexpensive microfluidic electrochemical sensor can aid in multiple diagnostics at the point of care (Fig. 4C).

Researchers [92] have developed a new electrochemical method for sensitive and reliable detection of nucleic acids in biological fluids. The advantages of the lipid membrane, especially its excellent antifouling ability, are employed to enhance the method's applicability in a complex environment, while the significant solid-state Ag/AgCl response of AgNPs is used to ensure the detection sensitivity of the method. The core of this method's workflow is the target-induced Y-shape structure formation, which recruits AgNPs to the electrode surface, producing considerable electrochemical

responses. Taking a liver cancer-related long non-coding RNA as a model target, the method exhibits high sensitivity, specificity, and reproducibility with a detection limit of 0.42 fM.

In another example, researchers [93] developed a versatile and highly sensitive electrochemical biosensing strategy for analyzing dengue virus (DENV) nucleic acids using a triple nanostructure-mediated dendritic hybridization chain reaction (HCR). The dendritic products formed by a series of hybridizations are combined with avidin-labelled horseradish peroxidase (avidin-HRP) to obtain an amperometric signal for ultrasensitive electrochemical detection of DENV. The method has a detection range of 1.6–1000 pM, a detection limit of 188 fM, and the ability to distinguish single-base mutations. By changing the recognition sequence of the initiator, the detection of different DENV nucleic acid fragments can be achieved with the same performance. Therefore, the method is well scalable to other nucleic acids and provides a good candidate for nucleic acid detection in early clinical diagnosis.

2.3.3. Colorimetric assays method

Colorimetric sensors are platforms that indicate the presence of a target through color change. Gold nanoparticle-based (AuNPs) colorimetric detection exhibits unique distance-dependent optical properties through gold nanoparticles' aggregation and dispersion behavior and can be identified with the naked eye [94–96]. Colorimetric assays have been used in POCT applications to benefit from portability, low cost, ease of preparation, and naked eye readings. Colorimetric assays can use the oxidation of peroxidase or peroxidase-like nanomaterials, aggregation of nanomaterials and addition of dye indicators to translate information about the presence of target nucleic acids into visible color changes [97]. Notably, gold nanoparticles are widely used in the establishment of colorimetric strategies. Due to the ability to induce rapid and significant color changes, there is a growing interest in developing colorimetric POCT platforms for on-site infectious disease diagnosis.

As an example, researchers [98] demonstrate the development of a face mask with a lyophilized CRISPR sensor for wearable, noninvasive detection of SARS-CoV-2 at room temperature within 90 min. They embedded colorimetric genetic circuits into cellulose substrates surrounded by a fluid wicking and containment assembly made of flexible elastomers. The devices are flexible, elastic and can rapidly wick in splashed fluids through capillary action, using a lacZ β-galactosidase operon as the circuit output to hydrolyze chlorophenol red-β-D-galactopyranoside (CPRG), a yellow-to-purple color change develops upon exposure to a target (Fig. 4D).

Researchers [99] presented a novel design of a colorimetric gene sensing platform based on the CRISPR/Cas system. In this strategy, programmable recognition of DNA by Cas12a/crRNA and programmable recognition of RNA with complementary targets by Cas13a/crRNA activate *trans*-ssDNA or -ssRNA cleavage. Target-induced *trans*-ssDNA or -ssRNA cleavage triggers changes in the aggregation behaviour of the designed AuNPs-DNA probe pairs, enabling naked-eye gene detection in less than 1 h. In another example, researchers [100] reported a colorimetric virus detection method based on the CRISPR/Cas9 system. In this method, RNA in virus lysates is directly recognized by the CRISPR/Cas9 system, and then streptavidin-horseradish peroxidase binds to biotin-PAMmer to induce colour changes by oxidation of 3,3′,5,5′-tetramethylbenzidine. SARS-CoV-2, pH1N1 and pH1N1/H275Y viruses could be successfully identified by visual inspection using this method.

Despite the outstanding performance of the above detection methods, disadvantages still exist. A comparison of these methods is presented (Table 2), including detailed information on some applications (including advantages and disadvantages).

3. Chip classification and structure

3.1. Centrifugal chip

The centrifugal microfluidic chip uses centrifugal force to drive liquid to flow to different areas for different chemical analysis reactions. The centrifugal microfluidic chip usually consists of a miniaturized flow pipeline, valve, reaction chamber, detector, and other functional components. In recent years, centrifugal chips have emerged endlessly and have been widely used in basic research in the field of nucleic acid detection.

Our group has been deeply cultivated in the field of the centrifugal microfluidic chip. We designed a pull-up centrifugal disc chip to drive the liquid flow with the power generated by the card pumping [20]. The chip has eight identical reaction units, and each unit has 4 sets of microchambers, which can complete the entire process from nucleic acid purification to detection. The chip implements a non-electrical driver detection, which can detect 6 kinds of pathogenic bacteria at the same time. The sensitivity is 200 bacteria/μL in cracking solution, which is of positive significance for clinical diagnosis. We also designed a centrifugal chip with 32 reaction chambers that can detect 16 targets at the same time, introduce CRISPR reactions into it [16,18], and develop The Cas12a-assisted straight-forward microfluidic equipment for analysis of nucleic acid (CASMEAN). We first applied it to the detection of *Pseudomonas aeruginosa*, which can be finished within 1.5 h, and has a 1000 CFU/mL detection limit and excellent specificity. After that, we also applied it to the DNA single nucleotide polymorphism detection. In 20 min, we completed the testing of three genotypes of the homozygous wild type, the homozygous mutant type, and the heterozygous mutant type with good preservation and accuracy. In addition, our group has recently developed an automated, integrated centrifugal chip that can complete the process from sample pre-processing to detection [17] (Fig. 5A). The chip is composed of 5 layers of PMMA material: substrate, microstructures, ball valve, and injection holes. We use this chip to perform RT-Raa-T7-CRISPR/CAS13A reaction to achieve high-specific accuracy and fast detection and typing of HBV in human blood samples.

In the detection of antibiotic resistance genes of Golden *Staphylococcus aureus*, researchers designed a centrifugal chip for the first time [101]. The chip has 30 independent reaction chambers that can restore frozen dry reagents, and it can conduct 5 groups of parallel testing simultaneously. In actual testing, the chip with RPA amplification can achieve a detection limit of 10 copies in less than 20 min, which has excellent performance. Researchers have designed a chip that can identify 6 influenza virus subtypes at the same time [102] (Fig. 5B). This centrifugal chip used for RT-LAMP amplification detection has 20 independent reaction chambers, which can be divided into seven layers, including two bottom caps and two pattern layers, two adhesive layers for adhesion, and a specially designed membrane valve layers that can be used for stabilizing pressure. For the detection of the five subtypes of influenza A and influenza B, the detection sensitivity of 100 copies can be achieved. It has excellent performance and is particularly hoped to monitor diseases in resource-scarce areas. In addition, for the test of SARS-COV-2, there are many applications of integrated disc chips. For example, the centrifuge chip that can be read out from the smartphone can complete the whole process of detection within 1 h [103], and the centrifugal disk chip can simultaneously detect seven human respiratory coronaviruses [104].

3.2. Chips with valves as the primary function

A key component in microfluidics is the microfluidic valve. Microfluidic valves can be used not only to direct flow at

Table 2
Comparation of the detecting methods based on microfluidics.

Detecting methods	Amplification methods	Microfluidic systems	Analyte	Performance	Advantages/disadvantages	Reference
Fluorescence	PCR	N/A	HPV DNA	18 fM 1 h	Superior sensitivity, low cost, easy to operate, rapid analysis	[85]
	RT-PCR	NanoPCR device	SARS-CoV-2 RNA	3.2 copies/μl 17 min		[26]
	CRISPR	Digital droplet chip	SARS-CoV-2 RNA	5 copies/μl	High background noise	[88]
Electrochemistry	LAMP-CRISPR/Cas12a	Multiplexed EC sensors within an LOC microfluidic chip	SARS-CoV-2 RNA	0.8 copies/μl 2 h	Rapid detection, easy to fabricate, low cost, portable and self-controlled	[22]
	N/A	N/A	RNA	0.42 fM		[92]
	HCR	N/A	DENV DNA	188 fM	Unstable and susceptible	[93]
Colorimetry	RT-RPA-CRISPR/Cas12a	wFDCF devices	SARS-CoV-2 RNA	500 copies 90 min	Portable, low cost, easy to prepare, naked eye readout	[98]
	CRISPR/Cas13a	N/A	Bacteria RNA	200 copies 1 h		[99]
	CRISPR/Cas9	N/A	SARS-CoV-2 RNA	140 pM 90 min	Unable to quantitatively detect, limited sensitivity	[100]

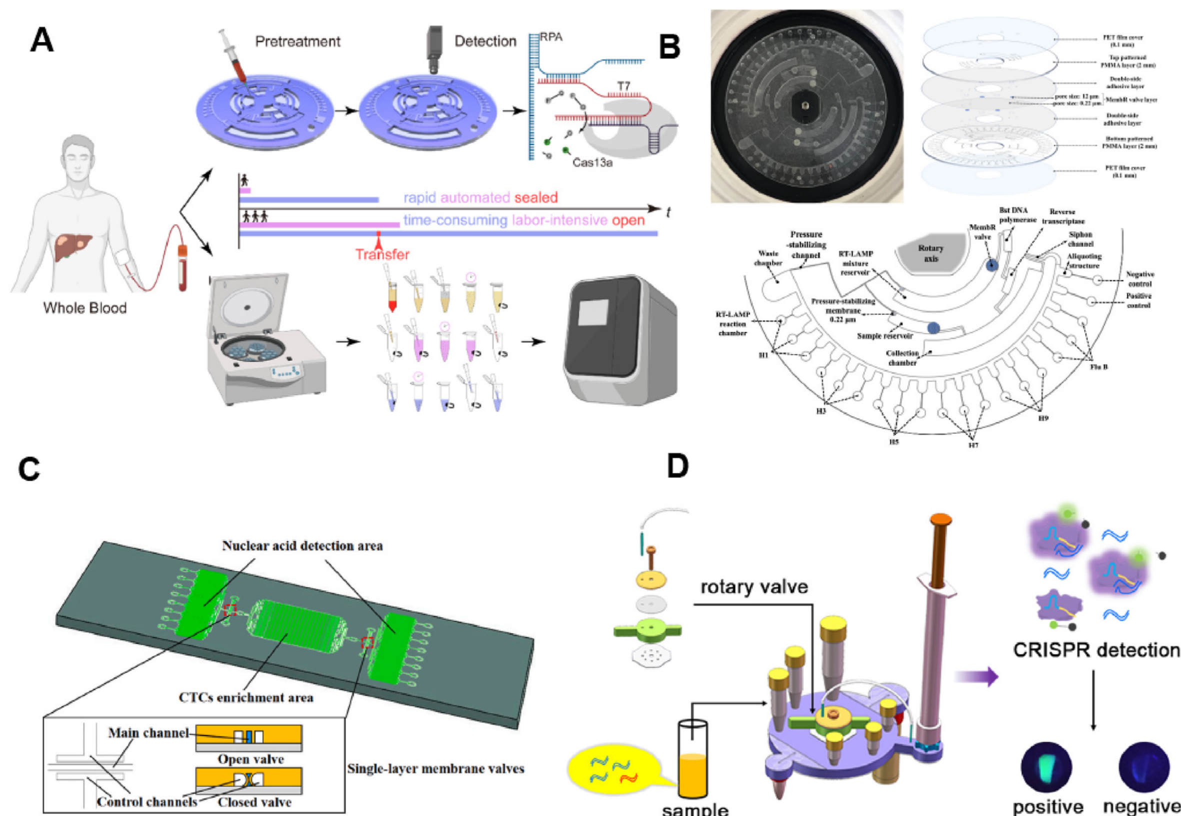

Fig. 5. Diagram of centrifugal chip and valves chip. (A) An automated, integrated centrifugal chip that can complete the process from sample pre-processing to detection. Reproduced with permission from Ref. [17]. (B) A centrifugal chip used for RT-LAMP amplification detection has 20 independent reaction chambers, which can identify 6 influenza virus subtypes at the same time. Reproduced with permission from Ref. [102]. (C) A multifunctional microfluidic device based on a monolayer membrane valve consisting of three parts, including a CTCs capture region, a monolayer membrane flap region, and a microchamber nucleic acid-based dPCR detection and analysis region. Reproduced with permission from Ref. [109]. (D) A rotary valve-assisted fluidic chip coupled with CRISPR/Cas12a for fully integrated nucleic acid detection. Reproduced with permission from Ref. [110].

