

Sample-to-answer sensing technologies for nucleic acid preparation and detection in the field

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Abstract

Efficient sample preparation and accurate disease diagnosis under field conditions are of great importance for the early intervention of diseases in humans, animals, and plants. However, in-field preparation of high-quality nucleic acids from various specimens for downstream analyses, such as amplification and sequencing, is challenging. Thus, developing and adapting sample lysis and nucleic acid extraction protocols suitable for portable formats have drawn significant attention. Similarly, various nucleic acid amplification techniques and detection methods have also been explored. Combining these functions in an integrated platform has resulted in emergent sample-to-answer sensing systems that allow effective disease detection and analyses outside a laboratory. Such devices have a vast potential to improve healthcare in resource-limited settings, low-cost and distributed surveillance of diseases in food and agriculture industries, environmental monitoring, and defense against biological warfare and terrorism. This paper reviews recent advances in portable sample preparation technologies and facile detection methods that have been / or could be adopted into novel sample-to-answer devices. In addition, recent developments and challenges of commercial kits and devices targeting on-site diagnosis of various plant diseases are discussed.

1 Introduction

Point-of-care (POC) sensing system is a portable analytical device providing clinical indices such as blood glucose level so patients and their care providers can take necessary actions immediately. It can serve as a reliable diagnostic platform for those living in resource-limited places and as daily care for urban populations in developed countries. An ideal POC sensing system is accurate, rapid, economical, and easy to operate for health monitoring and disease testing [1, 2].

Infectious diseases caused by viruses, viroids, bacteria, and other microbes have resulted in a significant loss of population and economy worldwide almost annually [3, 4]. Resource-limited

and remote regions such as small rural communities and most developing countries always take the brunt of disease outbreaks without early warning and effective treatments when they happen. Many living in these regions cannot effectively combat such circumstances due to the lack of healthcare infrastructures and feasible diagnostic kits allowing quick disease screening [5]. The worldwide COVID-19 pandemic has served as a timely reminder of the critical value of quick disease diagnosis at an early stage [6]. Overwhelmed centralized diagnostic facilities in the COVID-19 hotspots and the lack of access to such facilities in developing countries have highlighted the need for portable sample-to-answer diagnostic devices.

A tremendous amount of infectious diseases in not only humans, but also animals and plants can be analyzed based on detecting nucleic acids. The examples include, but are not limited to, SARS-CoV-2, H1N1, and H5N2 leading to severe diseases in humans, genetic biomarkers of tumor cells, *Salmonella spp*, Noroviruses, and *Toxoplasma gondii* contaminating food and drinking water, and huanglongbing (HLB) devastating citrus species [7-9]. Therefore, the ability to acquire ready-to-detect nucleic acids under field conditions is critical for POC sensing and sample-to-answer devices. Isolation of nucleic acids is implemented in two steps. First intracellular contents, including host and pathogen genomes, organelles, and proteins are released through various cellular lysis methods. Then, desired nucleic acids are separated from the resultant lysates and further purified. Sample-to-answer sensing devices targeting a genetic marker(s) are composed of three critical parts: (i) sample preparation, (ii) target amplification, and (iii) signal readout [2, 10, 11]. Ideally, such devices integrate necessary hardware and assay reagents onboard or in cartridges to minimize user intervention and the risk of contamination [12].

This paper reviews various technologies having great potential to be integrated into portable formats for sample-to-answer bioassays. First, it provides an overview of the in-field sample preparation for humans, animals, plants, and microbes with different approaches. These include sample lysis and nucleic acid purification. Next, it focuses on the nucleic acid amplification methods conducted on a chip or in an integrated system, followed by built-in signal readout principles (Fig. 1). Given the relative scarcity of reports focusing on in-field diagnosis of plant diseases. This review briefly summarizes a variety of portable commercial kits targeting plant diseases, highlighting the importance of this emerging field. Finally, the future perspectives of the portable and sample-to-answer devices are discussed, highlighting unmet needs and promising directions.

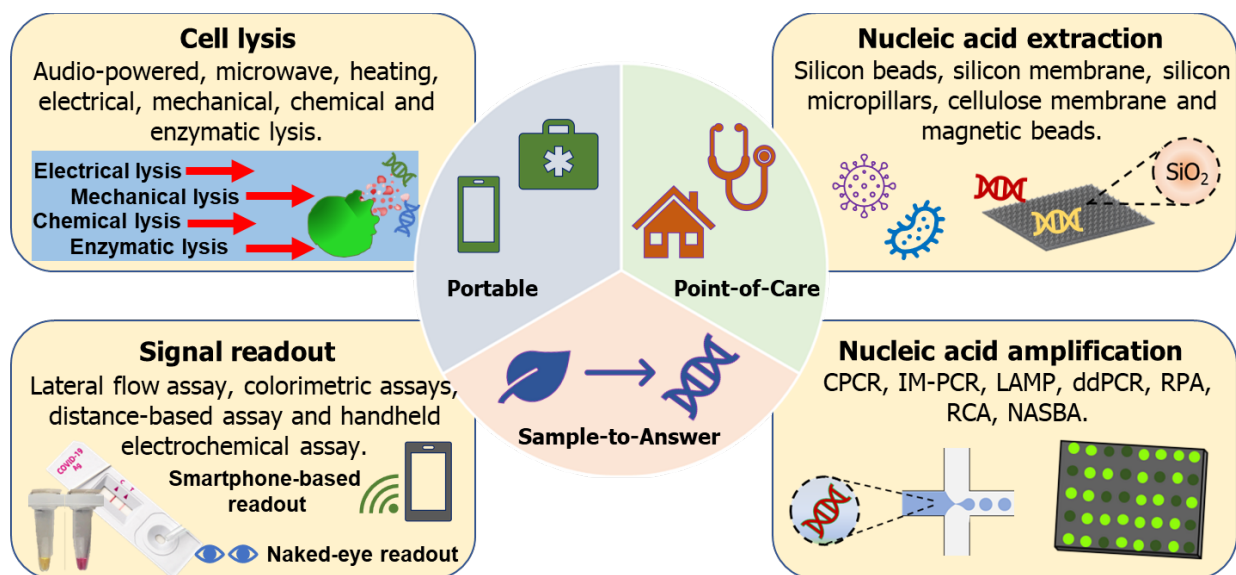


Fig. 1. Overview of portable and sample-to-answer diagnosis composed of four major parts: cell lysis, nucleic acid extraction, amplification, and signal readout.

2 Sample lysis methods

Preparation of biological specimens includes sample lysis and nucleic acid extraction. It is vital for downstream molecular analyses and processing, including genotyping, sequencing, CRISPR, drug screening, and disease diagnosis [13, 14]. Sample lysis breaks down cell walls and membranes and releases plenty of nucleic acids from samples varying in composition and species [15, 16]. However, it is a delicate process because excessive heat and forces resulting from mechanical and physical lysis can degrade the quality of products such as DNA, RNA, and proteins. Particularly, high throughput lysis of difficult-to-lyse samples (e.g., gram-positive bacteria, plant materials) require extra care [15]. Combining multiple lysis modes (or the use of commercialized kits) can alleviate these issues [17]. For in-field uses, sample lysis methods should be fast and easy to operate and consume small amount of sample and energy. This section highlights various sample lysis strategies including mechanical, physical, chemical, and biological approaches, as well as their combinations within a microfluidic chip or an integrated device.

2.1 Human and animal specimens

Pathogenic nucleic acids and various biomarkers related to cancers and infectious diseases among humans and animals are common analytical targets of disease diagnosis. Effective liberation of these desired substances from human and animal samples is critical. Lysis of human and animal cells and tissues is less demanding than plants due to the lack of fibrous cell walls. To date, several sample-to-answer devices integrated chemical lysis to process a variety of clinical samples, such as swab samples and bodily fluids (e.g., sputum, urine, and blood) [18-22]. For example, lab-on-a-disk platform is one of the developments capable of automatically executing sample-to-answer diagnoses by taking advantage of lysis buffers and varying rotation speed of the disk (Fig. 2A) [18, 20]. These devices performed on-disk sample lysis and liquefaction in a fully

automated manner for preparing pathogenic nucleic acids and downstream mass screening. In addition to lab-on-a-disk platforms, a sample-to-answer cartridge capable of processing clinical saliva and blood with lysis buffer was demonstrated [22]. The cartridge featured automated transfer and mixing of bodily fluids and buffers performed by built-in pipettor, the plunge pump, and two stepper motors. Chemical lysis can also be used for bacteria lysis in urine. Li et al. integrated a conical-shaped focusing magnet, a self-locking solenoid, and a slip ring with an electric field to manipulate magnetic beads to fully mix and extract bacteria lysate in bacteriuria (Fig. 2B) [19]. In some cases (e.g., sticky samples such as sputum), chemical lysis will be used together with heat to improve lysis efficiency [20].

Thermal lysis can also work perfectly by itself. Ye et al. lysed clinical vaginal and anal swab samples for the diagnosis of Group B *Streptococcus* (GBS) using on-disk thermal lysis system featuring a localized temperature module with disk rotation (Fig. 2C) [23].

Biological lysis, such as enzymes, also plays a critical role in sample lysis. Lafleur et al. developed a swab-to-result platform comprised dried enzyme, achromopeptidase, in the swab introduction inlet [24]. It allows rapid lysis of methicillin-resistant *Staphylococcus aureus* collected from nasal swab at room temperature.

Another frequently used method, ultrasound sonication, was also incorporated into a MEMS-fabricated piezoelectric microfluidic device [25]. With an oscillating microbubble array generated by sonication with an input voltage of 144 Vpp, ~98 % lysis of MCF-7 cells was achieved within 60 s.

The microfluidic chips/disks combining with lysis buffers, enzymes, or localized heating elements provide reliable platforms to process various clinical samples for rapid diagnosis under challenging conditions.

2.2 Plant specimens

In conventional cell lysis of plant tissues, a mechanical method is normally required to physically rupture fibrous cell walls, often with the help of liquid nitrogen or certain chemicals to facilitate the lysis process [26]. However, performing plant cell lysis in a microfluidic chip or a miniaturized device is challenging because mechanisms such as bead milling and high-pressure homogenizer (HPH) are hard to be scaled down or integrated. Additionally, the resultant thermal issues and degradation of cell products are critical [15].

Recently, physical lysis using miniaturized devices, such as microneedle patches, has drawn significant attention for its rapid sample preparation from plant leaves. Paul et al. used a 10 x 10 mm² microneedle patch covered with 15 x 15 needle arrays made of polyvinyl alcohol (PVA) to achieve rapid nucleic acid extraction simply by pressing the patch onto the leaf surface and rinsing it (Fig. 2D) [27, 28]. The microneedle patches were later interfaced with smartphone and demonstrated an in-field detection of tomato spotted wilt virus, providing a promising option for sample-to-answer applications [29]. The microneedle patch avoids thermal issues which large-scale physical lysis approaches suffer from. In addition, different formats of physical lysis methods were also reported. Equipment-free cell lysis using a 1.5 ml Eppendorf tube with a plastic pestle

in the presence of lysis buffer was also demonstrated as efficient for grinding *Arabidopsis thaliana* leaves [30]. The strategy was later adopted for sample preparation of tomato leaves and integrated with isothermal amplification technology for the detection of Tomato chlorotic spot virus (TCSV) [31]. Whatman FTA cards were also extensively utilized as a fast and simple strategy for cell lysis and nucleic acid extraction from various plant tissues under field conditions. Pressing the plant sample against the card realizes cell lysis and nucleic acids capture [32]. Although manual operation is still inevitable in this method, FTA cards played an important role in the recent advances of sample-to-answer devices [33]. Although studies on sample-to-answer platforms have been reported for various plant diagnoses [34, 35], studies featuring automated sample lysis modules are still rare [36, 37].

In addition to small-scale physical lysis, a strategy using magnetic ionic liquids (MILs) and ionic liquids (ILs) was also reported to isolate plant genomic DNA directly from plant leaves (e.g., *Arabidopsis thaliana*) [38]. The process of cell disruption and the following DNA extraction is expedited by statically incubating intact plant tissues with these chemicals. These chemical lysis techniques are especially suitable for miniaturized devices or microfluidic chips as no complex designs and external intervention are required. However, some critical pathogens, such as *Citrus tristeza virus* and Huanglongbing, are phloem-limited and can only be found in hard-to-lyse tissues such as barks, petioles, and midveins [39, 40]. It is still necessary to validate if these chemical lysis techniques are also applicable for the aforementioned tissues.

