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## Point-of-care nucleic acid tests: assays and devices

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The COVID-19 pandemic (caused by the SARS-CoV-2 virus) has emphasized the need for quick, easy-to-operate, reliable, and affordable diagnostic tests and devices at the Point-of-Care (POC) for homes/fields/clinics. Such tests and devices will contribute significantly to the fight against the COVID-19 pandemic and any future infectious disease epidemic. Often, academic research studies and those from industry lack knowledge of each other's developments. Here, we introduced DNA Polymerase Chain Reaction (PCR) and isothermal amplification reactions and reviewed the current commercially available POC nucleic acid diagnostic devices. In addition, we reviewed the history and the recent advancements in an effort to develop reliable, quick, portable, cost-effective, and automatic point-of-care nucleic acid diagnostic devices, from sample to result. The purpose of this paper is to bridge the gap between academia and industry and to share important knowledge on this subject.

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### 1. Introduction

Nucleic acid testing (NAT) techniques, which include the detection and quantification of a target nucleic acid, have become the most dominant and reliable techniques for detecting pathogens.<sup>1,2</sup> These tests include a few steps: sample collection, nucleic acid extraction, amplification of the target nucleic acid (either by PCR<sup>3</sup> or by isothermal amplification reactions<sup>4</sup>), and its monitoring, usually by optical or electrical means.<sup>5,6</sup> If the target nucleic acid is RNA, then, prior to amplification, RNA is reverse transcribed to cDNA and then analyzed.<sup>7</sup> Traditionally, the purification of nucleic acids includes a few steps: cell membrane rupture, binding of nucleic acids to an ion exchange column (usually positively charged silica particles), washing of unbound material from the column, and finally, elution of nucleic acids from the column.<sup>8</sup> In the 1990s, NATs embarked on a means to diagnose AIDS and hepatitis C virus (HCV) epidemics. German transfusion centers were the first to start in-house NAT testing in pools of up to 96 samples for HCV, hepatitis B virus (HBV), and human immunodeficiency virus-1 (HIV-1).<sup>9</sup> In 1990, Manz *et al.* published a study on the development of a silicon chip-based automatic device for the pretreatment, separation, and sensing of nucleic acids.<sup>10</sup> Until recently, NAT for infectious

diseases has almost exclusively been performed in centralized laboratories using heavy instrumentation, which requires skilled personnel.<sup>11</sup> A limit of detection (LOD) of  $\leq 100$  copies per ml of the pathogen is often needed for reliable detection, which requires amplification of the target nucleic acid through a polymerization process (although 1 copy of the target nucleic acid is sometimes sufficient for valid detection). Amplification take place by a polymerase chain reaction (PCR) mechanism or isothermal amplification. During the past few years, several systems for identifying pathogens at the POC, using portable equipment, have been developed; the most prominent are lateral-flow antigen-antibody assays and nucleic acid tests. Antigen-antibody tests, which rely on a recognition event between antibodies and a pathogen's antigen, are simple to use, quick, and require neither sophisticated instrumentation nor a skilled operator. However, they suffer from an insufficient detection limit and a high level of false results. On the other hand, most molecular diagnostic nucleic acid tests rely on amplification (PCR or isothermal nucleic acid amplification methods) of the target nucleic acid and thus require more sophisticated diagnostic equipment. These assays are extremely accurate and sensitive: they can potentially identify a single copy of a pathogen in a given sample. Traditionally, only skilled personnel could operate them (since nucleic acid extraction and amplification, which are required, are cumbersome). In the early 1990s, DNA purification was time-consuming, bulky, and required the use of toxic and hazardous reagents. However, the advent of solid phase extraction techniques and the availability of commercial kits for quick and reliable DNA extraction significantly contributed to the development of automated and miniaturized nucleic acid sensing