intersections but also to allow real-time adjustment of mixtures. Various valves give excellent expandability and functionality to microfluidic chips, such as membrane valves, rotary valves, ball valves, quake valves, so forth [105]. Microfluidic chips with good structure and performance will bring new vitality and infinite possibilities for nucleic acid detection functions [106,107].

Researchers [108]. developed a new microfluidic chip for nucleic acid detection using stretching acts as the driving force. The sample entered the chip by applying capillary force. The strain valve was opened under the action of tensile force, and the spring pump generated the power to drive the fluid to flow toward the detection chamber in a specific direction. The detection of Sars-CoV-2 was realized on the chip.

In another example, researchers [109] developed a multifunctional microfluidic device based on a monolayer membrane valve consisting of three parts, including a circulating tumor cells (CTCs)

capture region, a monolayer membrane flap region, and a microchamber nucleic acid-based dPCR detection and analysis region (Fig. 5C). The chip allows CTC capture, lysis, and genetic characterization on a single chip. CTCs are first captured in the CTC capture region and then cleaved using Proteinase K to release the nucleic acids. The CTCs lysates are then transferred to a nucleic acid detection region consisting of 12,800 microchambers for nucleic acid detection.

Researchers [110]. established a simple rotary valve-assisted fluidic chip coupled with CRISPR/Cas12a for fully integrated nucleic acid detection (Fig. 5D). All detection reagents are prestored on the fluidic chip. With the help of a rotary valve and syringe, fluid flow and agitation can be precisely controlled. Nucleic acid extraction, LAMP reactions and CRISPR assays can be completed in less than 80 min. Using Vibrio parahaemolyticus as the target, the chip can reach a detection sensitivity of 31 copies of target DNA per reaction.

3.3. Digital droplet chip

The microfluidic droplet chip is developed based on the single-phase microfluidic chip. Since Professor Rustem F. Ismagilov first designed the T-shaped microfluidic droplet chip, the microfluidic droplet chip has received extensive attention and research. Due to a series of potential advantages such as low sample consumption, fast mixing speed, simple operation, easy manipulation, and good repeatability, microfluidic droplet chip technology has been widely used in the field of high-throughput detection. With the development of droplet technology, the researchers divided the reaction solution into thousands of droplets and divided the reaction into many separate droplets, which can greatly improve the reaction sensitivity and realize a digital droplet-based nucleic acid assay.

3.3.1. Digital PCR chip

In recent years, researchers have developed a variety of digital droplet PCR (ddPCR) assays by introducing PCR into droplet microfluidic chips. Researchers developed a ddPCR using droplet chip technology to achieve multiplex screening of genes in transgenic maize lines [111] (Fig. 6A). This protocol adopted a single universal primer strategy to develop a single universal primer multiplex ddPCR (SUP-*M*-ddPCR). In the genetically modified screening assay for maize, a detection limit of 0.1% and a quantification limit of 0.01% are achieved with high specificity and a relative deviation of less than 25%, which has a good application prospect in the detection of GM food.

In the fight against SARS-CoV-2, ddPCR technology also played an important role. Researchers developed a ddPCR gene chip equipped with an imaging system [112]. The chip is designed as a three-layer structure, the upper and lower layers are made of transparent glass slides, and the middle is the PDMS layer with channels and microchambers. The entire chip has 20,000 independent chambers. The volume of each chamber is 0.81 nL, which can accommodate a total of about 16 μL of samples. In the detection of SARS-CoV-2 samples, the system achieved 99% accuracy, and the imaging system also reduced the imaging time by as much as 165 s, which has the possibility of further development of applications.

3.3.2. Digital isothermal amplification chip

The PCR process involves changes in different temperature ranges and has certain challenges for chip design. The isothermal amplification with constant temperature can reduce the difficulty of instrument and chip design. In recent years, more and more researchers have taken the isothermal amplification technologies into the microfluidic droplet platform to develop digital isothermal amplification technologies.

Researchers designed a droplet chip with a "cross" flow focusing junction design [113]. They combined this droplet chip with LAMP to detect *Salmonella typhimurium* and used the final fluorescence of the droplet to evaluate the result. The results show that this digital droplet LAMP method can detect the genes in the diluted 103−105 times in the pure *Salmonella typhimurium* with good sensitivity and specificity.

RPA technology is also a temperature amplification technology that has attracted much attention. Researchers did some research in this field [114,115]. They designed a "cross" junction droplet microfluidic chip with a microfluidic pico-injector. When the RPA reagent (excluding magnesium ions) forms a droplet, the magnesium ion is added from the pico-injector so that the RPA amplification reaction is performed in the droplet. For the detection of actual samples, this method shows a high signal-to-noise ratio and 100% accuracy. They also combines CRISPR/Cas13a with RPA and introduces them in the droplet chip, which also shows excellent performance. In the detection of HPV viruses, excellent sensitivity and specificity are displayed. At the same time, the detection time is greatly shortened, and the relative test results can be obtained in only 10 min.

3.3.3. Digital CRISPR and other chip

CRISPR technology has attracted much attention and has been extensively studied for nucleic acid detection. The target and crRNA can bind to the Cas protein to trigger specific *cis*-cutting and relevant non-specific *trans*-cutting to generate a lot of fluorescence signals. However, CRISPR-based assay mainly relies on reverse transcription and amplification to improve detection sensitivity. CRISPR-based assays are usually carried out in a reactor with a large volume of uL-level. The generated fluorescence is severely diluted and can be detected only when many targets are in the system. Digital CRISPR based on microdroplets was developed to detect RNA and DNA. Researchers designed a droplet chip to complete accurate digital CRIPR testing without amplification [10,116] (Fig. 6B). The chip structure is different from what we mentioned before. It uses a water phase entrance and two oil phase entrances. At the same time, the negative pressure formed by the piston syringe at the end exit promotes the liquid flow to form a droplet without a complex syringe pump. They have introduced the Cas12 and Cas13 systems into their designed chips and have achieved single molecular detection without any amplification. Compared with bulk CRISPR detection, the sensitivity of digital CRISPR has been increased by 50 times and 10000 times. At the same time, it only takes a few microliter reagents to consume, which is likely to be widely used in clinical situations. In addition to single index detection, the droplet chip also shows its multiple detection capabilities. Researchers have developed a droplet array chip platform CARMEN, which can be used for multiple pathogen detection [23] (Fig. 6C). After introducing the Cas13 system, more than 4500 targets can be detected on a single chip. At the same time, due to the strong multiple detection capacity, the detection cost has also dropped by about 300 times. The characteristics of multiple detection capabilities, low cost, and miniaturization of this method make it suitable for clinical diagnosis in the future.

In addition to CRISPR, the droplet chip is also combined with other technologies to achieve high-sensitivity nucleic acid detection. Our group has designed a simple "cross" junction droplet chip and introduced hybrid detection technology into it [19]. The attomolar sensitivity to the HPV virus is achieved without amplification (Fig. 6D). The DNA molecular circuit is also combined with the droplet chip and achieves the femtomolar sensitivity for miRNA detection [117].