2.3 Others – Microbial and fungal specimens

The lysis and the nucleic acids extraction from microbial, including bacteria, parasites, and fungi, is also crucial and closely related to human health and quality of life [41]. Among the listed technologies, physical lysis is still the major strategy dealing with hard-to-lyse samples. For example, audio-powered device was proven to be an effective strategy to liberate nucleic acids from *Mycobacterium marinum* and *Staphylococcus epidermidis* [42]. Simply driven by a smartphone with low electrical power consumption, the electromechanical cell lysis platform achieved 20 – 60 % of the efficiency of the benchtop bead beater within 10 min. Electric cell lysis (ECL) technology is another strategy of physical lysis that combines a nano-spike array to form a low-cost, high-power-density chip for effective cell lysis [43]. With local electric field enhancement due to the spike array, cell lysis can be realized with low voltage input (2 V) within 12 ms.

To process bacteria with cell walls, biological lysis with enzymes can support physical lysis to improve the lysis efficiency. For instance, Han et al. presented a self-contained cartridge with pre-stored reagents and enzymes for automated cell lysis and DNA extraction (Fig. 2E) [44]. Reagents were transferred through a microfluidic chip using pressurized air stored inside the cartridge followed by inserting the cartridge into the device for quick DNA extraction of *Escherichia coli* (*E. coli*) in 3 min. In addition, Usvaliev et al. also proved that the lysis efficiency of *E. coli* can be remotely enhanced by the oscillatory motion resulting from dopamine-functionalized, rod-shaped magnetic nanoparticles under the influence of an external non-heating,

low-frequency magnetic field (68.5 mT) [45]. Due to the remote manipulability of magnetic nanoparticles under an external field, it has a great potential to be incorporated into a portable device for sample-to-answer diagnosis.

In addition to the platforms mentioned above, commercial cell lysis elements are potential options that can be utilized independently or incorporated into an autonomous device for sample-to-answer diagnosis in the field. A commercial kit from Lyse-It (Bel Air, MD), composed of two deposited gold triangles with a “bow-tie” geometry was utilized in the presence of microwave irradiation (450 Watts) for rapid sample preparation of Gram-negative bacteria within 60 s [46]. Similarly, a miniaturized bead-beating chamber, OmniLyse (Claremont BioSolutions, Upland, CA), has been proven to effectively liberate nucleic acids not only from bacteria and spores but in out-of-the-earth environments such as low-biomass synthetic Mars analog soils and microgravity space for real-time and *in-situ* genetic analysis (e.g., nanopore sequencing) [47-50]. A variety of sample lysis strategies (audio-powered, microwave, heating, electrical, mechanical lysis) have been demonstrated with miniaturized and (semi-)automated assemblies, as summarized in Table 1.

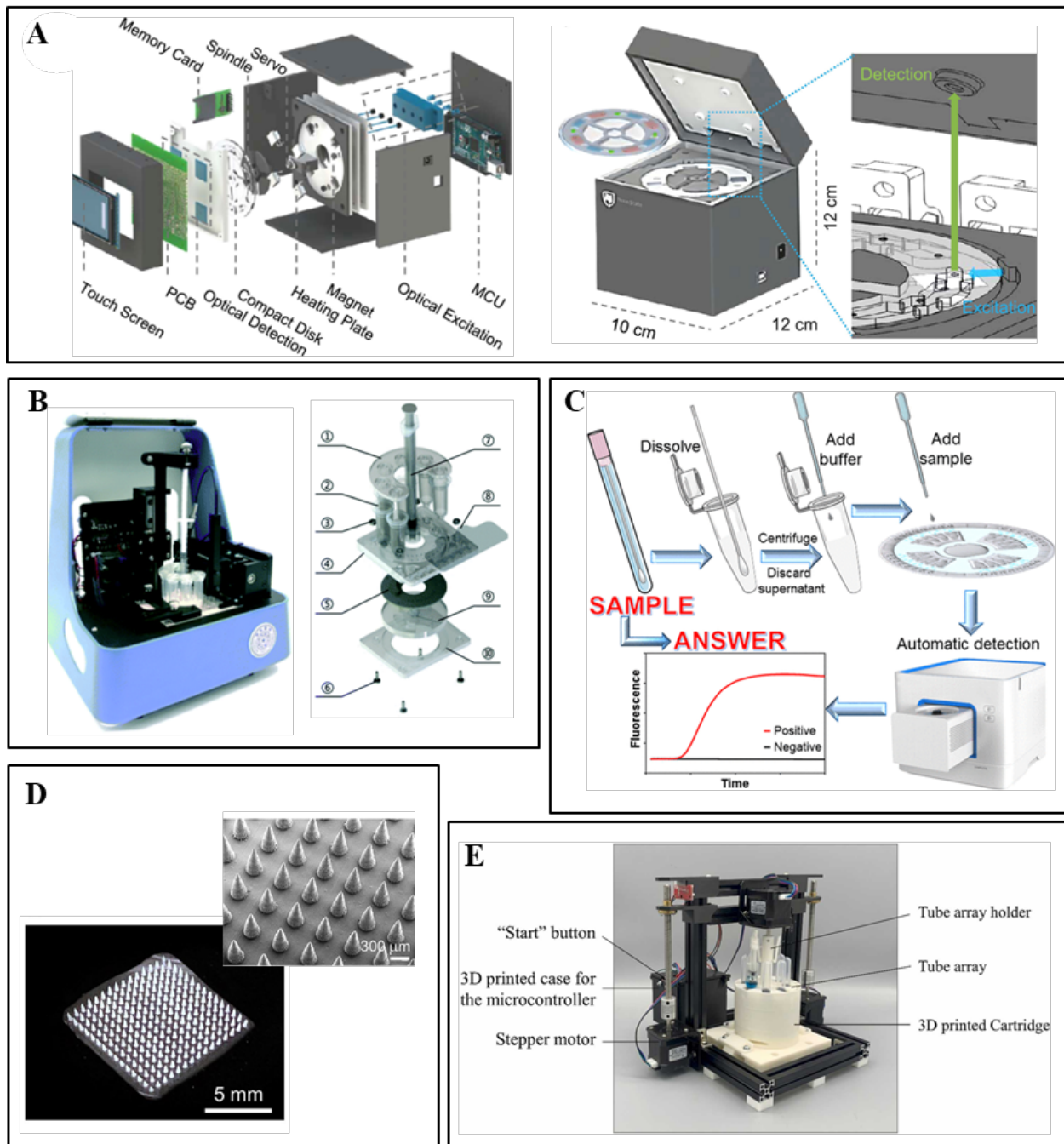


Fig. 2. Sample lysis approaches using portable and miniaturized systems. (A) Sample-to-answer diagnosis of malaria using integrated lab-on-a-disk system. Adapted from [18] with permission from Elsevier. (B) A self-contained fluidic system for multiplex detection of bacteriuria. A cassette comprises a conical-shaped focusing magnet, a self-locking solenoid, and a slip ring with an electric field for bacteria lysate extraction. Adapted from [19] with permission from The Royal Society of Chemistry. (C) A lab-on-a-disk system for sample-to-answer diagnosis of Group B Streptococcus (GBS) using clinical vaginal and anal swabs. Adapted with permission from [23]. Copyright 2020 American Chemical Society. (D) A microneedle patch for rapid extraction of plant DNA. Adapted with permission from [27]. Copyright 2019 American Chemical Society. (E) The machine is equipped with a disposable, pressure-driven, and self-contained cartridge for automatic

bacterial nucleic acid extraction. Adapted from [44] with permission from Elsevier.

Table 1. Comparison of portable cell lysis techniques for point-of-care diagnosis

Method	Specimen Input	Target Species	Processing Time	Pros	Cons	Ref.
Bead beating + lysis buffer	Cell suspension in broth	Bacteria / RNA	10 min	Higher efficiency and yield compared to mechanical or chemical lysis only	Necessary RNA protecting reagents (RNAlater) impairs cell lysis, lysis buffer inhibiting PCR reaction	[17]
Lysis buffer	Artificial human blood sample	Ring-stage parasite / DNA	< 10 min	Low cost and easy operation	Low lysis efficiency, lysis buffer inhibiting PCR reaction	[18]
Ultrasound sonication	Cell suspension in PBS buffer	human breast cancer MCF-7 cells / DNA	1min	Extremely short lysis time and no lysis buffer required	High input voltage	[25]
Microneedle puncturing	Plant leaves	Bacteria / DNA	< 1 min	Short lysis time, easy operation, and no lysis buffer required	Fabrication of microneedle patch required	[27]
Audio-powered bead-beating	Bacteria suspension in TE buffer	Bacteria / DNA	50% lysis efficiency achieved within 10 min	Low cost, low power input and no lysis buffer required	Relatively low yield with hard-to-lyse bacteria	[42]
Electrical lysis	Cell suspension in PBS buffer	HeLa cells / DNA and RNA	120 ms	Low power input, extremely short lysis time, high lysis efficiency, and no lysis buffer required	Fabrication of nano-spikes chip required	[43]

3 Nucleic acid extraction

Extraction of high-quality nucleic acids is very critical for downstream molecular assays. It involves basic steps such as denaturation of nucleoprotein, inactivation of enzymes (RNase, DNase, etc.), removal of unwanted contaminants, nucleic acid collection, and resuspension [51, 52]. Processing time, recovery and purity of nucleic acids, ease, and safety of the procedure are usually emphasized and significantly affect the downstream analyses such as PCR and sequencing [53, 54]. After the first time that DNA was successfully isolated from leukocytes by Friedrich Miescher in the 1860s, better extraction techniques have been developed to satisfy the increasing demands [52, 55]. Nowadays, many extraction methods have switched to non-toxic chemicals, such as non-chaotropic and enzyme-based reagents, and solid-phase materials for safer applications due to their flexibility in incorporating with point-of-care platforms [56, 57]. Solid-phase extractions using auxiliaries such as silicon material-based membranes, cellulose matrices, and surface-modified magnetic beads have been developed [55]. Proteins, tannic acid, and lipids, among others in biological samples, can inhibit the amplification of nucleic acids. Nucleic acid extraction and isolation procedures are especially critical for the success of downstream analyses such as genotyping and sequencing. This section will focus on extraction strategies based on silicon materials, cellulose fibers, or magnetic beads that have a good potential of being adopted for sample-to-answer devices.

3.1 Silicon materials-based extraction

Nucleic acids are negatively charged and bind to silicon and silicon derivatives such as silica gels, glass particles, and glass microfibers in the presence of chaotropic agents at high concentrations in a low pH environment. The bound nucleic acids can be released from these materials in a low-salt buffer at higher pH [55, 58, 59]. Chemical modification on a high surface-to-volume ratio surface is a common strategy to increase capture affinity between silicon-based materials and nucleic acids. For example, the amino-coated micro-pillar array offers a high surface-to-volume ratio, and it has been proven capable of achieving up to 20% extraction efficiency for low concentrations of RNAs within a microfluidic device [60]. A microfluidic platform with a packed column of mesoporous silica beads was developed for effective isolation and enrichment of pre-amplified rolling cyclic amplification (RCA) amplicons (Fig. 3A) [61]. They achieved a critical limit of detection with just 5 μ L of a sample by optimizing silica beads packing and sample flow rate. In another study, a silica membrane (Qiagen 69504) was employed for rapid nucleic acid isolation, resulting in a high recovery rate (62 ± 24 %) at low pH with high concentrations of chaotropic salts (Fig. 3B) [62].

Binding efficiency of the nucleic acids can be improved by modifying the surface chemistry of silicon-based substrates. Han et al. used a non-chaotropic reagent, dimethyl adipimidate (DMA), to covalently and reversibly bind to the amine groups of nucleic acids within a self-powered and disposable microfluidic system [63]. DNA/DMA complexes were later captured onto 3-Aminopropyltriethoxysilane (APTES)-treated slide glass via covalent binding between DMA and

amine groups of APTES. Bound DNA was then purified and collected in storage chambers by slow buffer elution, where the entire process was completed within 10 min with the help of switchable syringes and actuation elements.

Chitosan, a linear polysaccharide, is another frequently used material with biocompatibility, negligible toxicity, and cost-effectiveness. It has long been applied as an adsorbent or a functional coating in conjunction with silicon-based / cellulose-based materials in developing sample preparation / nucleic acid extraction processes for POC diagnosis [64]. For instance, a rapid nucleic acid extraction method (90 s) using chitosan-functionalized silicon dioxide capillaries was demonstrated for the diagnosis of Zika virus [65]. Target nucleic acids in a complex biofluid quickly bound to that functionalized surface at low pH (pH 5.5) and were later released into a PCR mixture (pH 8.5) due to the charge-switched characteristic of chitosan. These silicon-based materials offer effective and low-cost methods for nucleic acid extraction.

3.2 Cellulose fibers-based extraction

Cellulose-based matrices are other commonly used materials for nucleic acid extraction. Due to the hydroxylated groups, cellular matrices possess inherent physical affinity (electrostatic/hydrophilic forces, hydrogen bonding) and serve as the basis for additional surface modifications [55, 66, 67]. Similar to silicon-based materials in the previous section, surface area and chemical modifications are also important for cellulose-based matrices for nucleic acid extraction and storage [30, 68].