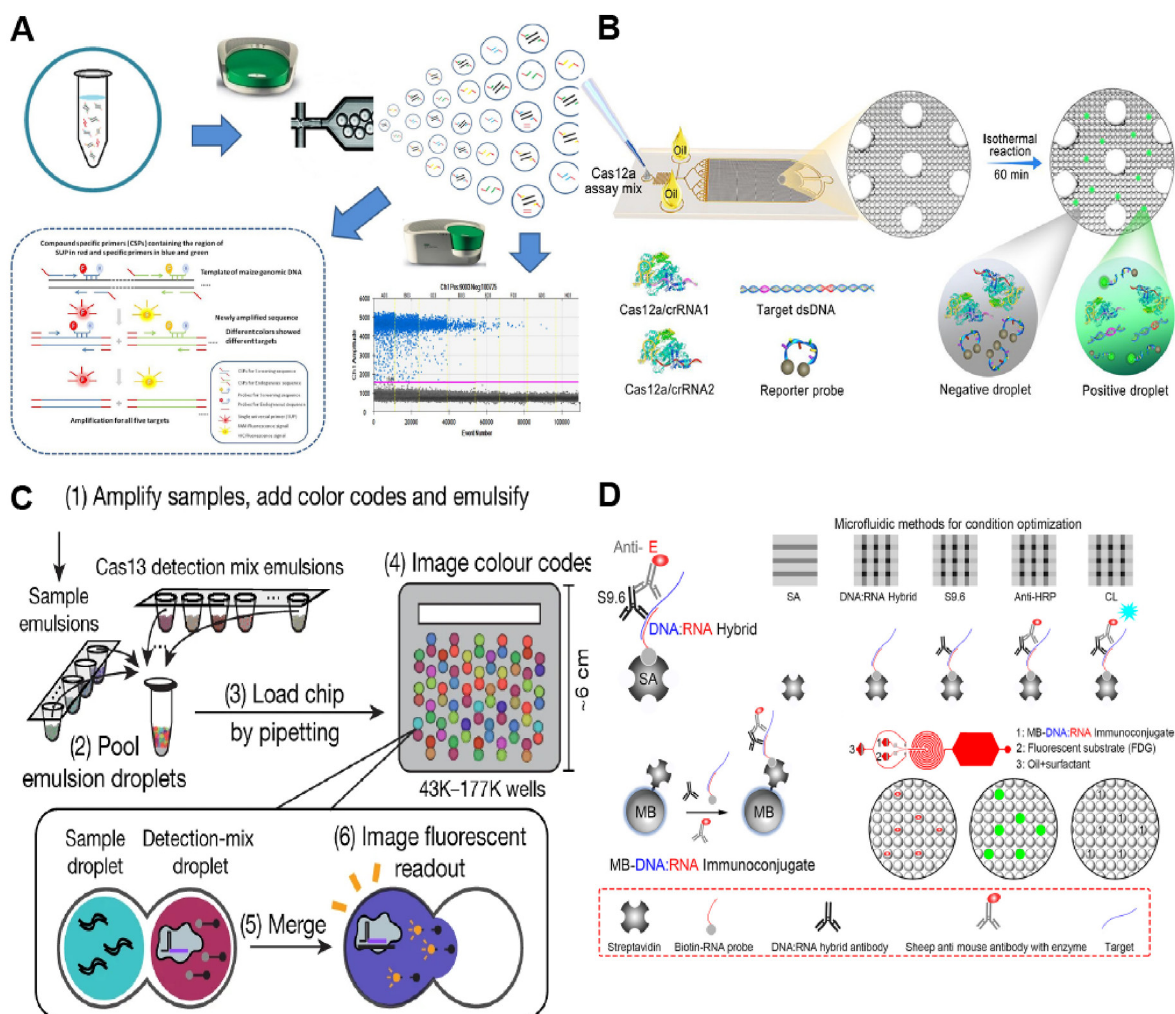

Fig. 6. Diagram of digital droplet chip. (A) A ddPCR chip can achieve multiplex screening of genes in transgenic maize lines. Reproduced with permission from Ref. [111]. (B) A droplet chip with a water phase entrance and two oil phase entrances, the negative pressure formed by the piston syringe at the end exit to promote liquid flowing to form droplets. Reproduced with permission from Ref. [116]. (C) A droplet array chip platform CARMEN can detect 4000–5000 targets simultaneously. Reproduced with permission from Ref. [23]. (D) A "cross" junction droplet chip with hybrid detection technology to realize attomolar sensitivity of HPV virus without amplification. Reproduced with permission from Ref. [19].

3.4. Paper-based chip

Paper is a promising material for constructing microfluidics for diagnosis. Cellulose paper is a ubiquitous, lightweight, biodegradable and inexpensive material that can be a natural platform for microfluidics [118] (Fig. 7A). Cellulose paper-based microfluidics can handle 0.1–100 μL of liquid through millimeter-scale fluidic channels [119]. As one of the popular applications of paper-based materials, lateral flow assay (LFA) is a user-friendly diagnostic tool [120–124].

Our group previously reported on a simple, fast, low-cost, robust and multiplexed barcode paper-based inspection (BPA) that is compatible with mobile devices [125]. The use of an inkjet printer and XYZ dispensing platform enabled the mass production of a highly accurate and efficient barcode paper-based analysis device. We designed a new set of barcodes and developed an application to read the new codes. The BPA system can be used to detect blood-borne infections, drug residues in milk, and multiplex nucleic acids. The entire testing process and read-out of results can be completed in less than 10 min (Fig. 7B).

In addition, researchers [126] developed a novel flowmetry strip-based assay to improve the sensitivity and specificity of LbCas12a-mediated nucleic acid detection. The modified crRNA is incorporated into a paper-based LFA that can detect targets with ultra-high sensitivity within 30 min. Pang et al. [127] developed CRISPR-Cas12a-mediated surface-enhanced Raman scattering (SERS) LFA to improve the sensitivity and specificity of LFA-based nucleic acid detection (Fig. 7C). By combining the ultra-sensitive SERS tag with the target-specific signal amplification capability of CRISPR-Cas12a, HIV-1 dsDNA can be quantified directly with a LOD of 0.3 fM without any pre-amplification step, which is nearly 4 orders of magnitude lower than CRISPR-Cas12a. Compared with the traditional colorimetric LFA method. The whole detection process can be completed in less than 1 h. Simple and inexpensive paper strips have great potential for immediate detection of nucleic acid targets.

The development of paper-based electroanalytical strips as powerful diagnostic tools has attracted a lot of attention in the sensor community. In particular, its application to nucleic acid detection in complex matrices has been evaluated by researchers [128] in combination with paper electrodes for two major nucleic acid detection methods based on target/probe hybridization, namely, a signal on and a signal off. The method uses single-stranded DNA associated with the H1047R (A3140G) missense

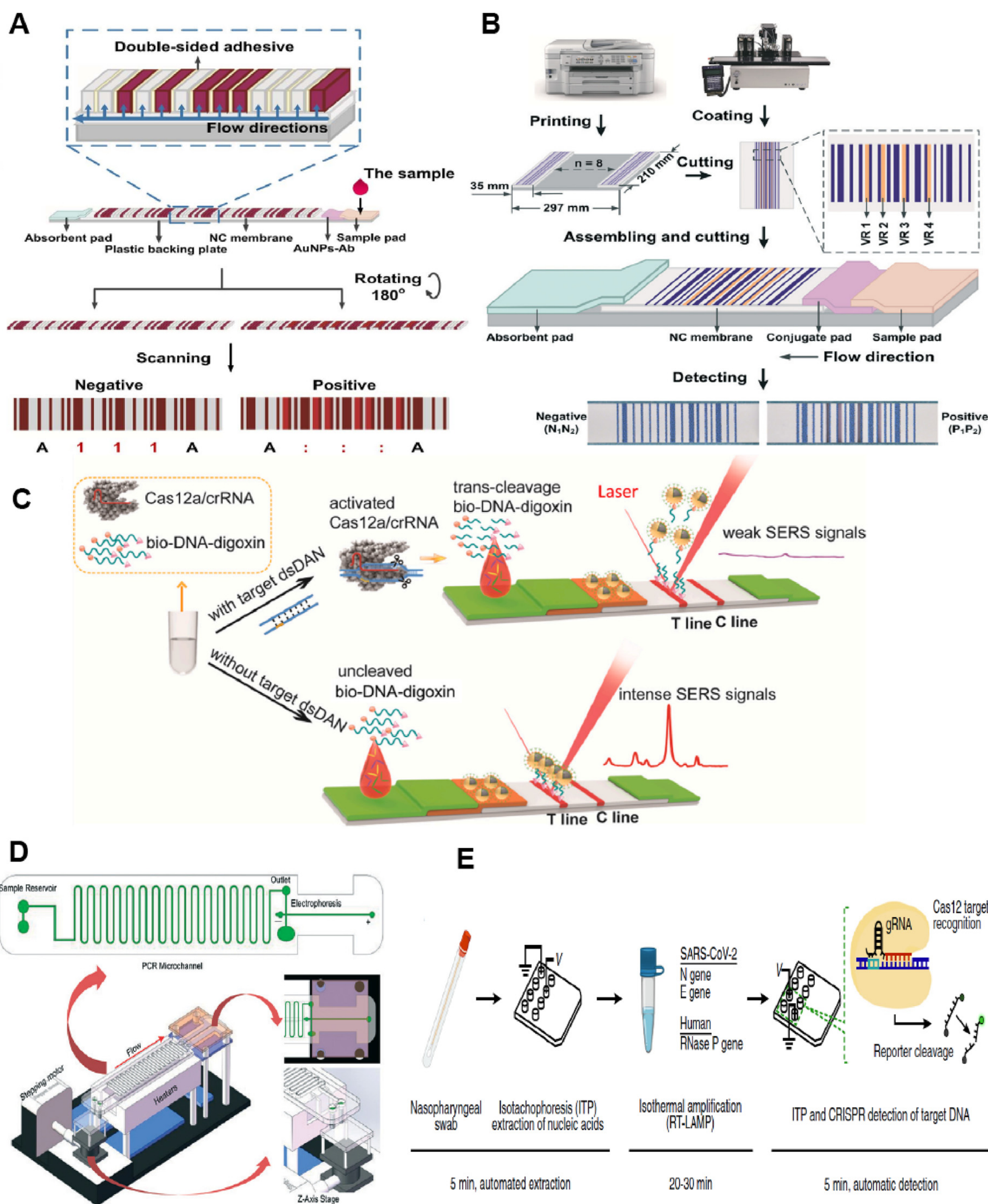

Fig. 7. Diagram of paper-based chip and ME chip. (A) The paper-based barcode assay system. Reproduced with permission from Ref. [118]. (B) Multiplexed barcode paper-based inspection that is compatible with mobile devices. Reproduced with permission from Ref. [125]. (C) CRISPR-Cas12a-mediated SERS to improve the sensitivity and specificity of LFA-based nucleic acid detection. Reproduced with permission from Ref. [127]. (D) A portable all-in-one microfluidic device for rapid diagnosis of pathogens based on an integrated CF-PCR and electrophoresis biochip. Reproduced with permission from Ref. [135]. (E) A ME device combining microfluidics, on-chip electric field control, and CRISPR to rapidly detect SARS-CoV-2 RNA in clinical samples. Reproduced with permission from Ref. [136].

mutation in exon 20 of breast cancer as a model target.

In another example, researchers [129] presented a 3D microfluidic paper-based electrochemical device for POCT applications in nucleic acid amplification testing. The devices use gold plasma coated wires to integrate electroanalytical readings using in situ self-assembled layers on the wire before assembly to the paper-based device. They also include a sandwich hybridization assay area where sample incubation, rinsing, and detection steps are integrated using removable laminated filter paper for time-sequence-based reactions. These devices use glass fibre matrices to store RPA reagents and perform isothermal amplification.

Table 3
Microfluidics platform comparation for nucleic acid analysis.

Microfluidic platform	Analyte	Amplification technique	LOD	Time	Signal readout	Advantages/disadvantages	Sample-process integration	Reference
Centrifugal chip	Bacteria DNA	RPA	10 copies	20 min	Fluorescence	Automation, multiple detection, multiplexing, simple fluid control, no active valves or pumps, high integration; One-time use, high cost of single test, difficult fabrication	No	[101]
	Influenza virus RNA	RT-LAMP	50-100 copies	45 min	Colorimetry		Yes	[102]
	SARS-CoV-2 RNA	RT-LAMP	100 copies	1 h	Fluorescence		No	[103]
	Coronaviruses RNA	RT-LAMP	10 copies/μL	40 min	Fluorescence		No	[104]
	Bacteria RNA	LAMP	200 CFU/μL	1 h	Fluorescence		Yes	[20]
	HBV DNA	RPA-CRISPR/Cas13a	1 aM	1 h	Fluorescence		Yes	[17]
	Bacteria DNA	RAA-CRISPR/Cas12a	10^3 CFU/mL	1.5 h	Fluorescence		No	[18]
Valves chip	SARS-CoV-2 RNA	RT-LAMP	N/A	N/A	Colorimetry	Complex fluid control, multi-functional integration, wide choice of materials, simple manufacturing and low cost;	No	[108]
	DNA	ddPCR	100 copies/μL	N/A	Fluorescence		Yes	[109]
	Bacteria DNA	LAMP-CRISPR/Cas12a	30 copies	80 min	Fluorescence	Requires pumps and other equipment to drive the fluid, complex structure, limited automation	Yes	[110]
Digital droplet chip	DNA	ddPCR	8 copies	N/A	Fluorescence	Ultra-high sensitivity, single molecule detection, absolute quantification, background-free fluorescence detection, high throughput, rapid generation of large numbers of microdroplets, digital information; Multiple processing steps, low sampling efficiency, requires complex image and data processing, time consuming	No	[111]
	SARS-CoV-2 DNA	ddPCR	10 copies/μL	N/A	Fluorescence		No	[112]
	Bacteria DNA	ddLAMP	25 copies	N/A	Fluorescence		No	[113]
	HPV DNA	ddRPA-CRISPR/Cas13a	10 copies/μL	30 min	Fluorescence		No	[114]
	Virus DNA	CRISPR/Cas12a	17.5 copies/μL	N/A	Fluorescence		No	[116]
	SARS-CoV-2 RNA	CRISPR/Cas13a	6 copies/μL	N/A	Fluorescence		No	[10]
	Virus DNA	CRISPR/Cas13a	1 copy/μL	N/A	Fluorescence		No	[23]
	HPV DNA	N/A	2×10^3 copies/mL	N/A	Fluorescence		No	[19]
	microRNA	isothermal amplification	1 fM	3 h	Fluorescence		No	[117]
Paper-based chip	ASFV DNA	CRISPR/Cas9	150 copies	1 h	Naked eyes	Naked-eye readout, fast, no amplification, convenient, inexpensive, easy to manufacture, field-deployable, no need for external instruments; Poor quantification ability, low sensitivity, low accuracy, low integration	No	[120]
	SARS-CoV-2 RNA	RT-LAMP-CRISPR/Cas12a	3-300 copies	30 min	Fluorescence Naked eyes		No	[126]
	HIV DNA	CRISPR-cas12a	0.3 fM	1 h	SERS Naked eyes		No	[127]
	DNA	N/A	6 nM	N/A	Electrochemistry		No	[128]
	DNA	RPA	0.06 pM	95 min	Electrochemistry		No	[129]
Microelectrophoresis chip	Bacteria DNA	CF-PCR	125 CFU/μL	10 min	Fluorescence	Microsample detection, low reagent consumption, high integration, sample concentration/purification, fast; Requires external electric field, high detection cost	No	[135]
	SARS-CoV-2 RNA	RT-LAMP-CRISPR-cas12a	10 copies/μL	35 min	Fluorescence		Yes	[136]
	Bacteria DNA	ISDPR	12.3 pM	1 h	Fluorescence		No	[137]

3.5. Microelectrophoresis chip

ME chip is a well-established separation technique at the microscale. It provided a robust tool for POCT. It has some advantages, such as lower sample and reagent requirements, lower risk of contamination, faster analysis time, and is suitable for high-throughput analysis. The portability of micro devices makes them ideal for forensic fieldwork or patient POCT, as mechanical and electronic structures can be integrated into the device, thus automating operations that usually require manual labour. These microfluidic devices allow ME chips to be miniaturized and portable, with fast analysis and low cost. Researchers from different

fields have applied ME to various clinical, biomedical, and forensic assays [130–132]. The most significant potential of ME continues to be the multi-step integration capability of its analytical procedures and the portability of POC testing [133]. The effective POC platform is designed to miniaturize and automate sample handling, enabling minimally trained personnel to perform the tests required for diagnosis in a wide range of operating environments with minimal risk of contamination. Researchers [134] introduced a method coupling PCR with ME for detecting high-risk HPV16 and HPV18. The device has an LED and a fluorescence detector that can perform a complete analysis in less than 3 min and detect multiple samples simultaneously. The ME method described in this paper has been successfully applied to HPV detection, and the sequencing results have shown good reliability. The ME allows HPV detection on a small chip, enabling automated and high-throughput analysis.

Current CF-PCR usually requires external precision syringe pumps and complex operations, researchers [135] developed a portable all-in-one microfluidic device for rapid diagnosis of pathogens based on an integrated CF-PCR and electrophoresis biochip (Fig. 7D). The device contained a polycarbonate microchannel, two parallel heating blocks for amplification, and a CCD camera for imaging. The new method achieved automatic sample injection into the chip, with no need for an external precision syringe pump. The all-in-one device can successfully detect three periodontal pathogens in a few minutes. In another example, researchers [136] combine microfluidics, on-chip electric field control, and CRISPR to rapidly detect SARS-CoV-2 RNA in clinical samples (Fig. 7E). In this design, isotachophoresis (ITP) was employed to extract and purify RNA from nasopharyngeal samples, followed by reverse transcriptase-LAMP. The authors then used electric fields to control and accelerate their CRISPR-Cas 12 enzymatic assay. The method takes about 35 min from sample to result, a significant improvement over existing nucleic acid-based diagnostic methods for COVID-19. In addition, researchers [137] integrated ME with isothermal strand-displacement polymerase reaction (ISDPR) to detect methicillin-resistant strains rapidly and sensitively. The amplified products were separated rapidly from other DNAs by ME, and the detection limit was as low as 12.3 pM. It is a label-free, ultrasensitive, and rapid method. We have compared the microfluidic platforms mentioned in this paper for nucleic acid detection (Table 3), including advantages and disadvantages, as well as details of some applications.

4. Conclusion

Various novel microfluidic detection techniques have been developed for nucleic acid detection and have a broad application prospect in the biomedical area. Many complicated chips are still in the laboratory research stage and are difficult to be developed as commercial kits. There still needs a lot of optimization in the process from laboratory to commercialization of microfluidic chips. Commercial highly integrated nucleic acid testing chip and supporting instrument is still expensive and deployed in extensive medical facilities. Rapid nucleic acid testing products for home use are also quite expensive when compared to antigen tests or widely used glucometers, so most of them are distributed as generous packages to employees by prosperous enterprises. To extend their application range to the broad grassroots and household market, further reducing the cost of chips is necessary. The development of rapid nucleic acid extraction reagents and amplification reagents with excellent adaptability to interference should be prioritized. These researches will significantly simplify the detection process, chip design, and manufacturing processes and thus have important significance for the reduction of the detection cost.

Besides the cost, an important direction in the future is the development of amplification-free nucleic acid detection technology, especially the new strategies based on CRISPR, which can release a large number of signaling molecules without nucleic acid amplification. It can greatly reduce the requirement for complicated heating and cooling systems and complex chip design and expand the application scenarios of nucleic acid detection. Ultrasensitive digital detection is another research orientation. Established digital PCR and digital CRISPR show the extreme limit of detection and absolute quantification. However, they require extensive off-chip sample preparation and multiple instrumentations to realize monodispersing and digital detection. The highly miniaturized microfluidic chip provides an ideal tool for the integration of complex digital detection processes. Reducing manual manipulation can greatly improve the accuracy of absolute quantification.

In conclusion, nucleic acid detection has many application scenarios, such as rapid home use tests and digital laboratory analysis. Nucleic acid testing is systematic engineering, including sample processing, nucleic acid extraction and detection steps. The realization of these applications in the future is inseparable from the development of microfluidic chip technology. With the progress in the study of chip material, chip driving force and control valve design, matching reagents, and supporting instrument construction, microfluidic chip technology will become an essential platform for fast, sensitive, and automated diagnosis in health care. The emergence of various flexible materials allows wearable devices to be more portable, highly integrated, and have excellent biocompatibility [138–140]. These excellent properties will be well suited for miniaturization and popularization of nucleic acid detection. Miniaturized devices combined with nucleic acid testing have increasingly become the trend and the mainstay of pandemic disease response.

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

No data was used for the research described in the article.

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References

[1] S. Chen, Y. Sun, F. Fan, S. Chen, Y. Zhang, Y. Zhang, X. Meng, J.M. Lin, Present status of microfluidic PCR chip in nucleic acid detection and future perspective, TrAC Trends Anal. Chem. (Reference Ed.) 157 (2022), 116737. https://doi.org/10.1016/j.trac.2022.116737.
[2] X. Dong, L. Liu, Y. Tu, J. Zhang, G. Miao, L. Zhang, S. Ge, N. Xia, D. Yu, X. Qiu,