Chitosan was incorporated into cellulose-based matrices such as Fusion 5 filter papers (GE Healthcare, PA, USA) for on-chip extraction of nucleic acids from pretreated human whole blood (Fig. 3C) [69]. Benefiting from the synergistic effect between physical entanglement within the fiber matrix of filter paper and the electrostatic adsorption from chitosan, up to 98% and 95% extraction efficiency for K562 human genomic DNA and bacteriophage λ -DNA, respectively, were achieved. The same combination was also adopted for quick, on-chip extraction of viral RNA from pretreated human blood plasma for the serotype-specific detection of the dengue virus [70]. Chitosan and glutaraldehyde were also synergistically formed a membrane on Whatman chromatography paper for quick DNA extraction of *Chlamydia Trachomatis* in a portable cartridge [71].

In addition to chitosan, other polymeric materials such as 3-aminopropyl trimethoxysilane (APTMS), polyethyleneimine (PEI), and polydiacetylenes (PDA) have been successfully utilized to enhance the nucleic acid binding capacity of paper. It was also reported that nucleic acids were retained after washing [30, 72]. Particularly, unmodified Whatman No.1 filter papers were not only able to bind with DNA and RNA but enabled direct amplification from unmodified paper disks in PCR reactions. Due to its unique chromatic property, PDA is frequently used as a colorimetric indicator.

However, it can also facilitate the binding of nucleic acids within a micropatterned paper device by exploiting the ionic affinity between the negatively-charged phosphate backbone of dsDNA and the positively-charged PDA vesicles [72, 73]. FTA cards also contain chemicals that

lyse cells, denature proteins, and protect nucleic acids. They are suitable for collecting and purifying nucleic acids lysed from a wide range of biological samples. Being capable of capturing and concentrating directly from liquid (whole blood, bio-suspensions) and solid (plants) specimens, FTA cards can be integrated with portable sample-to-answer devices [74]. All the above reports demonstrated successful extraction of nucleic acids from various clinical samples and the feasibility in incorporating cellulose-based materials with on-chip molecular assays. Thus, cellulose-based matrices are a promising alternative to silicon-based counterparts at low cost.

3.3 Magnetic beads-based extraction

Magnetic beads/powders have been extensively used to capture and manipulate nucleic acids within miniaturized systems due to the easy operation and high recovery of nucleic acids [75]. Recently, magnetic bead manipulation, aliquoting, and pumping have been successfully integrated into autonomous formats, such as lab-on-a-disk systems, for sample-to-answer diagnoses [76, 77]. For example, Li et al. developed a centrifugal microfluidic platform enabling automatic serum separation, nucleic acid extraction and hepatitis B virus detection using human whole blood (Fig. 3D) [78]. Integrated with frequency-tunable double rotation axes system and optimized chamber design, pre-stored buffers and magnetic beads were properly delivered and mixed with target fluids, resulting in high DNA recovery ($\sim 90\%$) and low beads loss ($6.3 \pm 0.47\%$) within 15 min. A fluidic handling system was developed for sample-to-answer detection of influenza A virus from clinical swab samples (Fig. 3E) [79]. Automatic transport, mixing, and extraction were achieved by using commercial magnetic beads in conjunction with a series of mechanical control elements resulting in quick preparation of PCR-ready nucleic acids in 5 min. Many other systems used magnetic beads for highly effective extraction through complex mechatronic manipulations to achieve sample-to-answer detection of bacteriuria and influenza A viruses [19, 80].

Chitosan has been routinely used to improve nucleic acid capturing. For instance, chitosan-modified magnetic microspheres were utilized for pH-induced human DNA extraction and release on a microfluidic chip for a sample-to-answer LAMP assays (Fig. 3F) [81]. Similar mechanism was employed in detecting circulating antibodies in human blood with ELISA [82]. Surface grafting of specific oligonucleotide sequences is another common strategy. Tripathy et al. demonstrated a sensitive SARS-CoV-2 nucleic acid detection with in situ LAMP, assisted by sequence-specific magnetocapture [83]. Use of magnetic beads modified with an oligonucleotide complementary to the target nucleic acid resulted in selective extraction and a sample-to-answer diagnosis with a sensitivity down to 2.5 copies/ μL . Tsai et al. adopted a similar strategy for diagnosing SARS-CoV-2 with an integrated microfluidic platform [84]. Magnetic beads functionalized with specific RNA probes complementary to three target genes enabled highly accurate detection of SARS-CoV-2 RNA.

Magnetic beads-based extraction techniques have drawn significant attention owing to their flexibility in surface modification, convenient beads manipulation, and high efficiency for nucleic acid extraction. They can be readily integrated with automated, sample-to-answer microfluidic devices. Nucleic acid extraction strategies reviewed in this section are summarized in Table 2.

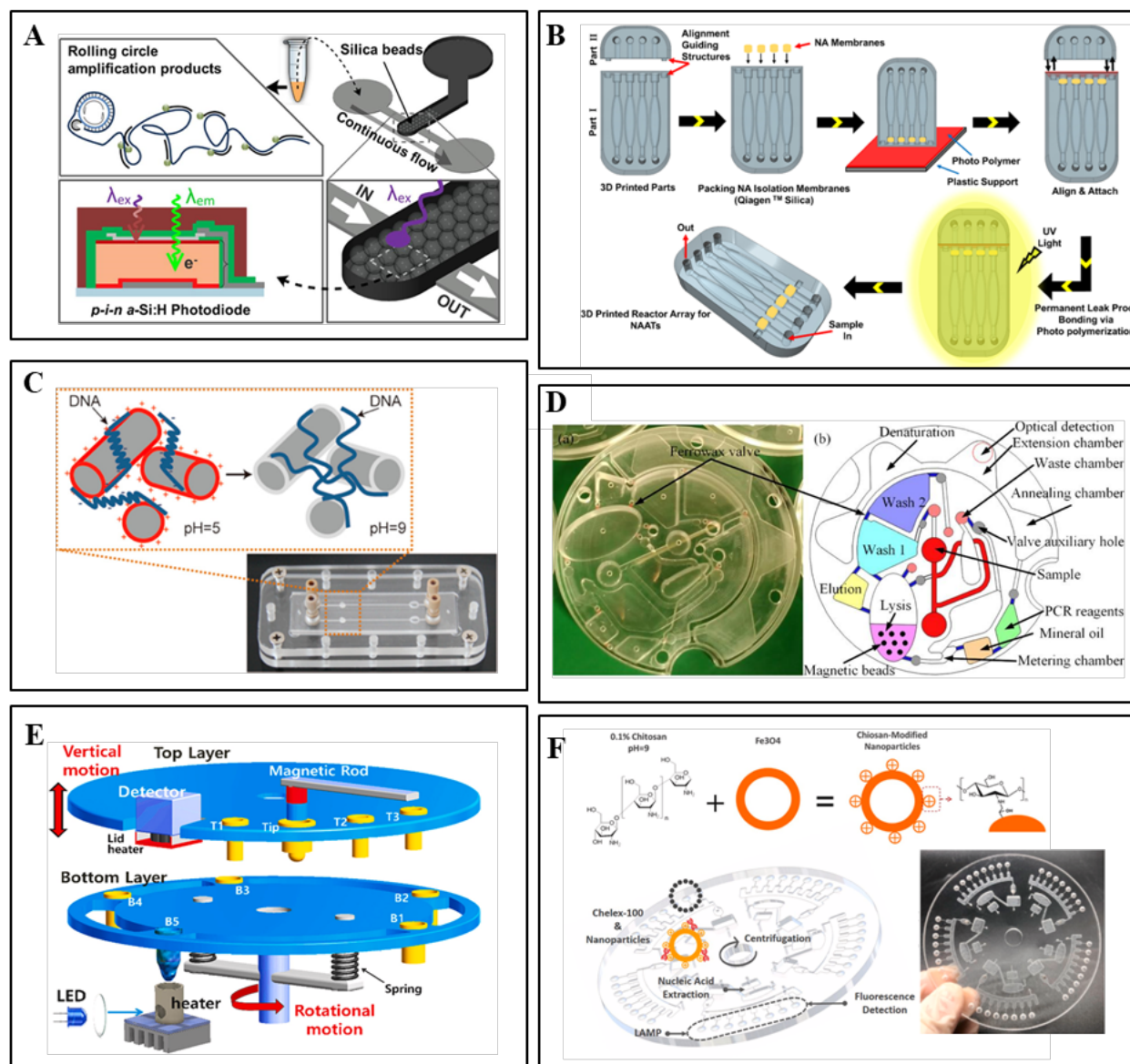


Fig. 3. Nucleic acid extraction approaches using portable and miniaturized systems. (A) A silica bead-based microfluidic platform enabling an on-chip and size-selective trapping of amplification products. Adapted from [61] with permission from Elsevier. (B) An assembly of multifunctional 3D printed reactor array for point-of-care molecular diagnostics. 3D printed parts are packed with silica membranes (Qiagen 69504) for rapid nucleic acid isolation. Adapted from [62] with permission from Elsevier. (C) Chitosan-modified Fusion 5 filter paper demonstrated highly efficient extraction of nucleic acids from K562 human genomic DNA and bacteriophage λ -DNA. Adapted with permission from [69]. Copyright 2017 American Chemical Society. (D) A centrifugal microfluidic platform for sample-to-answer hepatitis B virus detection from blood. The platform contains extraction chambers wherein magnetic beads, extraction reagents, and lysis buffer are prestored. Adapted with permission from [78]. Copyright 2019 American Chemical Society. (E) A sample-to-answer microfluidic device capable of automated transporting, mixing, and washing with commercialized magnetic beads for quick nucleic acid extraction. Adapted from [79] with permission from Elsevier. (F) A sample-to-answer microfluidic chip using chitosan-

modified magnetic microspheres for pH-induced nucleic acid extraction. Cell lysis, nucleic acid extraction, and the LAMP assays were all accomplished on chip. Adapted from [81] with permission from Elsevier.

Table 2. Comparison of portable extraction techniques for point-of-care diagnosis

Method	Target Species	Processing Time	Elution Buffer	Recovery Efficiency / Sample yield	Ref.
Cellulose filter paper	Plant, animal, and microbial samples / DNA, RNA	3 s – 1 min	PCR or LAMP reaction buffers	Not applicable	[30]
Silicon micropillars	Extracellular vesicles / RNA	~42 min	RNase-free water	20% RNA binding efficiency	[60]
Silica membrane	<i>P. Falciparum</i> and <i>Neisseria meningitidis</i> / DNA	Not available	LAMP reaction buffer	62 ± 24% nucleic acid yield	[62]
Chitosan-functionalized silicon dioxide capillaries	Zika virus / RNA	~25 min	PCR reaction buffer	Up to 240–280 ng RNA adsorption for single capillary	[65]
Chitosan-modified filter paper	K562 human genomic DNA, bacteriophage λ -DNA	5 min	No elution buffer required (<i>in situ</i> PCR)	98% capture efficiency for K562 human genomic DNA, and 95% capture efficiency for bacteriophage λ -DNA	[69]
Magnetic beads	Hepatitis B virus / DNA	6 min	Elution buffer from a commercial kit	Not applicable	[78]
RNA-conjugated magnetic beads	SARS-CoV-2 / RNA	10 min	LAMP reaction buffer	Not applicable	[84]

4 Nucleic acid amplification

Nucleic acid amplification is essential in detecting nucleic acids from a wide range of species, especially with low-titer samples. There are some key factors that determine the amplification outcomes: (i) primer efficacy; (ii) reaction temperature control; and (iii) concentration and species of reactants. A successful outcome should exhibit significant amplification, a wide dynamic range, and a low detection limit [85-88]. In this section, major amplification strategies integrated into portable, sample-to-answer devices are investigated and classified in terms of isothermal, non-isothermal, and digital amplification methods.

4.1 Non-isothermal nucleic acid amplification

Polymerase chain reaction (PCR) and quantitative PCR (qPCR) are powerful tools for nucleic acid amplification and detection. Replacing tissue culture and serology, PCR has been the gold standard in laboratories conducting molecular biology, environmental monitoring, and clinical diagnosis due to their reliability and reproducibility [89-91]. Over the past decades, portable and cost-effective microfluidic devices with built-in temperature control elements have been developed to realize multiple POC diagnoses, such as cell capture, cell lysis, isolation, and amplification [92].

Zai et al. demonstrated a sample-to-answer platform featuring a closed, single-use microfluidic cartridge that enables sample lysis and direct qPCR amplification of viral templates collected from swab specimens (Fig. 4A) [93]. The core technique of the heating module relies on a thermoelectric unit (i.e., a Peltier device) and thermal conductive block located below the PCR chamber. A PID controller with a sensor was placed on the PCR heat block for precise control of heating and cooling processes for PCR amplification. The optimized temperature module enabled a rapid PCR protocol with 35 cycles completed in < 20 min.

PCR technology has also been extensively integrated with other portable platforms, such as lab-on-a-disk systems, for the rapid diagnoses of various pathogens. For instance, “RespiDisk,” enabled a fully automated reverse transcriptase PCR (RT-PCR) assays for multiplex detection of up to 19 respiratory tract infection pathogens in a single run [94]. Featuring a centrifugal microfluidic platform and advanced liquid control technology, this air-heated RespiDisk automated all procedures by executing predefined protocols and achieved high throughput diagnosis (36 samples x 19 assays) in 200 min. Such “lab-on-a-disk” platforms can be easily customized to meet specific requirements and run on the player with predefined protocols for sample-to-answer applications [95].

Some chemicals, such as calcium oxide, were used as power-free heating sources, providing an economic strategy for portable amplification [96]. In this device, calcium oxide mixture served as a heat source where the temperature was regulated by a phase change material (water). An embedded aluminum thermal waveguide and a capillary tube with properly designed geometry enabled a fixed temperature at the bottom and a stable temperature gradient within the PCR chamber.

In another system, preheated liquid was utilized for realizing ultrafast PCR in a microfluidic

device (Fig. 4B) [97]. Relying on diffusion and ultrafast flow switches in the device, circulation of preheated liquids quickly thermalized the PCR chamber and achieved 30-cycle thermal cycling in 2 min.

Surface acoustic wave (SAW) technology has emerged as an alternative method for a wide range of biomedicine applications, including on-chip nucleic acid amplification [98, 99]. The SAW energy can be converted to generate different functions (e.g., pumping, mixing, and heating of droplets) to the liquid droplet in contact with patterned piezoelectric (PZT) substrate on a microfluidic chip for POC diagnosis [100, 101]. Vernon et al. developed an interdigitated transducers (IDT)-patterned PZT microfluidic chip to realize rapid and precise temperature control using Raspberry Pi and PID control for PCR applications [102].

A photothermal mechanism has provided another strategy for ultrafast thermal cycling on portable devices. Recently, an ultrafast on-chip PCR platform featuring a nanoplasmonic pillar array (NPA) was reported for rapid and precise molecular diagnostics (Fig. 4C) [103]. Photothermal heating based on dense electromagnetic hotspots enabled ultrafast PCR cycling, taking only 3.5 min for 30 cycles between 98 and 60 °C. This mechanism has also been exploited in combination with different particle geometries (dendritic nanoforests, nanoislands, triangular nanoparticles, etc.) [104-106]. Plasmonic photothermal effect demonstrates exceptional efficiency and has a great potential for rapid and high-throughput POC diagnostics. PCR-based amplifications have been successfully miniaturized and integrated with portable devices. However, relatively complex designs and fabrications, the problems of the lag phase in heating-cooling steps, and the inhomogeneous temperature profile remain to be improved.

4.2 Isothermal nucleic acid amplification

Despite the efforts to develop a miniaturized PCR module, the lag phase in the heating-cooling cycle and nonuniform temperature distribution within the reaction chamber remained challenging. Miniaturized isothermal amplification methods integrated with portable devices were investigated to avoid these problems. These include modified PCR-based methods (ion-mediated PCR (IM-PCR) and convective PCR (CPCR)) and some typical isothermal amplifications such as strand displacement amplification (SDA), helicase-dependent amplification (HDA), recombinase polymerase amplification (RPA), rolling circle amplification (RCA), nucleic acid sequence-based amplification (NASBA), multiple displacement amplification (MDA), and loop-mediated isothermal amplification (LAMP) and so forth [107].

Some PCR-based methods, such as IM-PCR and CPCR, can be conducted at a constant temperature throughout the amplification. In IM-PCR, switching electrical potential triggered buffer ionization and changed the pH value at a constant temperature, resulting in a favorable environment for DNA denaturation/annealing/extension. An alkaline condition promotes denaturation of dsDNA, whereas the neutral pH reforms dsDNA from the denatured ssDNA [108]. Another study reported that selectivity and specificity of DNA amplification were improved due to the selective function of quantum biological electron transfer (QBET) between DNA bases and metal ions (valence $1+ \sim 3+$) [109].

In the so-called CPCR method, an enclosed reactor drove reagents cyclically for melting, annealing, and extending steps through the Rayleigh-Bénard convection generated by spatially distributed heat sources and achieved quick nucleic acid amplification [110]. A sample-to-answer and real-time CPCR system was demonstrated combining the FTA membrane and a capillary tube loaded with PCR mix for the quick detection of influenza A virus [111]. Efficient thermal cycling introduced by thermal convection and in-tube centrifugation enabled rapid PCR (< 30 min) with good sensitivity (1.0 TCID₅₀/mL). CPCR powered by solar energy or combining reactors with different geometries (e.g., closed-loop reactor, and disk reactor) was also adopted for a variety of POC diagnosis [112-114].

SDA can restrictively nick unmodified strands using specialized restriction enzymes (e.g., HincII, *N.Bst*NBI) within the temperature range from 37°C to 65°C. With strand displacement enzyme (e.g., *Bst* polymerase), the nicking site is regenerated after each displacement step for repeated cycles of nicking and extension. The downstream strands are then displaced and free to anneal to primers for the subsequent exponential amplification [115, 116]. An integrated microfluidic platform with a lateral flow assay and isothermal SDA (iSDA) method was developed for methicillin-resistant *Staphylococcus aureus* (MRSA) detection [24]. Without the initial denaturation step, ten target copies of Achromopeptidase (ACP)-lysed samples were detected at 50°C within 30 min throughout the amplification. Zeng et al. reported an ultrasensitive photoelectrochemical sensing protocol that uses target-triggered palindromic molecular beacon (PMB) reaction accompanying SDA and demonstrated ultralow detection limit (29 fM) of kanamycin [117].

HDA, another isothermal amplification method, performs the separation of complementary strands of dsDNA by exploiting the activity of a DNA helicase in a temperature range from 45°C to 65°C. Without delicate temperature cycling, the method enables an isothermal production of single-stranded templates for the following hybridization and extension using DNA polymerase [118, 119]. Horst et al. developed a low-cost paper microfluidic device integrated with sample lysis from patient swabs, nucleic acid extraction, thermophilic HDA (tHDA), and visual detection of *Neisseria gonorrhoeae* [120]. Combining on-chip DNA precipitation, washing, amplification, and detection, the amplification was completed in 45 min at 65°C on a heat block, resulting in a low detection limit (10 cells per device).

The RPA-based method is useful for rapid, specific, and cost-effective identification of molecular diagnosis due to its minimal requirements in sample preparation and low reaction temperature (25 – 42°C) [121]. Olazarra et al. presented an RPA-based POC platform featuring multiplexed and qualitative genotyping of four single nucleotide polymorphisms (SNPs) directly from saliva [122]. By exploiting on-chip heating resistor networks regulated by a PID control system, RPA (37°C) was automatically achieved in 25 min, where the amplicons were then introduced to the surface of the giant magnetoresistive nanosensor for hybridization. Recently, a power-free RPA biosensor was demonstrated for the ultrasensitive detection of SARS-CoV-2 [123]. Human body temperature was successfully utilized for rapid amplification (< 20 min) on a skin patch-like sensor with a multi-microelectrode array.

RCA-based amplification has emerged as a simple isothermal amplification technique having high tolerance against interferents without the requirements of extensive assay optimization [124]. Soares et al. demonstrated a microfluidic device combining pre-amplified RCA amplicons and a miniaturized amplicon trapping area with silica beads [61]. Beginning with padlock probe ligation, purified nucleic acids were isothermally amplified with Φ 29 DNA polymerase buffer mix followed by subsequent circle-to-circle amplification and beads resuspension procedures for 30 min at 37°C. Integrated with miniaturized *p-i-n a-Si:H* photodiodes, the critical limit of detection (< 0.5 fM) was achieved within the portable device.

NASBA-based method emerged as an isothermal and transcription-based amplification particularly suited for amplifying RNAs such as rRNA, tmRNA, mRNA, and genomic RNA, or DNA subjected to a denaturation step. Standard NASBA protocol includes denaturation at 65°C to remove secondary structures from RNAs, followed by incubation with enzymes for specific amplification at 41°C [119, 125]. Chung et al. developed a microfluidic platform for the detection of murine norovirus in oysters [126]. Combining surface-modified microbeads and NASBA (41°C), the chip carried out concentration and on-chip amplification with a critical limit of detection (100 pfu). NASBA was also broadly adopted for the detection of SARS-CoV-2 with the use of a commercialized kit (ID NOW, Abbott, IL, USA) and saliva / nasopharyngeal swabs as sample input [127-129].

Capable of the whole genome amplification (WGA), the MDA-based isothermal method includes the binding of random hexamers to single-stranded DNA isothermally operated with strand displacement reaction using Φ 29 DNA polymerase [130]. Exploiting the high activities and low error rate of Φ 29 DNA polymerase, MDA enables a great number of the DNA fragments up to 70 kb without sequence bias and is thus well-suited for SNP testing and genotyping. [131-133]. To date, many MDA-based microfluidic platforms enabling whole-genome sequencing and analysis of single cells on a nanoliter scale have been developed to address the need for precision medicine for cancers and other diseases [134, 135].

LAMP, first reported by Notomi et al. in 2000, has emerged as the most commonly used isothermal method for sensitive and rapid diagnosis [136]. Combining *Bst* DNA polymerase and two pairs of primers (inner and outer primers) recognizing six regions on the target sequence, LAMP enables an isothermal amplification (63–65°C) with up to 10^9 -fold within an hour [137]. This method can be easily modified for RNA-based detection by adding reverse transcriptase and is especially suited for early diagnosis in resource-limited settings [138]. Liu et al. developed a fully integrated microfluidic disk capable of automatically conducting sample preparation (cell lysis and nucleic acid extraction), target amplification (LAMP), and signal readout (built-in color sensor) for the diagnosis of foodborne bacteria [139]. The device enabled multiplex and simultaneous detection of 5 types of bacteria in 70 min with a detection limit down to 10 copies/ μ L. More recently, Jankelow et al. reported a fully integrated system comprising a 2-stage microfluidic cartridge for rapid sample-to-answer detection of Zika virus in a droplet of whole blood [140]. Using a battery-powered thermoelectric heater, the RT-LAMP assay was completed in 22 min with a sensitivity as low as 2.7×10^2 copies/ μ L.

Considering the portability of the whole system and easy operability, isothermal nucleic acid amplification conducted with simple heating elements provides great potential to develop low-cost, sample-to-answer devices for clinical diagnosis under critical conditions.

4.3 Digital nucleic acid amplification

Digital amplification assay is a technique where the sample is partitioned into several small compartments containing a discrete unit of the whole biological system for subsequent amplification and analysis [141]. The sensitivity and precision of digital amplifications are significantly constrained by the sparse partitioning and high volume (μL) of individual partitions. The digital strategy incorporated with isothermal amplification was extensively studied for absolute quantification and single-cell gene expression analysis due to its high sensitivity, quantification precision, and unambiguous signal outcomes (Fig. 4D) [142, 143]. With microwell arrays or droplet generators for single-cell compartmentalization, nucleic acid amplification such as PCR, LAMP, and RPA can be carried out individually with molecular beacon probes, resulting in clear and accurate readout. This is a powerful and reliable technique for the molecular analysis, especially for the early effects of carcinogens and cellular mutations in a diluted and small volume of samples [107, 144].

In digital non-isothermal amplification, two general approaches are used for massive cell partitioning. One is a microarray composed of physically separated chambers (cdPCR), and the other is droplet-based emulsions (ddPCR). In the latter, chemicals and target nucleic acids are encapsulated in water droplets and separated by an oil-based continuous phase. Both methods can provide compartmentalized entities with down to femtoliter scale [141].

In the chamber-based digital assay, Yin et al. recently presented a self-priming digital PCR chip featuring 48,192 wells of 0.2 nL well volume for direct and multiplex diagnosis of epidermal growth factor receptor (EGFR) mutation through screening of circulating tumor DNA (ctDNA) [145]. Requiring no DNA extraction, the chip enabled the direct introduction of plasma for simultaneous detection of four targets using single fluorescence with LoD of 5 copies/ μL . This strategy greatly streamlined the process and reduced the cost of multiplex sensing. Similarly, Ning et al. demonstrated a low-cost self-digitization PCR chip with 10,000 picoliter-scale chambers for the absolute quantification with a dynamic range of 10^4 copies of EGFR genomic DNA [146]. Utilizing two glass slides and a micropatterned PDMS film to form a vacuum “accumulator” and a hydration “reservoir”, the integrated void served as a power-free vacuum source for high-throughput sample loading and partitioning without significant liquid loss.