Rapid PCR powered by microfluidics: a quick review under the background of COVID-19 pandemic, TrAC Trends Anal. Chem. (Reference Ed.) 143 (2021), 116377. https://doi.org/10.1016/j.trac.2021.116377.
[3] B. Udugama, P. Kadhiresan, H.N. Kozlowski, A. Malekjahani, M. Osborne, V.Y.C. Li, H. Chen, S. Mubareka, J.B. Gubbay, W.C.W. Chan, Diagnosing COVID-19: the disease and tools for detection, ACS Nano 14 (2020) 3822–3835. https://doi.org/10.1021/acsnano.0c02624.
[4] Y. Zhang, Q. Liu, D. Wang, S. Chen, X. Wang, S. Wang, Genotyping and detection of common avian and human origin-influenza viruses using a portable chemiluminescence imaging microarray, SpringerPlus 5 (2016) 1–13. https://doi.org/10.1186/s40064-016-3482-9.
[5] Y. Zhang, Q. Liu, D. Wang, S. Chen, S. Wang, Simultaneous detection of oseltamivir- and amantadine-resistant influenza by oligonucleotide microarray visualization, PLoS One 8 (2013) 1–8. https://doi.org/10.1371/journal.pone.0057154.
[6] M.J. Mina, K.G. Andersen, COVID-19 testing: one size does not fit all, Science 371 (2021) 126–128. https://doi.org/10.1126/science.abe9187.
[7] H.M. Yoo, I.H. Kim, S. Kim, Nucleic acid testing of sars-cov-2, Int. J. Mol. Sci. 22 (2021) 1–25. https://doi.org/10.3390/ijms22116150.
[8] B. Xiao, R. Zhao, N. Wang, J. Zhang, X. Sun, A. Chen, Recent advances in centrifugal microfluidic chip-based loop-mediated isothermal amplification, Trends Anal. Chem. 158 (2022), 116836. https://doi.org/10.1016/j.trac.2022.116836.
[9] J.G. Carter, L.O. Iturbe, J.L.H.A. Duprey, I.R. Carter, C.D. Southern, M. Rana, C.M. Whalley, A. Bosworth, A.D. Beggs, M.R. Hicks, J.H.R. Tucker, T.R. Dafforn, Ultrarapid detection of SARS-CoV-2 RNA using a reverse transcription-free exponential amplification reaction, RTF-EXPAR, Proc. Natl. Acad. Sci. U.S.A. 118 (2021). https://doi.org/10.1073/pnas.2100347118.
[10] T. Tian, B. Shu, Y. Jiang, M. Ye, L. Liu, Z. Guo, Z. Han, Z. Wang, X. Zhou, An ultralocalized Cas13a assay enables universal and nucleic acid amplification-free single-molecule RNA diagnostics, ACS Nano 15 (2021) 1167–1178. https://doi.org/10.1021/acsnano.0c08165.
[11] X. Xiang, Y. Shang, J. Zhang, Y. Ding, Q. Wu, Advances in improvement strategies of digital nucleic acid amplification for pathogen detection, TrAC Trends Anal. Chem. (Reference Ed.) 149 (2022), 116568. https://doi.org/10.1016/j.trac.2022.116568.
[12] M. De Falco, M. De Felice, F. Rota, D. Zappi, A. Antonacci, V. Scognamiglio, Next-generation diagnostics: augmented sensitivity in amplification-powered biosensing, TrAC Trends Anal. Chem. (Reference Ed.) 148 (2022), 116538. https://doi.org/10.1016/j.trac.2022.116538.
[13] D. Gao, Z. Ma, Y. Jiang, Recent advances in microfluidic devices for foodborne pathogens detection, TrAC Trends Anal. Chem. (Reference Ed.) 157 (2022), 116788. https://doi.org/10.1016/j.trac.2022.116788.
[14] Y. Chen, Y. Liu, Y. Shi, J. Ping, J. Wu, H. Chen, Magnetic particles for integrated nucleic acid purification, amplification and detection without pipetting, TrAC Trends Anal. Chem. (Reference Ed.) 127 (2020), 115912. https://doi.org/10.1016/j.trac.2020.115912.
[15] Y. Chen, N. Zong, F. Ye, Y. Mei, J. Qu, X. Jiang, Dual-CRISPR/Cas12a-Assisted RT-RAA for Ultrasensitive SARS-CoV - 2 Detection on automated centrifugal microfluidics, Anal. Chem. 94 (2022) 9603–9609. https://doi.org/10.1021/acs.analchem.2c00638.
[16] Y. Chen, Y. Mei, X. Jiang, Universal and high-fidelity DNA single nucleotide polymorphism detection based on a CRISPR/Cas12a biochip, Chem. Sci. 12 (2021) 4455–4462. https://doi.org/10.1039/d0sc05717g.
[17] N. Zong, Y. Gao, Y. Chen, X. Luo, X. Jiang, Automated centrifugal microfluidic chip integrating pretreatment and molecular diagnosis for hepatitis B virus genotyping from whole blood, Anal. Chem. 94 (2022) 5196–5203. https://doi.org/10.1021/acs.analchem.2c00337.
[18] Y. Chen, Y. Mei, X. Zhao, X. Jiang, Reagents-loaded, automated assay that integrates recombinase- aided amplification and Cas12a nucleic acid detection for a point-of-care test, Anal. Chem. 92 (2020) 14846–14852. https://doi.org/10.1021/acs.analchem.0c03883.
[19] L. Mou, H. Hong, X. Xu, Y. Xia, X. Jiang, Digital hybridization human papillomavirus assay with attomolar sensitivity without amplification, ACS Nano 15 (2021) 13077–13084. https://doi.org/10.1021/acsnano.1c02311.
[20] L. Zhang, F. Tian, C. Liu, Q. Feng, T. Ma, Z. Zhao, T. Li, X. Jiang, J. Sun, Lab on a Chip inspired by the spinning top for sample-to-answer, Lab Chip 18 (2018) 610–619. https://doi.org/10.1039/c7lc01234a.
[21] S.S. Chandrasekaran, S. Agrawal, A. Fanton, A.R. Jangid, B. Charrez, A.M. Escajeda, S. Son, R. Mcintosh, H. Tran, A. Bhuiya, M.D. de León Derby, N.A. Switz, M. Armstrong, A.R. Harris, N. Prywes, M. Lukarska, S.B. Biering, D.C.J. Smock, A. Mok, G.J. Knott, Q. Dang, E. Van Dis, E. Dugan, S. Kim, T.Y. Liu, , IGI Testing Consortium, E.A. Moehle, K. Kogut, B. Eskenazi, E. Harris, S.A. Stanley, L.F. Lareau, M.X. Tan, D.A. Fletcher, J.A. Doudna, D.F. Savage, P.D. Hsu, Rapid detection of SARS-CoV-2 RNA in saliva via Cas13, Nat. Biomed. Eng. 6 (2022) 944–956. https://doi.org/10.1038/s41551-022-00917-y.
[22] D. Najjar, J. Rainbow, S.S. Timilsina, P. Jolly, H. De Puig, M. Yafia, N. Durr, H. Sallum, G. Alter, J.Z. Li, X.G. Yu, D.R. Walt, J.A. Paradiso, P. Estrela, J.J. Collins, A lab-on-a-chip for the concurrent electrochemical detection of SARS-CoV-2 RNA and anti-SARS-CoV-2 antibodies in saliva and plasma, Nat. Biomed. Eng. 6 (2022) 968–978. https://doi.org/10.1038/s41551-022-00919-w.
[23] C.M. Ackerman, C. Myhrvold, S.G. Thakku, C.A. Freije, H.C. Metsky, D.K. Yang, S.H. Ye, C.K. Boehm, J. Kehe, T.G. Nguyen, A. Carter, A. Kulesa, J.R. Barnes, V.G. Dugan, D.T. Hung, P.C. Blainey, Massively multiplexed nucleic acid detection with Cas13, Nature 582 (2020). https://doi.org/10.1038/s41586-020-2279-8.
[24] D. Kong, X. Wang, C. Gu, M. Guo, Y. Wang, Z. Ai, S. Zhang, Y. Chen, W. Liu, Y. Wu, C. Dai, Q. Guo, D. Qu, Z. Zhu, Y. Xie, Y. Liu, D. Wei, Direct SARS-CoV-2 nucleic acid detection by y-shaped DNA dual-probe transistor assay, J. Am. Chem. Soc. 143 (2021) 17004–17014. https://doi.org/10.1021/jacs.1c06325.
[25] D. Wang, S. He, X. Wang, Y. Yan, J. Liu, S. Wu, S. Liu, Y. Lei, M. Chen, L. Li, J. Zhang, L. Zhang, X. Hu, X. Zheng, J. Bai, Y. Zhang, Y. Zhang, M. Song, Y. Tang, Rapid lateral flow immunoassay for the fluorescence detection of SARS-CoV-2 RNA, Nat. Biomed. Eng. 4 (2020) 1150–1158. https://doi.org/10.1038/s41551-020-00655-z.
[26] J. Cheong, H. Yu, C.Y. Lee, J. uk Lee, H.J. Choi, J.H. Lee, H. Lee, J. Cheon, Fast detection of SARS-CoV-2 RNA via the integration of plasmonic thermocycling and fluorescence detection in a portable device, Nat. Biomed. Eng. 4 (2020) 1159–1167. https://doi.org/10.1038/s41551-020-00654-0.
[27] L. Zhang, Y. Zhang, C. Wang, Q. Feng, F. Fan, G. Zhang, X. Kang, X. Qin, J. Sun, Y. Li, X. Jiang, Integrated microcapillary for sample-to-answer nucleic acid pretreatment, amplification, and detection, Anal. Chem. 86 (2014) 10461–10466. https://doi.org/10.1021/ac503072a.
[28] M.N. Emaus, M. Varona, D.R. Eitzmann, S.A. Hsieh, V.R. Zeger, J.L. Anderson, Nucleic acid extraction: fundamentals of sample preparation methodologies, current advancements, and future endeavors, TrAC Trends Anal. Chem. (Reference Ed.) 130 (2020), 115985. https://doi.org/10.1016/j.trac.2020.115985.
[29] N. Ali, R.D.C.P. Rampazzo, A. Di, T. Costa, M.A. Krieger, Current nucleic acid extraction methods and their implications to point-of-care diagnostics, BioMed Res. Int. 2017 (2017). https://doi.org/10.1155/2017/9306564.
[30] M. Flaender, R. den Dulk, V. Flegeau, J. Ventosa, G. Delapierre, J. Berthier, A.G. Bourdat, Grinding Lysis (GL): a microfluidic device for sample enrichment and mechanical lysis in one, Sensor. Actuator. B Chem. 258 (2018) 148–155. https://doi.org/10.1016/j.snb.2017.11.082.
[31] J.H. Wang, C.H. Wang, G. Bin Lee, Sample pretreatment and nucleic acid-based detection for fast diagnosis utilizing microfluidic systems, Ann. Biomed. Eng. 40 (2012) 1367–1383. https://doi.org/10.1007/s10439-011-0473-4.
[32] A. Sarafraz-Yazdi, A. Amiri, Liquid-phase microextraction, TrAC Trends Anal. Chem. (Reference Ed.) 29 (2010) 1–14. https://doi.org/10.1016/j.trac.2009.10.003.
[33] W. Ouyang, J. Han, One-step nucleic acid purification and noise-resistant polymerase chain reaction by electrokinetic concentration for ultralow-abundance nucleic acid detection, Angew. Chem. Int. Ed. 59 (2020), 10981. https://doi.org/10.1002/anie.201915788. –10988.
[34] W. Pan, X. Wang, X. Ma, Y. Chu, S. Pang, Y. Chen, X. Guan, B. Zou, Y. Wu, G. Zhou, Postsynthetic modification of the magnetic zirconium-organic framework for efficient and rapid solid-phase extraction of DNA, ACS Appl. Mater. Interfaces 13 (2021) 50309–50318. https://doi.org/10.1021/acsami.1c12622.
[35] L.M. Dignan, M.S. Woolf, C.J. Tomley, A.Q. Nauman, J.P. Landers, Multiplexed centrifugal microfluidic system for dynamic solid-phase purification of polynucleic acids direct from buccal swabs, Anal. Chem. 93 (2021) 7300–7309. https://doi.org/10.1021/acs.analchem.1c00842.
[36] H. Li, H. Li, Z. Chen, J. Lin, On-chip solid phase extraction coupled with electrophoresis using modified magnetic microspheres as stationary phase, Sci. China, Ser. B: Chem. 52 (2009) 2287–2294. https://doi.org/10.1007/s11426-009-0285-7.
[37] X. Wang, X.Z. Hong, Y.W. Li, Y. Li, J. Wang, P. Chen, B.F. Liu, Microfluidics-based strategies for molecular diagnostics of infectious diseases, Mil. Med. Res. 9 (2022) 1–27. https://doi.org/10.1186/s40779-022-00374-3.
[38] P. Li, M. Li, D. Yue, H. Chen, Solid-phase extraction methods for nucleic acid separation. A review, J. Separ. Sci. 45 (2022) 172–184. https://doi.org/10.1002/jssc.202100295.
[39] X. Zhu, J. Zhao, A. Hu, J. Pan, G. Deng, C. Hua, C. Zhu, Y. Liu, K. Yang, L. Zhu, A novel microfluidic device integrated with chitosan-modified capillaries for rapid ZIKV detection, Micromachines 11 (2020) 186. https://doi.org/10.3390/mi11020186.
[40] K. Lee, J.H. Kang, H.M. Kim, J. Ahn, H. Lim, J.J. Lee, W.J. Jeon, J.H. Lee, K.B. Kim, Direct electrophoretic microRNA preparation from clinical samples using nanofilter membrane, Nano Converg 7 (2020) 1–11. https://doi.org/10.1186/s40580-019-0212-3.
[41] C. Tang, Z. He, H. Liu, Y. Xu, H. Huang, G. Yang, Z. Xiao, S. Li, H. Liu, Y. Deng, Z. Chen, H. Chen, N. He, Application of magnetic nanoparticles in nucleic acid detection, J. Nanobiotechnol. 18 (2020) 1–19. https://doi.org/10.1186/s12951-020-00613-6.
[42] P. Li, M. Li, F. Zhang, M. Wu, X. Jiang, B. Ye, Z. Zhao, D. Yue, Q. Fan, H. Chen, High-efficient nucleic acid separation from animal tissue samples via surface modified magnetic nanoparticles, Separ. Purif. Technol. 262 (2021). https://doi.org/10.1016/j.seppur.2021.118348.
[43] F. Chen, S. yeong Lee, K. Han, T.Y. Lee, N,N-Dimethyl-4,4′-azodianiline functionalized magnetic nanoparticles for enhanced sensitivity of nucleic acid amplification tests, Sensor. Actuator. B Chem. 332 (2021), 129461. https://doi.org/10.1016/j.snb.2021.129461.
[44] X. Zhao, Y. Huang, X. Li, W. Yang, Y. Lv, W. Sun, Full integration of nucleic acid extraction and detection into a centrifugal microfluidic chip employing chitosan-modified microspheres, Talanta 250 (2022), 123711. https://doi.org/10.1016/j.talanta.2022.123711.