More platforms with chamber arrays on different scales can be fabricated by exploiting similar microfabrication techniques such as micro-molding of flexible elastomer and thermoplastic polymers [147], nanoimprint lithography [148], and soft lithography [149].

The second partitioning strategy for digital assays utilizes water-phase droplet generation in a continuous oil phase. The cross-junction design on the microfluidic chip was extensively used for the generation of reaction droplets [150]. The cross-junction enables an aqueous stream being squeezed by ambilateral oil streams to form monodisperse droplets with reactive cores

encapsulated by inertia shells [151]. The number of cross-junction can be properly adjusted and integrated with alginate, Ca-EDTA, and oil to construct water–water–oil double emulsions to fit specific needs (e.g., 3D liver model) [152]. Unlike ddPCR requiring nearly identical droplet size, Yen et al. have proposed a statistical analysis of nonuniform droplet distributions based on the Poisson distribution of analytes in droplets [153]. This method benefits ddPCR users without standard lab settings because no microchip fabrication and cross-junction is required for bulk emulsification. Instead, general shaking or stirring works for a rapid and nonuniform droplet partitioning.

In addition to digital PCR, the digital assay has been widely integrated with other isothermal amplification methods, including LAMP, RPA, and MDA, for absolute quantification and single-molecule analysis [154-156]. Such isothermal protocols are of great interest while operating in resource-limited settings, as no delicate thermal cycling and robust control are needed. Digitization strategies for sample partitioning and subsequent amplification include the generation of droplets within emulsions [157, 158], the SlipChip platform [159, 160], and microvalve-based arrays of multi-layered structure enabling rapid and high throughput analysis using LAMP and RPA [161]. Recently, Ruan et al. developed a digital-MDA microfluidic chip enabling parallel and highly-performance whole-genome sequencing of single cells [154]. Exceptional identification of single-nucleotide variations with an allele dropout rate of 5.2% and copy number variants with the smallest bin of 150 kb was performed. Kreutz et al. demonstrated a self-digitization (SD) LAMP platform for the quantitative detection of human papillomavirus (HPV) genes [162]. The proposed microfluidic chip achieved automatic and large-scale loading and partitioning, covering 640 ~ 10,240 microchambers with down to 0.05 nL of chamber volume. Utilizing the intrinsic viscoelastic property driven by the geometry of chip channels and chambers, the technique provided simultaneous self-digitization without the need for precise lab-based instrumentations. In addition, a novel digitation strategy was developed using commercial track-etched polycarbonate (PCTE) membrane to effectively form droplets with a facile peel-off process due to embedded pores [163]. To date, digital amplification strategies have combined automatic sample emulsification/partitioning, miniaturized heating elements, and signal readout modules within portable systems enabling sample-to-answer and high-throughput assays. For instance, Wan et al. demonstrated a handheld digital LAMP platform for the detection of *Trypanosoma brucei* in human blood [164]. The device combining droplet manipulation techniques using electrowetting force and real-time temperature control systems detected 40 copies in 2 μ L droplets without any bulky instrumentations. A similar digital-LAMP platform combining smartphone-based image analysis was demonstrated for sensitive diagnosis of EGFR L858R gene mutation directly from spiked plasma samples [165]. Another sample-to-answer digital-LAMP chip integrated with rapid nucleic acid extraction based on “immiscible phase filtration assisted by surface tension” was developed for the detection of low-abundance ctDNA and mutations [166]. Spiral mixing channel and cross-junction design were exploited for droplet generation and the sample mixing and partitioning under negative pressure, whereas a smartphone-based microdroplet imaging device was demonstrated for portable applications.

These methods provide reliable alternatives for point-of-care diagnosis with their simple, cost-effective, and high throughput features, which are especially suited for applications in rural places without standard digitation settings. A number of nucleic acid amplification strategies have been demonstrated with miniaturized and (semi-)automated assemblies, as summarized in Table 3. They have the potential to be integrated into various platforms for sample-to-answer diagnosis in the field.

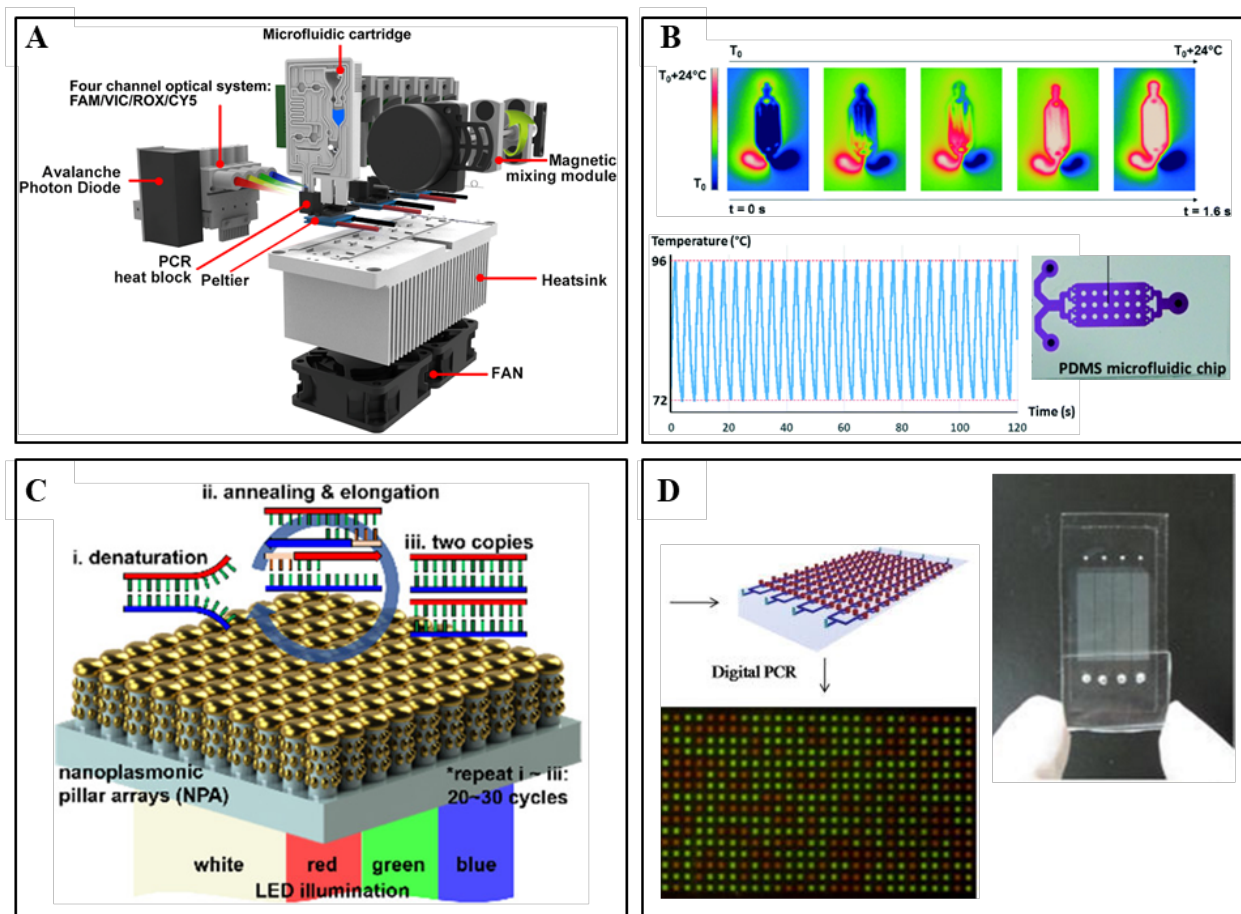


Fig. 4. Nucleic acid amplification approaches using portable and miniaturized systems. (A) Sample-to-answer system comprising single-use microfluidic cartridge for sample lysis, nucleic acid extraction, and direct qPCR amplification for the detection of viruses. Adapted from [93] with permission from The Royal Society of Chemistry. (B) A microfluidic thermalization chamber consisting of an alternative injecting assembly enables an ultrafast flow switch of preheated liquids. Adapted from [97] with permission from The Royal Society of Chemistry. (C) An ultrafast on-chip PCR platform featuring the nanoplasmonic pillar array (NPA) and LED light sources as a light-to-heat converter. Adapted with permission from [103]. Copyright 2020 American Chemical Society. (D) A self-priming compartmentalization chip with a great number of microwells connected to the main channel enables on-chip single-cell digital PCR. Reprinted from [142] with permission from AIP Publishing.

Table 3. Comparison of portable amplification techniques for point-of-care diagnosis.

Method	Sample type	Target nucleic acids	Heating/cooling Elements	Incubation Time	Reaction Temperature	Limit of Detection / Dynamic range	Ref.
Close-loop CPCR ^a	Bacteria culture	<i>S. aureus</i> / DNA	Ceramic heating plate w/ PID controller and a stainless-steel loop	11-25 min, depends on target copies	Denaturation: 95 °C Annealing and extension: 60–85°C	Linear dynamic range 10 ¹ ~10 ⁶ copies/μL Limit of Detection: 1 copy/μL	[114]
RPA ^b	Saliva	Human saliva / DNA	Heating resistor networks w/ PID controller	25 min	37 °C	33 ng/μL of genomic DNA demonstrated. Ideal range: 2–200 ng/μL	[122]
NASBA ^c	Glands of infected oysters	Murine norovirus / RNA	Not available	< 4 h	41 °C	100 CFU per oyster	[126]
LAMP ^d	Bacteria culture	<i>S. aureus</i> , <i>Salmonella</i> , <i>Shigella</i> , enterotoxigenic <i>E. coli</i> , and <i>P. aeruginosa</i> bacterial / DNA	An aluminum plate w/ a polyimide heating membrane	~50 min	65 °C	<i>Sau</i> and <i>Sal</i> samples: 10 ² copies/μL <i>Sty</i> , <i>Pae</i> , and <i>Eco</i> samples: 10 ¹ copies/μL	[139]
Digital PCR	Cell culture	EGFR genomic DNA of lung cancer cell	Mastercycler nexus flat, PCR apparatus	77 min	Potential DNA contamination digesting: 50 °C	Dynamic range: 10 ¹ to 10 ⁴ copies/μL	[146]

					Taq DNA polymerase activation: 95 °C 95 °C–60 °C *45 cycles		
Digital LAMP	Human erythroblast leukemia cell line (HEL)	JAK2 V617F DNA	A hot plate	1 h	63 °C	Dynamic range: 10 ¹ to 10 ⁴ copies/μL	[158]
Digital RPA	Spiked milk	<i>E. coli</i> O157:H7, Listeria monocytogenes and Salmonella enterica DNA	A hot plate	30 min	39 °C	10 cells/10 μL milk samples	[161]
Digital LAMP	Spiked toilet wastewater	<i>E. coli</i> , <i>E.</i> <i>faecalis</i> , and <i>Salmonella</i> <i>Typhi</i> / genomic DNA	A hot plate	40 min	65 °C	Dynamic range: 11 to 1.1 × 10 ⁵ copies/μL	[163]
Digital LAMP	Blood of infected mice	<i>Trypanosoma</i> <i>brucei</i> / genomic DNA	Positive- temperature- coefficient (PTC) heater	1 h	65 °C	20 copies/μL	[164]

^a CPCR, convective polymerase chain reaction. ^b RPA, recombinase polymerase amplification, ^c NASBA, nucleic acid sequence-based amplification. ^d LAMP, loop-mediated isothermal amplification.

5 Signal readout

A signal transducer is responsible for recognizing target and reporting measurable signal. Colorimetric, fluorescent, electrochemical, acoustic, magnetic, or other signal outputs are generated in sample-to-answer microfluidic devices, enabling the sensitive detection of various biological targets [92]. These signals can be quantitative or qualitative and allow end users to read through naked eyes, miniaturized optical detection instrumentations, piezo-electric modules, mobile phones, distance-based readout, or other techniques.

5.1 Optical signal

Optical signal outputs, such as colorimetric [139], fluorescence [167], surface plasmon resonance (SPR) [168], and surface-enhanced infrared absorption spectroscopy assays (SEIRAS) [169], are extensively used in sensitive detection of various targets. With the high sensitivity and flexibility of being integrated into portable point-of-care systems, a variety of optical sensing strategies have been proposed. In this section, recent advances in optical sensing techniques integrated with sample-to-answer systems are reviewed.

In dye-based colorimetric assays, calcein and hydroxynaphthol blue (HNB) are frequently utilized as metal ion indicators for POC colorimetric detections [16, 170, 171]. They allow smartphone-based detection and are especially suitable in combination with LAMP assays due to the rapid accumulation of Mg^{2+} [172, 173]. For example, Ma et al. demonstrated an HNB-based LAMP platform enabling sample-to-answer detection of the H1N1 virus and MRSA bacteria *via* smartphone interface with wireless connectivity (Fig. 5A) [172]. Tetrabromophenol blue (TBPB) is another dye frequently used in conjunction with ethanol for equipment-free protein tests such as kidney function test and protein to creatinine ratio in urine due to color change resulting from nonspecific binding of TBPB to proteins [174-176]. SYBR green I specifically binds to double-stranded DNA and is commonly used as an indicator in nucleic acid quantification due to the high signal-to-noise ratio, short response time, and enhanced sensitivity [107, 177]. Many smartphone-based platforms for ultrasensitive POC diagnosis exploit SYBR green I [178-180].

Gold nanoparticle (AuNP) is another option usually used for sensitive detection of nucleic acid targets due to its significant color change resulting from the extent of particle aggregation [181, 182]. Combining surface modification of AuNP for specific binding, such as single-stranded DNA hybridization, avidin-biotin binding, and antibody-antigen pair, with lateral flow assay or smartphone-based image analysis are common strategies for self-contained POC diagnosis [183, 184]. Fluorescent nanoparticles combined with particle diffusometry analysis are an emerging technique for such assays [185, 186].

In chemiluminescence (CL)-based detection, polydiacetylene (PDA) is a powerful and unique group of polymers frequently used in nucleic acid-based detection due to its low cost, short response time, and biomimetic structure [187]. Most proposed PDA-based sensors were demonstrated in the form of a vesicle dispersed in aqueous solutions to impart fluidity to PDA film or a layered deposition coating onto the rigid material surfaces, enabling enhanced color shifting and better detection limit [188, 189]. To date, low-cost and equipment-free sensing platforms

combining smartphone-based detection and analysis have been developed for a variety of POC diagnoses, such as H1N1 and SARS-CoV-2 [190, 191]. Featuring its intrinsic chromatic properties involving a color shifting from blue to red towards various concentrations of targets, PDA can form a visible color change that can be captured and analyzed through a developed smartphone app wirelessly. In addition, the combination of hydrogen peroxide and luminol catalyzed by horseradish peroxidase (HRP) is another widely-use mechanism in CL [192]. Smartphone-based microfluidic platforms integrated with this strategy have recently been developed to realize a fully autonomous POC diagnosis [193]. Electro-chemiluminescence (ECL)-based optical signal is generated when applying an electrical potential onto the electrode where electro-sensitive chemicals are excited, resulting in the conjugation of the chemicals and nucleic acids [107]. ECL technique has also been successfully applied to cloth-based materials for the development of low-cost and ultra-flexible microfluidic devices where the colorimetric results can be collected *via* a portable CCD-based sensing system to realize POC testing [194-196].

Optical signals for nucleic acid assays can also be obtained using the SPR approach. Nguyen et al. demonstrated an SPR-based all-in-one platform enabling a rapid and non-labeled PCR amplification of *Salmonella spp.* within 30 min [197]. The Ag/Al coated optical fiber can detect the interaction between the evanescent wave and the bacterial nucleic acids leading to the power change of SPR fiber output. The SPR sensor signal increased along with the PCR cycle number because the refractive index of the amplicon is smaller compared to the sum of the component dNTP molecules. SPR (or localized SPR) has later been widely coupled with other smartphone-based platforms, enabling ultrasensitive POC diagnosis (Fig. 5B) [198-200].

Significant efforts have been made to make colorimetric assay more portable and accurate by integrating it with mobile device imaging and analysis. Although the consideration of the cost and accessibility may trade off critical capabilities such as sensitivity and specificity, the colorimetric strategy is still a great candidate as colorimetric transducers can be readily integrated into microfluidic devices within sample-to-answer systems [201].

5.2 Distance-based signal

Inspired by the design of thermometers, distance-based signal readout has been extensively integrated with microfluidic systems. In this sensing strategy, the detection results manifest as distance-based signals such as wicking color bands on paper or particle accumulation in an on-chip reservoir [202]. Distance-based readout within devices enables direct counting for endpoint quantification of nucleic acids without the need for cumbersome instrumentations [203].

By integrating a redox-coupled indicator system (HRP / H₂O₂) with a paper strip, the distance-based detection platforms were first developed in the 1990s for the visualized quantification of high-density lipoprotein (HDL) cholesterol and its similar derivatives in human whole blood [204, 205]. These self-contained sensing platforms consolidate the separation of whole blood and sensitive HDL detection into one step, creating a new paradigm. Many more distance-based platforms have been developed with improved portability and robustness for sensing visualization and quantification. Herein, recent advances in distance-based sensing techniques integrated into

portable systems for POC diagnosis are reviewed.

The distance-based approach is especially suitable for paper-based microfluidic platforms to realize equipment-free and visualized quantification. They have drawn great attention in POC diagnosis due to their ubiquitous, disposable, and low-cost features [206]. Chemical reactions leading to cumable colored particles deposited onto paper microfluidics is one of the common strategies. Abate et al. exploited cascade enzymatic reactions involving immunoglobulin G (IgG)-specific antibody, glucose oxidase (GOx), HRP, and 3,3'-diaminobenzidine (DAB) to develop a paper-based IgG sensor [207]. During the reaction, alkaline phosphatase-conjugated antibodies converted glucose-1-phosphate into glucose. Consequently, generated glucose turned colorless DAB into visible brown poly-DAB with the help of GOx and HRP pre-deposited in paper channels. The growing-colored poly-DAB stripe, which is proportional to IgG concentration, can be easily measured using the printed ruler on the device. A similar mechanism producing cumable colored complexes was widely applied to the detection of compounds related to chronic diseases (e.g., glucose and glutathione), antioxidants in food, bacteria (e.g., *Salmonella typhimurium*), and metal ions (e.g., nickel, copper, iron, mercury and potassium ions) [208-215].

Sensing platforms combining particle aggregation and on-chip nucleic acid amplification with fluorescein have also been extensively developed for visualization and direct quantification of target nucleic acids [216]. Wang et al. developed a paper-based platform featuring a smartphone-controlled portable thermal cycler and on-chip PCR [217]. It allowed direct and sensitive quantification of soil-transmitted helminth (1 aM – 1 fM) by measuring the rising distance resulting from SYBR green-intercalated double-stranded DNA (dsDNA). Instead of using intercalating dye for nucleic acid quantification, Kalish et al. developed a paper-based scale bar exploiting the hybridization between target DNA and surface-functionalized colored microspheres for quantification [218]. The colored microspheres were modified by conjugating short single-stranded DNA (ssDNA) partially complementary to the targets and formed measurable color bands inversely proportional to the concentration of target DNA. Aggregation strategies exploiting complementary ssDNA or DNA aptamer is especially suitable for POC applications as the sequences can be customized and adopted for a wide range of sensing targets as needed [219, 220].

Silicon-based microfluidics allows for more complex processes and has been extensively integrated with many distance-based sensing techniques [220]. Wu et al. demonstrated a microfluidic chip featuring a synergistic effect among hairpin complex H1H2 and GR-5 DNAzyme in the presence of magnetic microparticles (MMPs) and polystyrene microparticles (PMPs) for visualized quantification of lead ions (Pb^{2+}) [221]. A catalytic assembly was triggered by Pb^{2+} , leading to the generation of an MMPs-H1H2-PMPs complex that can be magnetically trapped. Consequently, the remaining free PMPs relevant to the amount of Pb^{2+} then flowed along the microchannel and accumulated at a particle dam. A similar mechanism was implemented and proved effective earlier in the detection of specific target oligonucleotides by the same group [222]. A novel sensing mechanism in combination with photothermal (PT) effects and antibody-conjugated Fe_3O_4 nanoparticles (NPs) was also proposed to enable a visual quantification of prostate-specific antigen (PSA) in human whole blood (Fig. 5C) [223]. By converting Fe_3O_4 NPs

into Prussian blue (PB) NPs, stronger PT effects were then induced under the exposure of near-infrared (NIR) radiation. Resulting heat generated a powerful driving force to push PSA-antibody-PB NPs in the microchannel to form a colored bar proportional to PSA concentration. Additionally, platinum nanoparticles (PtNPs)/H₂O₂ pair were employed for the efficient generation of O₂. [224]. Working as a pneumatic pump, O₂ pushes ink/colored mixtures through the microchannels of distance-based platforms for visual quantification. This strategy has been successfully applied in conjunction with hydrogel and aptamers and integrated into a microfluidic platform for a wide range of POC diagnoses such as *S. enterica*, *E. coli*, *L. monocytogenes* and Aflatoxin B₁ [225-227]. Qin's group was one of the pioneers of a similar "air-push" strategy for the detection of target proteins and DNAs with the integration of immunoassay and H₂O₂/catalase pair [228, 229].

Distance-based sensing approach has advantages such as signal clear visualization and non-instrumented analysis, which is less susceptible to the variation of user interpretation and is suitable for point-of-care diagnosis in resource-limited settings. However, some inherent problems, such as the assay reproducibility and instability of catalysts, still need to be addressed.

5.3 Electrochemical signal

In developing an effective electrochemical sensor, the affinity of target recognition elements and the chemical mechanism to generate electrical signals play a critical role [230]. This approach provides low sample consumption, rapid signal response, and flexibility to fit in miniaturized modules for fully automatic diagnosis. It also allows multiplex and label-free detection if designed properly [231]. Portable POC devices integrated with the electrochemical method have been extensively developed for sensitive diagnosis targeting multiple diseases, including cancers [230]. Additionally, these platforms were interfaced with mobile devices (e.g., smartphones) connecting with wireless communication to realize remote control and smartphone-based analysis (Fig. 5D) [232]. In this section, the mechanism and performance of electrochemical strategies in recent portable/smartphone-based POC diagnosis are reviewed.

Researchers have tried various target recognition elements, including aptamers, complementary ssDNA, and antibodies. For example, Low et al. exploited specific ssDNA for a smartphone-based detection of circulating microRNA-21 (miR-21) in saliva [233]. The ultrasensitive diagnosis was achieved (LoD: 1 pM) with modified electrodes and an autonomous circuit board where no benchtop instruments (e.g., laptop, electrochemical workstation) were involved. Antibodies were frequently used in combination with smartphone-based analysis for sensitive detection of different protein biomarkers (e.g., cancer antigen 125, serum-derived exosomes, breast cancer biomarker HER2, and SARS-CoV-2 nucleocapsid protein) [234-237]. This robust electrochemical technology has also been combined with an aptamer-functionalized interface to extend its clinical applications [238]. For example, Chakraborty et al. modified ZnO nanorods with DNA aptamers against carcinoembryonic antigen (CEA) to realize a label-free sensing platform [239]. With a smartphone-based potentiostat, a detection limit down to 1 fg/ml in human serum was demonstrated. Although the recognition layer is critical for detection specificity, it is not always necessary for the detection of some targets, such as metal ions, due to

their unique and differentiable redox properties [240, 241]. These recognition components performed well on a paper-based substrate for multiplexed and ultrasensitive detections targeting biomarkers of clinical significance, although they have not been fully automated without benchtop machines [242-246].

Apart from the recognition layer, chemical modification on electrodes is another powerful strategy to boost detection sensitivity. Screen-printed carbon electrodes (SPCE) became one of the most commonly used electrodes for this purpose because of its low cost, materials properties, and the ease of modification with chemicals [233, 241, 247, 248]. SPCE has been functionalized with various components, such as gold/silver/platinum nanoparticles, graphene, reduced graphene oxide, carbon nanotubes, to improve electrode performances [233, 237, 241, 247-249]. In addition, electrodes modified with nanohybrids comprising carbon-based nanomaterials, metal oxides (e.g., SnO₂, NiO, ZnO), and polymers (e.g., polyethylenimine, polypyrrole) demonstrated extraordinary performance in electrical conduction [241, 247, 248, 250]. These nanocomposites can be printed onto paper-based POC devices using screen printing for a continuous electrochemical interrogation [243, 244, 251, 252]. Electrochemical performance can be boosted in a flexible and tubular membrane-like sensor due to enhanced electron hopping and tunneling effects resulting from an enhanced electrical field and its particular geometry [253].

Smartphone-based POC platforms combining nucleic acid amplification and an electrochemical-based approach are of great interest. [230, 254]. Recently, Li et al. demonstrated a smartphone-based sensing platform combining LAMP and real-time electrochemical monitoring, enabling home-testing of SARS-CoV-2 (Fig. 5E) [255]. Covering Nafion film on the polyaniline-based electrode, this sensor demonstrated an excellent potentiometric response to pH change due to accumulated pyrophosphate. Combining on-chip thermolysis and Bluetooth module, this plug-and-play potentiometric pH sensor allowed a rapid and ultrasensitive outcome (2×10^2 copies per test within 25 min) in both endpoint detection and real-time pH monitoring on a smartphone at home. Paper-based POC devices with similar technology were also developed, consisting of on-chip sample preparation, LAMP, and electrochemical analysis [251, 256]. However, relatively bulky potentiostat and external heating devices have not yet been integrated into a one-piece package and interfaced with smartphones in these studies.