[45] Q. Lin, Z. Huang, X. Ye, B. Yang, X. Fang, B. Liu, H. Chen, J. Kong, Lab in a tube: isolation, extraction, and isothermal amplification detection of exosomal long noncoding RNA of gastric cancer, Talanta 225 (2021), 122090. https://doi.org/10.1016/j.talanta.2021.122090.
[46] R. Paul, A.C. Saville, J.C. Hansel, Y. Ye, C. Ball, A. Williams, X. Chang, G. Chen, Z. Gu, J.B. Ristaino, Q. Wei, Extraction of plant DNA by microneedle patch for rapid detection of plant diseases, ACS Nano 13 (2019) 6540–6549. https://doi.org/10.1021/acsnano.9b00193.
[47] H. Li, J. Feng, Y. Wang, G. Liu, X. Chen, L. Fu, Instant and multiple DNA extraction method by microneedle patch for rapid and on-site detection of food allergen-encoding genes, J. Agric. Food Chem. 69 (2021) 6879–6887. https://doi.org/10.1021/acs.jafc.1c01077.
[48] P. Agrawal, K.D. Dorfman, Microfluidic long DNA sample preparation from cells, Lab Chip 19 (2019) 281–290. https://doi.org/10.1039/C8LC01163J.
[49] M. Chung, D. Kim, A.E. Herr, Polymer sieving matrices in microanalytical electrophoresis, Analyst 139 (2014) 5635–5654. https://doi.org/10.1039/c4an01179a.
[50] X. Jiang, C. Zhao, X. Fan, W. Xu, R. Zhang, H. Xu, G. Wu, A DNA-modified hydrogel for simultaneous purification, concentration and detection of targeted cfDNA in human serum, RSC Adv. 9 (2019) 3407–3415. https://doi.org/10.1039/c8ra10138h.
[51] M.A. Cornejo, T.H. Linz, Multiplexed miRNA quantitation using injectionless microfluidic thermal gel electrophoresis, Anal. Chem. 94 (2022) 5674–5681. https://doi.org/10.1021/acs.analchem.2c00356.
[52] K. Wei, J. Zhao, Y. Qin, S. Li, Y. Huang, S. Zhao, A novel multiplex signal amplification strategy based on microchip electrophoresis platform for the improved separation and detection of microRNAs, Talanta 189 (2018) 437–441. https://doi.org/10.1016/j.talanta.2018.07.037.
[53] W. Lu, J. Wang, Q. Wu, J. Sun, Y. Chen, L. Zhang, C. Zheng, W. Gao, Y. Liu, X. Jiang, High-throughput sample-to-answer detection of DNA/RNA in crude samples within functionalized micro-pipette tips, Biosens. Bioelectron. 75 (2016) 28–33. https://doi.org/10.1016/j.bios.2015.08.016.
[54] J.S. Park, K. Hsieh, L. Chen, A. Kaushik, A.Y. Trick, T.H. Wang, Digital CRISPR/Cas-assisted assay for rapid and sensitive detection of SARS-CoV-2, Adv. Sci. 8 (2021) 1–7. https://doi.org/10.1002/advs.202003564.
[55] S.S. Chandrasekaran, S. Agrawal, A. Fanton, A.R. Jangid, B. Charrez, A.M. Escajeda, S. Son, R. Mcintosh, H. Tran, A. Bhuiya, M.D. de León Derby, N.A. Switz, M. Armstrong, A.R. Harris, N. Prywes, M. Lukarska, S.B. Biering, D.C.J. Smock, A. Mok, G.J. Knott, Q. Dang, E. Van Dis, E. Dugan, S. Kim, T.Y. Liu, J.R. Hamilton, E. Lin-Shiao, E.C. Stahl, C.A. Tsuchida, P. Giannikopoulos, M. McElroy, S. McDevitt, A. Zur, I. Sylvain, A. Ciling, M. Zhu, C. Williams, A. Baldwin, E.A. Moehle, K. Kogut, B. Eskenazi, E. Harris, S.A. Stanley, L.F. Lareau, M.X. Tan, D.A. Fletcher, J.A. Doudna, D.F. Savage, P.D. Hsu, Rapid detection of SARS-CoV-2 RNA in saliva via Cas13, Nat. Biomed. Eng. 6 (2022) 944–956. https://doi.org/10.1038/s41551-022-00917-y.
[56] R.L. Nouwairi, L.L. Cunha, R. Turiello, O. Scott, J. Hickey, S. Thomson, S. Knowles, J.D. Chapman, J.P. Landers, Ultra-rapid real-time microfluidic RT-PCR instrument for nucleic acid analysis, Lab Chip 22 (2022) 3424. https://doi.org/10.1039/d2lc00495j.
[57] H. Sun, Y. Liang, G. Li, X. Zhang, S. Wang, C. Huang, A droplet-based multi-volume microfluidic device for digital polymerase chain reaction, J. Alloys Compd. (2021), 159155. https://doi.org/10.1016/j.snb.2022.132473.
[58] N.H. Yunfeng Zai, Chao Min, b Zunliang Wang, Yongjun Ding, b Huan Zhao, Enben Su, A Sample-to-Answer, Quantitative real-time PCR system with low-cost, gravity-driven microfluidic cartridge for rapid detection of SARS-CoV-2, influenza A/B, and human papillomavirus 16/18, Lab Chip 22 (2022) 3436. https://doi.org/10.1039/D2LC00434H.
[59] H. Zhu, H. Zhang, Y. Xu, S. Laššáková, M. Korabečná, P. Neužil, PCR past, present and future, Biotechniques 69 (2020) 317–325. https://doi.org/10.2144/BTN-2020-0057.
[60] T.N.D. Trinh, N.Y. Lee, Advances in nucleic acid amplification-based microfluidic devices for clinical microbial detection, Chemosensors 10 (2022). https://doi.org/10.3390/chemosensors10040123.
[61] S. Sheu, Y. Song, J. Chen, A portable continuous-flow polymerase chain reaction chip device integrated with arduino boards for detecting colla corii asini, Micromachines Artic 13 (2022) 1289.
[62] F. Talebi, H. Ghafoorifard, S. Ghafouri-Fard, A. Jahanshahi, A straight-forward, inexpensive, low power continuous-flow μPCR chip using PCB-based heater electrodes with uniform temperature distribution, Sensors Actuators A Phys 333 (2022), 113220. https://doi.org/10.1016/j.sna.2021.113220.
[63] Z. Li, J. Liu, P. Wang, C. Tao, L. Zheng, S. Sekine, S. Zhuang, D. Zhang, Y. Yamaguchi, Multiplex amplification of target genes of periodontal pathogens in continuous flow PCR microfluidic chip, Lab Chip 21 (2021) 3159–3164. https://doi.org/10.1039/d1lc00457c.
[64] S. Lei, S. Chen, Q. Zhong, Digital PCR for accurate quantification of pathogens: principles, applications, challenges and future prospects, Int. J. Biol. Macromol. 184 (2021) 750–759. https://doi.org/10.1016/j.ijbiomac.2021.06.132.
[65] L. Zhang, R. Parvin, Q. Fan, F. Ye, Emerging digital PCR technology in precision medicine, Biosens. Bioelectron. 211 (2022), 114344. https://doi.org/10.1016/j.bios.2022.114344.
[66] Y. Sun, Y. Huang, T. Qi, Q. Jin, C. Jia, J. Zhao, S. Feng, L. Liang, Wet-etched microchamber array digital PCR chip for SARS-CoV-2 virus and ultra-early stage lung cancer quantitative detection, ACS Omega 7 (2022) 1819–1826. https://doi.org/10.1021/acsomega.1c05082.
[67] X. Cui, L. Wu, Y. Wu, J. Zhang, Q. Zhao, F. Jing, L. Yi, G. Li, Fast and robust sample self-digitization for digital PCR, Anal. Chim. Acta 1107 (2020) 127–134. https://doi.org/10.1016/j.aca.2020.02.010.
[68] X. Fang, Y. Liu, J. Kong, X. Jiang, Loop-mediated isothermal amplification integrated on microfluidic chips for point-of-care quantitative detection of pathogens, Anal. Chem. 82 (2010) 3002–3006. https://doi.org/10.1021/ac1000652.
[69] X. Fang, H. Chen, L. Xu, X. Jiang, W. Wu, J. Kong, A portable and integrated nucleic acid amplification microfluidic chip for identifying bacteria, Lab Chip 12 (2012) 1495–1499. https://doi.org/10.1039/c2lc40055c.
[70] M. Chaouch, Loop-mediated isothermal amplification (LAMP): an effective molecular point-of-care technique for the rapid diagnosis of coronavirus SARS-CoV-2, Rev. Med. Virol. 31 (2021). https://doi.org/10.1002/rmv.2215.
[71] J. Song, B. Cha, J. Moon, H. Jang, S. Kim, J. Jang, D. Yong, H.-J. Kwon, I.-C. Lee, E.-K. Lim, J. Jung, H.G. Park, T. Kang, Smartphone-based SARS-CoV-2 and variants detection system using colorimetric DNAzyme reaction triggered by loop-mediated isothermal amplification (LAMP) with clustered regularly interspaced short palindromic repeats (CRISPR), ACS Nano 16 (2022) 11300–11314. https://doi.org/10.1021/acsnano.2c04840.
[72] L. Bokelmann, O. Nickel, T. Maricic, S. Pääbo, M. Meyer, S. Borte, S. Riesenberg, Point-of-care bulk testing for SARS-CoV-2 by combining hybridization capture with improved colorimetric LAMP, Nat. Commun. 12 (2021) 1–8. https://doi.org/10.1038/s41467-021-21627-0.
[73] Y. Zhang, L. Zhang, J. Sun, Y. Liu, X. Ma, S. Cui, L. Ma, J.J. Xi, X. Jiang, Point-of-care multiplexed assays of nucleic acids using microcapillary-based loop-mediated isothermal amplification, Anal. Chem. 86 (2014) 7057–7062. https://doi.org/10.1021/ac5014332.
[74] D. Natsuhara, R. Saito, H. Aonuma, T. Sakurai, S. Okamoto, M. Nagai, H. Kanuka, T. Shibata, A method of sequential liquid dispensing for the multiplexed genetic diagnosis of viral infections in a microfluidic device, Lab Chip 21 (2021) 4779–4790. https://doi.org/10.1039/d1lc00829c.
[75] Q. Lin, X. Fang, H. Chen, W. Weng, B. Liu, J. Kong, Dual-modality loop-mediated isothermal amplification for pretreatment-free detection of Septin9 methylated DNA in colorectal cancer, Microchim. Acta 188 (2021) 307. https://doi.org/10.1007/s00604-021-04979-8.
[76] Y. Tian, T. Zhang, J. Guo, H. Lu, Y. Yao, X. Chen, X. Zhang, G. Sui, M. Guan, A LAMP-based microfluidic module for rapid detection of pathogen in cryptococcal meningitis, Talanta 236 (2022), 122827. https://doi.org/10.1016/j.talanta.2021.122827.
[77] X. Zhao, X. Li, W. Yang, J. Peng, J. Huang, S. Mi, An integrated microfluidic detection system for the automated and rapid diagnosis of high-risk human papillomavirus, Analyst 146 (2021) 5102–5114. https://doi.org/10.1039/d1an00623a.
[78] Y. Bai, J. Ji, F. Ji, S. Wu, Y. Tian, B. Jin, Z. Li, Recombinase polymerase amplification integrated with microfluidics for nucleic acid testing at point of care, Talanta 240 (2022), 123209. https://doi.org/10.1016/j.talanta.2022.123209.
[79] G. Cao, D. Huo, X. Chen, X. Wang, S. Zhou, S. Zhao, X. Luo, C. Hou, Automated, portable, and high-throughput fluorescence analyzer (APHF-analyzer) and lateral flow strip based on CRISPR/Cas13a for sensitive and visual detection of SARS-CoV-2, Talanta 248 (2022), 123594. https://doi.org/10.1016/j.talanta.2022.123594.
[80] J. Yin, Z. Zou, Z. Hu, S. Zhang, F. Zhang, B. Wang, S. Lv, Y. Mu, A "sample-in-multiplex-digital-answer-out" chip for fast detection of pathogens, Lab Chip 20 (2020) 979–986. https://doi.org/10.1039/c9lc01143a.
[81] H. Yue, M. Huang, T. Tian, E. Xiong, X. Zhou, Advances in clustered, regularly interspaced short palindromic repeats (CRISPR)-based diagnostic assays assisted by micro/nanotechnologies, ACS Nano 15 (2021) 7848–7859. https://doi.org/10.1021/acsnano.1c02372.
[82] M. Yuan, R. Ding, S. Chen, G. Duan, Advances in field detection based on CRISPR/Cas system, ACS Synth. Biol. 10 (2021) 2824–2832. https://doi.org/10.1021/acssynbio.1c00401.
[83] C. shu Zhu, C. yang Liu, X. yuan Qiu, S. si Xie, W. ying Li, L. Zhu, L. yun Zhu, Novel nucleic acid detection strategies based on CRISPR-Cas systems: from construction to application, Biotechnol. Bioeng. 117 (2020) 2279–2294. https://doi.org/10.1002/bit.27334.
[84] D. Sun, T. Fan, F. Liu, F. Wang, D. Gao, J.M. Lin, A microfluidic chemiluminescence biosensor based on multiple signal amplification for rapid and sensitive detection of E. coli O157:H7, Biosens. Bioelectron. 212 (2022), 114390. https://doi.org/10.1016/j.bios.2022.114390.
[85] Y. Zhang, Z. Li, W. Su, G. Zhong, X. Zhang, Y. Wu, B. Situ, Y. Xiao, X. Yan, L. Zheng, A highly sensitive and versatile fluorescent biosensor for pathogen nucleic acid detection based on toehold-mediated strand displacement initiated primer exchange reaction, Anal. Chim. Acta 1221 (2022), 340125. https://doi.org/10.1016/j.aca.2022.340125.
[86] J.S. Gootenberg, O.O. Abudayyeh, J.W. Lee, P. Essletzbichler, A.J. Dy, J. Joung, V. Verdine, N. Donghia, N.M. Daringer, C.A. Freije, C. Myhrvold, R.P. Bhattacharyya, J. Livny, A. Regev, E.V. Koonin, D.T. Hung, P.C. Sabeti, J.J. Collins, F. Zhang, Nucleic acid detection with CRISPR-Cas13a/C2c2, Science 356 (2017) 438–442. https://doi.org/10.1126/science.aam9321.
[87] J.S. Chen, E. Ma, L.B. Harrington, M. Da Costa, X. Tian, J.M. Palefsky, J.A. Doudna, CRISPR-Cas12a target binding unleashes indiscriminate single-stranded DNase activity, Science 360 (2018) 436–439. https://doi.org/10.1126/science.aar6245.
[88] X. Ding, K. Yin, Z. Li, M.M. Sfeir, C. Liu, Sensitive quantitative detection of SARS-CoV-2 in clinical samples using digital warm-start CRISPR assay, Biosens. Bioelectron. 184 (2021), 113218. https://doi.org/10.1016/

j.bios.2021.113218.
[89] Q. Hu, Y. Luo, X. Cao, Z. Chen, Y. Huang, L. Niu, Bioinspired electro-RAFT polymerization for electrochemical sensing of nucleic acids, ACS Appl. Mater. Interfaces 13 (2021) 54794–54800. https://doi.org/10.1021/acsami.1c17564.
[90] D. Ji, M. Guo, Y. Wu, W. Liu, S. Luo, X. Wang, H. Kang, Y. Chen, C. Dai, D. Kong, H. Ma, Y. Liu, D. Wei, Electrochemical detection of a few copies of unamplified SARS-CoV-2 nucleic acids by a self-actuated molecular system, J. Am. Chem. Soc. 144 (2022) 13526–13537. https://doi.org/10.1021/jacs.2c02884.
[91] P. Miao, H. Chai, Y. Tang, DNA hairpins and dumbbell-wheel transitions amplified walking nanomachine for ultrasensitive nucleic acid detection, ACS Nano 16 (2022) 4726–4733. https://doi.org/10.1021/acsnano.1c11582.
[92] X. Jiang, Q. Zhu, H. Zhu, Z. Zhu, X. Miao, Antifouling lipid membrane coupled with silver nanoparticles for electrochemical detection of nucleic acids in biological fluids, Anal. Chim. Acta 1177 (2021), 338751. https://doi.org/10.1016/j.aca.2021.338751.
[93] J. Fu, J. Wu, R. Zhang, Q. Wu, H. Ju, Electrochemical biosensing of DENV nucleic acid amplified with triplet nanostructure-mediated dendritic hybridization chain reaction, Sensor. Actuator. B Chem. 345 (2021), 130436. https://doi.org/10.1016/j.snb.2021.130436.
[94] J. Zhang, L. Mou, X. Jiang, Surface chemistry of gold nanoparticles for health-related applications, Chem. Sci. 11 (2020) 923–936. https://doi.org/10.1039/c9sc06497d.
[95] S. Chatterjee, X.Y. Lou, F. Liang, Y.W. Yang, Surface-functionalized gold and silver nanoparticles for colorimetric and fluorescent sensing of metal ions and biomolecules, Coord. Chem. Rev. 459 (2022), 214461. https://doi.org/10.1016/j.ccr.2022.214461.
[96] J. Zhang, Z. Huang, Y. Xie, X. Jiang, Modulating the catalytic activity of gold nanoparticles using amine-terminated ligands, Chem. Sci. 13 (2022) 1080–1087. https://doi.org/10.1039/d1sc05933e.
[97] J. Zhang, W. Zheng, X. Jiang, Ag+-gated surface chemistry of gold nanoparticles and colorimetric detection of acetylcholinesterase, Small 14 (2018) 1–8. https://doi.org/10.1002/smll.201801680.
[98] P.Q. Nguyen, L.R. Soenksen, N.M. Donghia, N.M. Angenent-Mari, H. de Puig, A. Huang, R. Lee, S. Slomovic, T. Galbersanini, G. Lansberry, H.M. Sallum, E.M. Zhao, J.B. Niemi, J.J. Collins, Wearable materials with embedded synthetic biology sensors for biomolecule detection, Nat. Biotechnol. 39 (2021) 1366–1374. https://doi.org/10.1038/s41587-021-00950-3.
[99] C. Yuan, T. Tian, J. Sun, M. Hu, X. Wang, E. Xiong, M. Cheng, Y. Bao, W. Lin, J. Jiang, C. Yang, Q. Chen, H. Zhang, H. Wang, X. Wang, X. Deng, X. Liao, Y. Liu, Z. Wang, G. Zhang, X. Zhou, Universal and naked-eye gene detection platform based on the clustered regularly interspaced short palindromic repeats/Cas12a/13a system, Anal. Chem. 92 (2020) 4029–4037. https://doi.org/10.1021/acs.analchem.9b05597.
[100] J. Moon, H.J. Kwon, D. Yong, I.C. Lee, H. Kim, H. Kang, E.K. Lim, K.S. Lee, J. Jung, H.G. Park, T. Kang, Colorimetric detection of SARS-CoV-2 and drug-resistant pH1N1 using CRISPR/dCas9, ACS Sens. 5 (2020) 4017–4026. https://doi.org/10.1021/acssensors.0c01929.
[101] S. Lutz, P. Weber, M. Focke, B. Faltin, J. Hoffmann, M. Claas, D. Mark, P. Munday, N. Armes, O. Piepenburg, F. Von Stetten, Microfluidic lab-on-a-foil for nucleic acid analysis based on isothermal recombinase polymerase amplification (RPA), Lab Chip 10 (2010) 887–893. https://doi.org/10.1039/b921140c.
[102] Y. Yao, X. Chen, X. Zhang, Q. Liu, J. Zhu, W. Zhao, S. Liu, G. Sui, Rapid detection of influenza virus subtypes based on an integrated centrifugal disc, ACS Sens. 5 (2020) 1354–1362. https://doi.org/10.1021/acssensors.9b02595.
[103] D. Barrett, G. Sandh, X. Yin, Lab on a Chip using a low-cost centrifugal microfluidic platform, Lab Chip 21 (2021) 2932–2944. https://doi.org/10.1039/d1lc00266j.
[104] H. Xiong, X. Ye, Y. Li, L. Wang, J. Zhang, X. Fang, J. Kong, Rapid differential diagnosis of seven human respiratory coronaviruses based on centrifugal microfluidic nucleic acid assay, Anal. Chem. 92 (2020) 14297–14302. https://doi.org/10.1021/acs.analchem.0c03364.
[105] B. Hu, J. Li, L. Mou, Y. Liu, J. Deng, W. Qian, J. Sun, R. Cha, X. Jiang, An automated and portable microfluidic chemiluminescence immunoassay for quantitative detection of biomarkers, Lab Chip 17 (2017) 2225–2234. https://doi.org/10.1039/c7lc00249a.
[106] Y. Zhang, J. Sun, Y. Zou, W. Chen, W. Zhang, J.J. Xi, X. Jiang, Barcoded microchips for biomolecular assays, Anal. Chem. 87 (2015) 900–906. https://doi.org/10.1021/ac5032379.
[107] S. He, Y. Zhang, P. Wang, X. Xu, K. Zhu, W. Pan, W. Liu, K. Cai, J. Sun, W. Zhang, X. Jiang, Multiplexed microfluidic blotting of proteins and nucleic acids by parallel, serpentine microchannels, Lab Chip 15 (2015) 105–112. https://doi.org/10.1039/c4lc00901k.
[108] X. Li, X. Zhao, W. Yang, F. Xu, B. Chen, J. Peng, J. Huang, S. Mi, Stretch-driven microfluidic chip for nucleic acid detection, Biotechnol. Bioeng. 118 (2021) 3559–3568. https://doi.org/10.1002/bit.27839.
[109] Y. Wang, W. Gao, M. Wu, X. Zhang, W. Liu, Y. Zhou, C. Jia, H. Cong, X. Chen, J. Zhao, EGFR mutation detection of lung circulating tumor cells using a multifunctional microfluidic chip, Talanta 225 (2021), 122057. https://doi.org/10.1016/j.talanta.2020.122057.
[110] H. Wu, S. Qian, C. Peng, X. Wang, T. Wang, X. Zhong, Y. Chen, Q. Yang, J. Xu, J. Wu, Rotary valve-assisted fluidic system coupling with CRISPR/Cas12a for fully integrated nucleic acid detection, ACS Sens. 6 (2021) 4048–4056. https://doi.org/10.1021/acssensors.1c01468.
[111] C. Niu, Y. Xu, C. Zhang, P. Zhu, K. Huang, Y. Luo, W. Xu, Ultrasensitive single fluorescence-labeled probe-mediated single universal primer–multiplex–droplet digital polymerase chain reaction for high-throughput genetically modified organism screening, Anal. Chem. 90 (2018) 5586–5593. https://doi.org/10.1021/acs.analchem.7b03974.
[112] J. Shen, J. Zheng, Z. Li, Y. Liu, F. Jing, X. Wan, Y. Yamaguchi, S. Zhuang, Lab on a Chip system with large field of view for a droplet digital, Lab Chip 21 (2021) 3742–3747. https://doi.org/10.1039/d1lc00532d.
[113] M. Azizi, M. Zaferani, S.H. Cheong, A. Abbaspourrad, Pathogenic bacteria detection using RNA-based loop-mediated isothermal-amplification-assisted nucleic acid amplifi cation via droplet microfluidics, ACS Sens. 4 (2019) 841–848. https://doi.org/10.1021/acssensors.8b01206.
[114] J.Q. Cui, F.X. Liu, H. Park, K. Wai, T. Leung, B. Zhong, S. Yao, Biosensors and Bioelectronics Droplet digital recombinase polymerase amplification (ddRPA) reaction unlocking via picoinjection, Biosens. Bioelectron. 202 (2022), 114019. https://doi.org/10.1016/j.bios.2022.114019.
[115] F.X. Liu, J.Q. Cui, H. Park, K.W. Chan, T. Leung, B.Z. Tang, Isothermal background-free nucleic acid quantification by a one-pot Cas13a assay using droplet microfluidics, Anal. Chem. 94 (2022) 5883–5892. https://doi.org/10.1021/acs.analchem.2c00067.
[116] H. Yue, B. Shu, T. Tian, E. Xiong, M. Huang, D. Zhu, J. Sun, Q. Liu, Droplet Cas12a assay enables DNA quantification from unamplified samples at the single-molecule level, Nano Lett. 21 (2021) 4643–4653. https://doi.org/10.1021/acs.nanolett.1c00715.
[117] G. Gines, R. Menezes, K. Nara, A. Kirstetter, V. Taly, Y. Rondelez, Isothermal digital detection of microRNAs using background-free molecular circuit, Sci. Adv. 6 (2020) 1–9.
[118] M. Yang, W. Zhang, J. Yang, B. Hu, F. Cao, W. Zheng, Y. Chen, X. Jiang, Skiving stacked sheets of paper into test paper for rapid and multiplexed assay, Sci. Adv. 3 (2017). https://doi.org/10.1126/sciadv.aao4862.
[119] K. Mao, X. Min, H. Zhang, K. Zhang, H. Cao, Y. Guo, Z. Yang, Paper-based microfluidics for rapid diagnostics and drug delivery, J. Contr. Release 322 (2020) 187–199. https://doi.org/10.1016/j.jconrel.2020.03.010.
[120] X. Wang, E. Xiong, T. Tian, M. Cheng, W. Lin, H. Wang, G. Zhang, J. Sun, X. Zhou, Clustered regularly interspaced short palindromic repeats/Cas9-mediated lateral flow nucleic acid assay, ACS Nano 14 (2020) 2497–2508. https://doi.org/10.1021/acsnano.0c00022.
[121] J. Zhang, Y. Li, F. Chai, Q. Li, D. Wang, L. Liu, B.Z. Tang, X. Jiang, Ultrasensitive point-of-care biochemical sensor based on metal-AIEgen frameworks, Sci. Adv. 8 (2022), eabo1874. https://doi.org/10.1126/sciadv.abo1874.
[122] F. Chai, D. Wang, L. Zhu, W. Zheng, X. Jiang, Dual gold nanoparticle/chemiluminescent immunoassay for sensitive detection of multiple analytes, Anal. Chem. 94 (2022) 6628–6634. https://doi.org/10.1021/acs.analchem.2c01177.
[123] D. Wang, C. Ge, L. Wang, X. Xing, L. Zeng, A simple lateral flow biosensor for the rapid detection of copper(II) ions based on click chemistry, RSC Adv. 5 (2015) 75722–75727. https://doi.org/10.1039/c5ra11752f.
[124] D. Wang, C. Ge, K. Lv, Q. Zou, Q. Liu, L. Liu, Q. Yang, S. Bao, A simple lateral flow biosensor for rapid detection of lead(ii) ions based on G-quadruplex structure-switching, Chem. Commun. 54 (2018) 13718–13721. https://doi.org/10.1039/c8cc06810k.
[125] M. Yang, W. Zhang, W. Zheng, F. Cao, X. Jiang, Inkjet-printed barcodes for a rapid and multiplexed paper-based assay compatible with mobile devices, Lab Chip 17 (2017) 3874–3882. https://doi.org/10.1039/c7lc00780a.
[126] L.T. Nguyen, B.M. Smith, P.K. Jain, Enhancement of trans-cleavage activity of Cas12a with engineered crRNA enables amplified nucleic acid detection, Nat. Commun. 11 (2020) 1–13. https://doi.org/10.1038/s41467-020-18615-1.
[127] Y. Pang, Q. Li, C. Wang, S. zhen, Z. Sun, R. Xiao, CRISPR-cas12a mediated SERS lateral flow assay for amplification-free detection of double-stranded DNA and single-base mutation, Chem. Eng. J. 429 (2022), 132109. https://doi.org/10.1016/j.cej.2021.132109.
[128] S. Cinti, G. Cinotti, C. Parolo, E.P. Nguyen, V. Caratelli, D. Moscone, F. Arduini, A. Merkoci, Experimental comparison in sensing breast cancer mutations by signal on and signal off paper-based electroanalytical strips, Anal. Chem. 92 (2020) 1674–1679. https://doi.org/10.1021/acs.analchem.9b02560.
[129] S. Khaliliazar, A. Toldrà, G. Chondrogiannis, M.M. Hamedi, Electroanalytical paper-based nucleic acid amplification biosensors with integrated thread electrodes, Anal. Chem. 93 (2021) 14187–14195. https://doi.org/10.1021/acs.analchem.1c02900.
[130] S. Davaran, M. Sadeghinia, Z. Jamalpoor, V. Raeisdasteh Hokmabad, M. Doosti-Telgerd, A. Karimian, Z. Sadeghinia, J. Khalilifard, A. keramt, F. Moradikhah, A. Sadeghinia, Multiple functions of microfluidic platforms: characterization and applications in tissue engineering and diagnosis of cancer, Electrophoresis 41 (2020) 1081–1094. https://doi.org/10.1002/elps.201900341.
[131] F. Luo, Y. Lu, Z. Li, G. Dai, Z. Chu, J. Zhang, P. He, Q. Wang, Probe-lengthening amplification-assisted microchip electrophoresis for ultrasensitive bacteria screening, Sensor. Actuator. B Chem. 325 (2020), 128784. https://doi.org/10.1016/j.snb.2020.128784.
[132] Y. Gu, B. Zhuang, J. Han, Y. Li, X. Song, X. Zhou, L. Wang, P. Liu, Modular-based integrated microsystem with multiple sample preparation modules for automated forensic DNA typing from reference to challenging samples, Anal. Chem. 91 (2019) 7435–7443. https://doi.org/10.1021/acs.analchem.9b01560.
[133] A. Wuethrich, J.P. Quirino, A decade of microchip electrophoresis for clinical