5.4 Magnetic signal

Magnetic signal readout offers good stability, high signal-to-noise ratio, and simplified procedures of magnetic nanobeads (MNBs) and has been applied to the detection of a variety of biomolecules, including pathogens [257, 258], proteins [259-261], and small molecules [262, 263]. Biorecognitions including the antibody-antigen interaction, aptamer-target recognition, and molecularly imprinting polymers-based recognition, play a critical role in MNBs-based sensing methods such as nuclear magnetic resonance (NMR), magnetorotation, optomagnetic approach, giant magnetoresistive effect (GMR) and so on [264]. Some notable MNBs-based approaches integrated with portable systems for POC diagnosis are discussed here.

The interaction between the target analytes and the bioreceptors forms agglutination in

various volumes/geometries leading to a property change in the dynamics of the MNB [264]. Based on the rotational dynamics of the aggregates responding to an external oscillating magnetic field, the number of target molecules can be quantified [265]. Sharma et al. developed a magnetic tunneling junction (MTJ)-based platform comprising specialized portable electronic and microfluidic setups, enabling nM-level sensing of bacteria and viruses [266]. This platform optimized hybridization and magnetic labeling of natural DNA, resulting in highly selective and specific detection and genotyping. A similar MTJ-based strategy was previously exploited for sensing magnetic particles at low concentrations by measuring their Brownian relaxation frequency [267], optimization of DNA hybridization, and magnetoresistive biorecognition [268-270].

NMR is another technique frequently integrated into miniaturized devices for POC testing. A miniaturized system combining μ NMR and targeted magnetic nanoparticles was developed for measuring multiple cancer biomarkers in both circulating tumor cells (CTC) and fine needle biopsies of solid epithelial cancers (Fig. 5F) [271]. Collected fresh samples were labeled with antibodies and selectively targeted by tetrazine-nanoparticles for quantifying epithelial cellular adhesion molecule (EpCAM), EGFR, human epidermal growth factor receptor 2 (HER-2), and vimentin by the μ NMR system. Other miniaturized platforms using NMR-based techniques have been reported for the identification of drug-resistant *Mycobacterium tuberculosis* (MTB) strains [272], the detection of *Salmonella* in milk [273], and phenotyping of 13 bacterial species [274].

In agglutination assays, the formation of magnetic bead clusters due to the specific binding of biomarkers have been studied and exploited for ultrasensitive optomagnetic detection [275]. Uddin et al. demonstrated a rotational disk platform for the detection of thrombin using aptamer-coated magnetic nanobeads and microbeads (MNBs and MMBs) [276]. With the magnetic field excitation, an increasing optomagnetic signal can be detected, indicating a gradual formation of MNB agglutination over time due to the increasing amount of thrombin. In addition, strong magnetic field pulses facilitated particle agglutination (i.e., magnetic incubation), enabling a low detection limit of 25 pM with 15.5 min of reaction. A similar mechanism integrated with the disk platform was exploited in the detection of C-reactive protein and peripheral blood mononuclear cells in whole blood [277] and prostate-specific antigen in a 50% serum [278].

Tian et al. demonstrated a microfluidic platform combining self-assembled magnetic nanoparticle and graphene oxide (MNP-GO) nanotags for optomagnetic detection of *E. coli* 16S rDNA sequence [279]. The aggregation of target RCA amplicons and MNP-GO nanotags led to an enhanced optomagnetic signal due to high shape anisotropy related to aggregation size and the number of target amplicons. The proposed platform enabled an ultrasensitive detection of *E. coli* 16S rDNA sequence (2 pM) within 90 min, indicating an extraordinary optomagnetic property of MNP-GO nanocomposite. Furthermore, microfluidic-based optomagnetic approaches combining various nucleic acid amplifications were reported in recent publications including the detection of synthetic DNA strands targeting type B-influenza virus [280, 281], tuberculosis [282] using RCA, and Newcastle disease virus and dengue serotype 2 virus using LAMP [283, 284].

The magnetic technology is versatile and applicable to a variety of purposes beyond sensing

assays, such as a highly accurate cytometer for the quantification of pancreatic cancer cells [285]. The magnetic-based thermometer is one of the novel inventions regarded as “Next-Generation Optomagnetic Devices” [286]. Due to their biological inertness, physical stability, and lack of interfering magnetic signals in biomaterials, MNBs-based platforms would be favorable for developing a more efficient POC diagnosis.

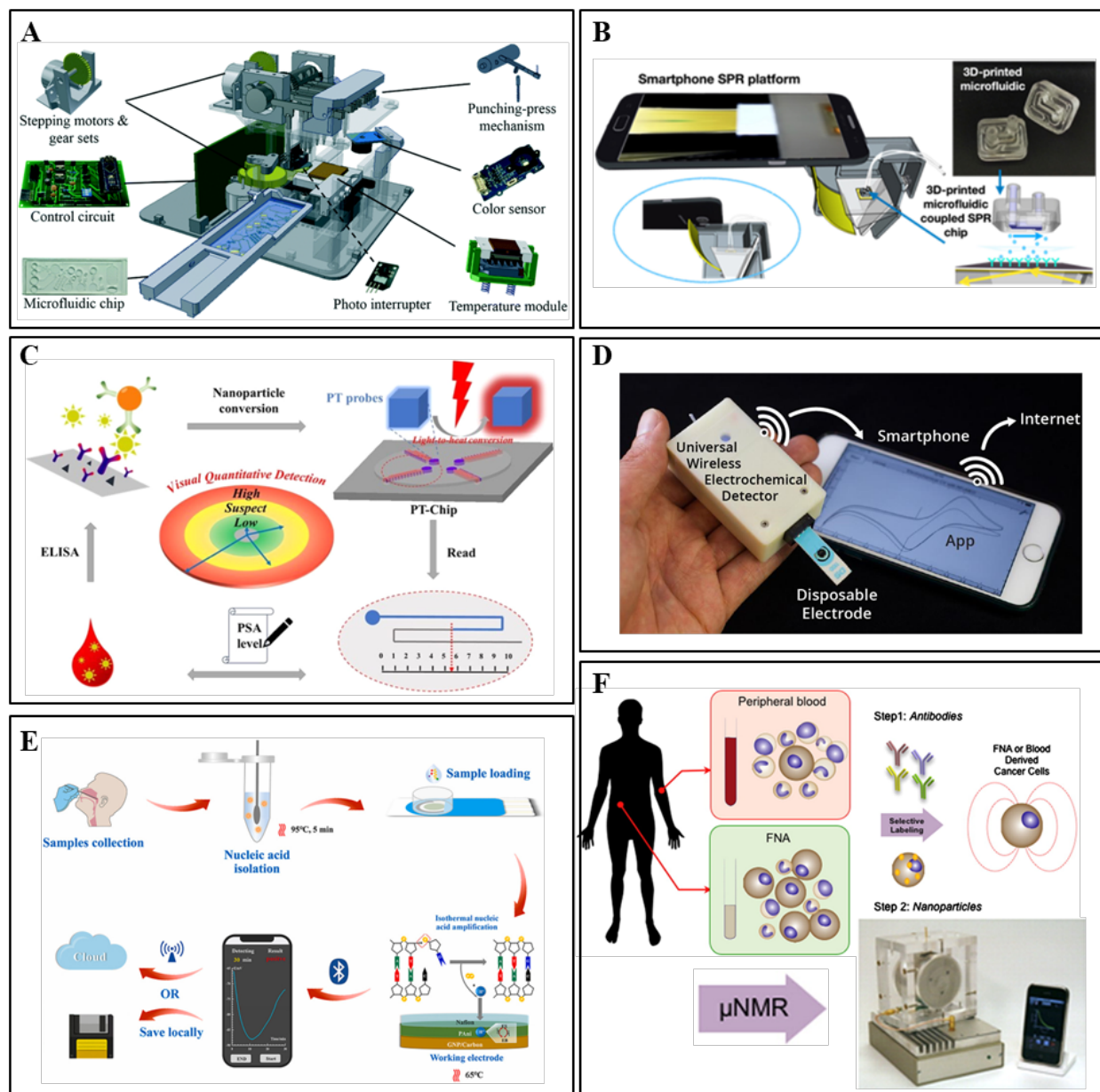


Fig. 5. Nucleic acid amplification approaches using portable and miniaturized systems. (A) An HNB-based LAMP platform enabling sample-to-answer detection of H1N1 virus and MRSA bacteria *via* smartphone interface with wireless connection. Adapted from [172] with permission from The Royal Society of Chemistry. (B) A cost-effective 3D-printed unibody microfluidic SPR chip integrated into with smartphone-based iSPR system. Adapted from [200] with permission from Elsevier. (C) Nanomaterial-mediated bar-chart microfluidic chip exploiting photothermal

effect for visual quantification. Adapted with permission from [223]. Copyright 2021 American Chemical Society. (D) A smartphone-based electrochemical sensing platform integrated with open-Source potentiostat enables wireless detection. Adapted with permission from [232]. Copyright 2018 American Chemical Society. (E) A smartphone-based sensing platform combining LAMP and real-time electrochemical monitoring for ultrasensitive diagnosis of SARS-CoV-2. Adapted from [255] with permission from Elsevier. (F) A portable platform combining μ NMR strategy and magnetic nanoparticle labeling to detect and characterize tumor cells and metastatic tumor. Adapted from [271] with permission from Elsevier.

Table 4. Comparison of portable and integrated devices for point-of-care diagnosis of nucleic-acid targets

Amplification method	Sample type	Target	Signal type	Reaction mechanism	Sensing system	Limit of Detection / Time-to-result	Ref.
RT-LAMP	Spiked oropharyngeal swab	SARS-CoV-2 / armored RNA particles	Fluorescence	Byproduct of RT-LAMP, magnesium pyrophosphate, activating fluorescence of Calcein dye	LED light source, fluorescence filter, objective lens, electric autofocus system and a CMOS camera	2 copies per reaction / Sample-to-answer time: ~ 70 min	[167]
RT-LAMP	Spiked whole blood	HIV-1 / RNA	Visible test bands	Amplicons, containing FAM- and biotin-tagged loop primers, captured by anti-FAM antibody and visualized by streptavidin-conjugated gold nanoparticles on the test line	Visual detection based on test and control bands	2.3×10^7 copies/mL of whole blood / ~ 90 min for visual interpretation of results	[183]
PCR	Bacteria culture	<i>Salmonella</i> spp. / DNA	Surface plasmon resonance	Amplicons reducing refractive index on the SPR fiber sensor surface	Output power measured by an optical power meter	Detection limit: Not applicable / DNA amplification time ~ 30 min	[197]
RT-LAMP	Human blood	Hepatitis C virus / RNA	Electrochemical	Byproduct of RT-LAMP, magnesium pyrophosphate, reducing pH	Ion-sensitive field effect transistor sensing released protons	10 copies/mL / 30 min	[254]

RT-LAMP	Nasopharyngeal swabs	SARS-CoV-2 pseudovirus / RNA	Electrochemical	Byproduct of RT-LAMP, magnesium pyrophosphate, reducing pH	Signals measured by potentiometric sensor and wirelessly transmitted to smartphone	2×10^2 copies/test / 25 min	[255]
RCA	Spiked urine samples	<i>E. coli</i> , <i>P. mirabilis</i> , and <i>P. aeruginosa</i> / DNA	Optomagnetic	Agglutination of amplicons and magnetic nanoparticles (MNPs) slowing MNP's response under AC magnetic field	Resulting modulation of the transmitted laser light detected by a photodetector	~50 pM of synthetic monomer target / Total assay time: ~ 4 h	[258]
PCR	Bacteria culture	<i>L. monocytogenes</i> , <i>S. typhimurium</i> , and hepatitis E virus / DNA	Magnetoresistive	Capture of target DNA by complementary probe on magnetic tunneling junction (MTJ) sensor surface, followed by MNP tagging, inducing magnetoresistance	Resulting voltage change across the MTJ sensor measured by a lock-in amplifier	< 1 nM for natural DNA / DNA hybridization time < 6000 s	[266]
PCR	Spiked sputa	<i>M. tuberculosis</i> / DNA	Nuclear magnetic resonance (NMR)	Hybridization of PCR-amplified target DNA and microbeads and MNPs with complementary probes resulting in acceleration of the decay of NMR signal	The change of transverse relaxation rate (ΔR_2) measured by a μ NMR electronics	1 nM ssDNA in 1 μ L sample volume and 10^2 – 10^3 CFU mL ⁻¹ for whole MTB cell-spiked MTB-negative sputa / Total assay time: ~	[272]

2.5 h

6 Commercial sample-to-answer kits for plant disease diagnosis and their challenges

Human societies have heavily relied on plants as indispensable resources for food, medicine, lumber, landscaping, carbon storage, and many more [287-289]. Particularly, food crops are essential for meeting the food demands of the rising world population [290, 291]. However, the threat of various plant diseases to agricultural industries has been increasing in the recent years. For instance, citrus greening disease (also called Huanglongbing) once caused a huge disaster to Florida's citrus industry, with an estimated economic loss of up to \$4.5 billion within five years [292]. To our knowledge, reports specifically reviewing commercial kits for in-field diagnosis of plant diseases are still rare, especially when compared with those targeting human diseases, which increased in the past few years due to the outbreak of COVID-19 [293-296]. Thus, this section focuses on in-field plant disease detection and relevant commercial kits, including their applicability and cost.

Many commercial tools have been designed and utilized to address the key bottleneck of in-field plant disease diagnosis in a sample-to-answer manner. These tools enable quick screening of diseases in the field without lab instruments and well-trained personnel involved, leading to more cost-effective disease management. As shown in Table 5, a variety of commercially available kits for plant disease detection are listed based on the key factors that matter in actual on-site diagnoses, such as time to result, ease of use, and cost per assay. Examples in this section include the ones detecting non-nucleic acid targets, as these are extensively used in plant diagnosis.

Lateral flow immunoassay (LFIA) is one of the most extensively used approaches in the field, featuring simple operation and rapid response. The principle is based on antibody/antigen binding, where antibodies are designed to recognize specific antigens from pathogens of interest. The antigens in extracted saps are captured by antibodies deposited on test strips. A dye (e.g., colloidal gold) then enables the captured antigen to be visualized [297]. Kits usually come with a tool for physically mashing the sample, a specialized buffer for dilution and reaction enhancement, and an LFIA test strip/cassette with specific antibodies for yes / no response. Pocket Diagnostic (Abingdon Health, York, UK) developed a series of LFIA tests for detecting *Erwinia amylovora*, potato virus Y, *Ralstonia solanacearum*, and *Phytophthora*. With these kits, plant samples are manually pulverized by shaking the buffer bottle with grinding balls inside. The resulting saps are ready for the LFIA test [298-301]. Another common product is Agdia ImmunoStrip (Agdia, IN, USA). Unlike the Pocket Diagnostic's kits, it features buffer-filled mesh bags for crushing samples with a blunt object. Agdia has extended the application to more than 40 viral diseases, covering many plants such as tomato, citrus, and tobacco [302-304]. Other companies also developed similar products to target a wide range of pathogens, including bacteria and fungi [301, 305, 306].

Electronic nose (e-nose) is a powerful alternative that offers non-invasive plant inspection by analyzing volatile organic compounds (VOCs) emitted by the plants. Over the past few decades, e-nose devices have been developed and applied in various industries requiring precise monitoring and analysis of VOC (e.g., food and agricultural industry) [307]. Currently, a number of e-nose devices serving plant diagnosis purposes are commercially available (Table 5). For example,

Bloodhound ST214 and Cyranose 320 are early e-nose products capable of differentiating VOC profiles emitted by healthy and diseased cucumber, pepper, onion, tomato, and blueberries [308-310]. Models that are more portable and functional, such as Portable Electronic Nose 3 (PEN3) and zNose (Model 4300), have been adopted for a wide range of applications in the fields (e.g., botany, forestry, and agriculture) where fungal, bacterial infections and pest infestations cause problems [308, 311-314].

Commercial molecular assay kits have been developed and applied to on-site diagnosis of plant diseases (Table 5). In these kits, amplification and analysis of nucleic acids are simplified and integrated into a single instrument to facilitate in-field applications. An early product from Cepheid (Sunnyvale, CA, USA), the SmartCycler system allowed multiplex testing and was previously used for in-field detection of Pierce's disease of grapes without extraction of DNA by performing PCR with sap. The same model was later applied to citrus Mal secco disease and dieback of *Parrotia persica* [315-317]. More recently, SmartCycler was deployed to the field trials evaluating penicillin's therapeutic effect in huanglongbing (HLB)-affected citrus [318]. Cepheid's other product, the GeneXpert system, was considered an improved version of the sample-to-answer PCR device. Known for its multitasking cartridge with pre-loaded reagents and built-in fluidics, nucleic acid extraction, thermal cycling, and interpretation of the results are integrated and automatically executed with minimal human intervention [319, 320]. OptiGene Genie I, II & III integrated isothermal amplification (e.g., LAMP) in a handheld, battery-powered device. With the isothermal technology, these models are specifically suitable for outdoor use and have been utilized for on-site diagnosis of various plant diseases such as *Erwinia amylovora*, *Pepper chat fruit viroid*, *Phytophthora infestans*, *Sweet potato feathery mottle virus*, *Sweet potato chlorotic stunt virus* [321-324]. Despite the portability and diagnostic efficiency, commercialization of LAMP-based sample-to-answer devices has been limited and not as popular as seen on their PCR-based counterparts in the past few decades due to licensing required from the inventors [138].

Sample-to-answer devices featuring PCR-based detection (e.g., Palm PCR, Biofire Filmarray, Luminex ARIES System, 3M Integrated Cyclor, and Roche cobas Liat) are commercially available for on-site applications [325-329]. However, these devices are predominantly used for human disease diagnosis. This is probably due to the increasing threat of human infectious diseases caused by fatal viruses such as SARS-CoV-2 and Ebola Virus Disease (EVD) [330-332].

On-site plant disease diagnosis still depends on off-device sample preparation prior to the assays such as the production of crude saps and purification of nucleic acids where human intervention is required. Although some of the kits mentioned above are designed to have a higher tolerance to inhibitors in test samples, quality of the inputs that one can prepare in the field is still the key factor dominating detection sensitivity and accuracy. The same requirement applies to portable devices with PCR-based technologies. Without an automatic and hand-free sample preparation of difficult-to-lyse plants, the applicability of these devices to field settings could be limited. To overcome the bottleneck and reduce the cost of plant disease management, cellulose paper is considered a suitable medium featuring affordability and easy-to-fabricate/modify. It has long been reported that paper can quickly retain, dry, and store nucleic acids and reagents, which

facilitates the extraction process and makes it possible for distant transportation without a cold chain [75]. In addition, the usability of paper can always be expanded by treating it with functional chemicals (e.g., Chitosan) or DNA for enhancing extraction efficiency (e.g., electrostatic attraction) or other purposes (e.g., selective binding) [75, 333].

In the future, sample-to-answer devices should integrate with sequencing technologies such as next-generation sequencing (NGS) and third-generation sequencing (TGS) [334]. Although more challenges (e.g., stability of nucleic acids, data acquisition, and analysis of complex genomes) can emerge while trying to make this happen, such capability would revolutionize on-site diagnosis processes and advance the low-cost and high-throughput disease management [309, 334].

Table 5. Comparison of commercial sample-to-answer kits and devices for plant disease diagnosis

Product	Test type	Key components	Sample input	Pathogens/Diseases	Time to result/Response time	Cost per assay ^a	Ref.
Pocket Diagnostic Lateral Flow Test	Lateral flow immunoassay	A buffer bottle with milling beads, a pipette, and a test cassette	Crude lysate of plant tissue	<i>Erwinia amylovora</i> <i>Phytophthora spp.</i> <i>Ralstonia solanacearum</i> Potato virus Y	< 10 min	~\$6-11	[298-301]
Agdia ImmunoStrip	Lateral flow immunoassay	A buffer-filled mesh bag and a test strip	Crude lysate of plant tissue	41 plant viruses 7 plant bacteria and 2 fungi	5-30 min	~\$6-15	[302-304]
Bioreba AgriStrip	Lateral flow immunoassay	An extraction bag, an extraction buffer, a cuvette and a test strip	Crude lysate of plant tissue	16 plant viruses 3 plant bacteria and 1 fungus	10-15 min	~\$5 ^b	[306]
PlantMedia ALERT-LF	Lateral flow immunoassay	Not available	Crude lysate of plant tissue	<i>Phytophthora spp.</i> <i>Pythium spp.</i> <i>Rhizoctonia spp.</i> <i>Botrytis spp.</i>	< 5 min	~\$27-33	[305]
LOEWE FAST Kits	Lateral flow immunoassay	A test cassette, a single-use pipette and a pre-filled	Crude lysate of plant tissue	20 plant viruses 9 plant bacteria and fungi	5-10 min	~\$5	[301]

		sample buffer tube					
Bloodhound ST214	E-nose	A medium size benchtop machine featuring 14 organic polymer sensor arrays	Top air (headspace sampling)	<i>Oidium neolycopersici</i>	Not available	-	[308- 310]
Cyranose 320	E-nose	A handheld terminal featuring a composite sensor array with 32 electrodes and conducting nanoparticles	Top air (headspace sampling)	<i>Burkholderia cepacia, Botrytis cinerea, Colletotrichum, gloeosporioides, Alternaria sp., grey mould, anthracnose, Alternaria rot</i>	2 min	-	[308- 310]
PEN3	E-nose	A portable device featuring a 10-fold array of thick film metal oxide gas sensors	Top air (headspace sampling)	<i>F. Culmorum Erwinia amylovora Pseudomonas syringae pv Agrobacterium vitis Botrytis sp. Penicillium sp. Rhizopus sp.</i>	< 1 min	-	[308, 311- 313]

zNose 4300	E-nose	A portable device featuring surface acoustic wave (SAW) sensor and ultra-fast gas chromatography (GC) capabilities	Top air (headspace sampling)	<i>Oidium neolycopersici</i>	< 1 min	-	[314]
SmartCycler	Real Time PCR	I-CORE: Fan + Circuitry + Heating plates + Optical blocks	Crude lysate of plant tissue, extracted nucleic acids	<i>Xylella fastidiosa</i> <i>Phytophthora ramorum</i> <i>Phoma tracheiphila</i>	20-40 min	-	[315-318]
OptiGene Genie II & III	Isothermal amplification	Ceramic substrate with resistive coating High-precision thermistor Block with 4-zone independent digital PID and heated lid	Crude lysate of plant tissue, extracted nucleic acids	<i>Erwinia amylovora</i> Pepper chat fruit viroid <i>Phytophthora infestans</i> Sweet potato feathery mottle virus Sweet potato chlorotic stunt virus	30-45 min	-	[321-324]

^a Calculated based on the companies' website information in March 2023. ^b Adapted from Bioreba Price list 2019.

7 Future outlook and conclusion

In this review, recent advances of microfluidic devices and point-of-care testing are studied. These portable systems are composed of several functions, including sample preparation, nucleic acid amplification, and resultant signal readouts. Most devices perform each process within the microfluidic platform from crude samples, such as saliva and whole blood, without additional user intervention. Additionally, there are a number of strategies to improve performances of each function. For sample lysis, on-chip lysis methods such as enzymatic and chemical lysis are enabled by specific enzymes or chemicals. A heating element or external electrical field deployed can further facilitate the lysis reactions, shortening reaction time. Magnetic beads, silicon-based materials, or cellulose fiber materials are frequently used for assisting nucleic acid extraction in portable systems due to their unique analyte-binding properties. Reports on an automated or one-step protocols for the detection of plant diseases are limited, compared to those for biomedical applications. In fact, automatic sample preparation of plant samples in microfluidic chips or handheld devices is challenging due to the solid cell walls, and general on-chip sample lysis strategies have minimal effects on plant cells. Therefore, developing a plant sample preparation unit is of great interest. For nucleic acid amplification methods, PCR has been successfully demonstrated using either a precise temperature control module or others such as Rayleigh-Bénard thermal convection. Although these approaches work reliably, heat dissipation issues within microfluidics elements are still challenging. Instead, LAMP-based strategies are simpler, require less energy, and need no thermocycler during the heating steps. LAMP can be implemented in portable systems with a smaller footprint and cost, suitable for resource-limited settings.

A fully automated sample-to-answer device with minimal user intervention, a simple user interface, and good network connectivity, is highly desired for many applications. Such a device would find widespread uses in places outside of laboratories, where lack of instrument resources and the need for extensive user training and knowledge have precluded its adaptation. Additionally, high price of most commercial devices is another bottleneck for their deployment beyond large institutions such as hospitals, research laboratories, government agencies, and food and agricultural industries. Recent advance in 3D printers will likely facilitate designing and prototyping of key components, reducing the development cost and time. Use of cellulose-based membranes for nucleic acid extraction and LAMP for amplification and signal readout will also help simplify the device design and reduce its cost. The authors hope this review will encourage further exploration and progress toward the development of user-friendly, fully automated, and portable sample-to-answer technology for in-field disease diagnosis of human, animal, and plants.

8 Conflicts of interest

The authors declare that there are no conflicts of interest associated with the current work.

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expressed in this paper are those of the authors and do not necessarily reflect the views of the National Science Foundation.

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