diagnostics − a review of 2008−2017, Anal. Chim. Acta 1045 (2019) 42−66. https://doi.org/10.1016/j.aca.2018.08.009.
[134] Z. Fan, X. Feng, W. Zhang, X. Zhang, J.M. Lin, Rapid detection of high-risk HPV16 and HPV18 based on microchip electrophoresis, J. Pharm. Anal. 10 (2020) 329−333. https://doi.org/10.1016/j.jpha.2020.04.003.
[135] Z. Li, R. Ju, S. Sekine, D. Zhang, S. Zhuang, Y. Yamaguchi, All-in-one microfluidic device for on-site diagnosis of pathogens based on an integrated continuous flow PCR and electrophoresis biochip, Lab Chip 19 (2019) 2663−2668. https://doi.org/10.1039/c9lc00305c.
[136] A. Ramachandran, D.A. Huyke, E. Sharma, M.K. Sahoo, C. Huang, N. Banaei, B.A. Pinsky, J.G. Santiago, Electric field-driven microfluidics for rapid CRISPR-based diagnostics and its application to detection of SARS-CoV-2, Proc. Natl. Acad. Sci. U.S.A. 117 (2020) 29518−29525. https://doi.org/10.1073/pnas.2010254117.
[137] Y. Lu, F. Luo, Z. Li, G. Dai, Z. Chu, J. Zhang, F. Zhang, Q. Wang, P. He, Ultrasensitive microchip electrophoretic detection of the mecA gene in methicillin-resistant Staphylococcus aureus (MRSA) based on isothermal strand-displacement polymerase reaction, Talanta 222 (2021), 121686. https://doi.org/10.1016/j.talanta.2020.121686.
[138] D. Wang, R. Dong, X. Wang, X. Jiang, Flexible electronic catheter based on nanofibers for the in vivo elimination of circulating tumor cells, ACS Nano 16 (2022) 5274−5283. https://doi.org/10.1021/acsnano.1c09807.
[139] L. Mou, J. Qi, L. Tang, R. Dong, Y. Xia, Y. Gao, X. Jiang, Highly stretchable and biocompatible liquid metal-elastomer conductors for self-healing electronics, Small 16 (2020) 1−9. https://doi.org/10.1002/smll.202005336.
[140] L. Tang, L. Mou, W. Zhang, X. Jiang, Large-scale fabrication of highly elastic conductors on a broad range of surfaces, ACS Appl. Mater. Interfaces 11 (2019) 7138−7147. https://doi.org/10.1021/acsami.8b20460.