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devices (from sample to result). DNA extraction can be performed manually by a skilled laboratory operator or by automated systems (such as Qiagen's QIA Symphony SP/AS, TissueLyzer II, EZ2 Connect, EZ1 Advanced XL, QIAcube Connect TissueRuptor II, QIAcube HT, ThermoFisher's AutoMater Express, and KingFisher systems as well as Geneture's AMY 32 and AMY 96). Several techniques have been developed for nucleic acid amplification with PCR;<sup>8,12,13</sup> however, cycling through a series of defined temperatures is the most popular. A few isothermal techniques,<sup>14–16</sup> which amplify the target nucleic acid, also exist. Several reviews have been published lately that discuss POC diagnostic systems for SARS\_CoV\_2 and other pathogens.<sup>11,17–22</sup> In recent years, many commercially available automated nucleic acid detection systems have been developed. Here, we will review recent developments and instruments, explain different nucleic acid amplification techniques used for diagnostics, present several state-of-the-art commercially available nucleic acid detection systems, and discuss the technology behind each one. In addition, we will analyze different methods to miniaturize the existing bench-top laboratory nucleic acid testing instruments in order to develop portable POC detection systems. First, we will present PCR and isothermal amplification techniques in detail; then we will introduce nine POC commercially available testing devices and compare their mechanisms. Finally, we will discuss future developments in this field.

## 2. DNA amplification

### 2.1 Polymerase chain reaction (PCR)

The polymerase chain reaction is a technique to exponentially amplify nucleic acids (DNA and RNA). PCR was invented in Cetus laboratories (later bought by Roche), by Kary B. Mullis and co. in 1983 followed by a publication in *Science* in 1985.<sup>23</sup> Since then, it has become the most common method for nucleic acid amplification. It requires a set of two primers, which are DNA molecules of *ca.* 20 bases; they are designed specifically to bind to and initiate amplification of a target DNA molecule by a DNA polymerase (Fig. 1).

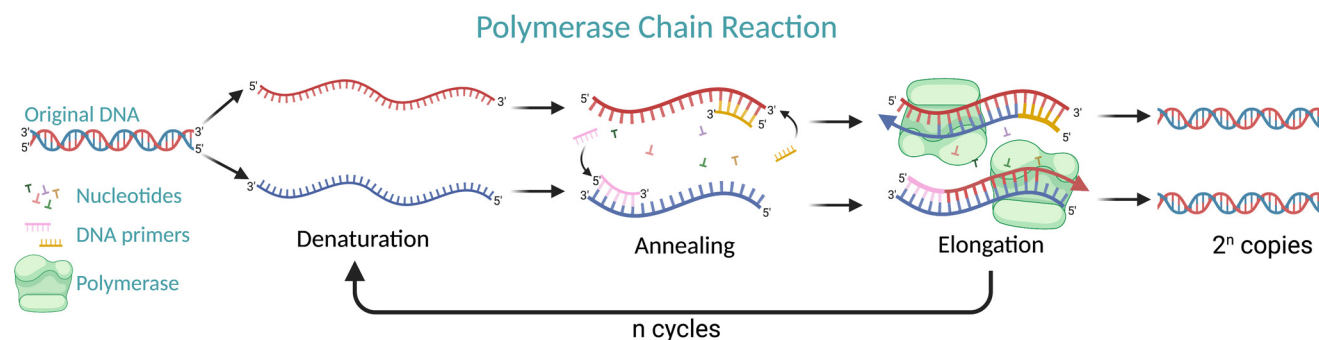
Thermal cycling involves repeated heating and cooling steps. Each successful PCR cycle, which involves three steps (denaturation, annealing, and extension of the target DNA), doubles the amount of DNA with which it began. Normally, for a reliable PCR test, 30–40 cycles of amplification are required, a process that takes minutes to hours. One of the aims of the POC testing devices is to reduce the time required to obtain a reliable result.

**2.1.1. Denaturation.** This step involves heating the sample to almost a water boiling temperature (95 °C), which denatures the double-stranded DNA (breaking the hydrogen bonds between the two DNA strands). The two separated single strands serve as the initiators for synthesizing two new complete double strands of DNA.

**2.1.2. Annealing.** DNA primers bind to their complementary sequences on the target DNA strands. The primers set the starting point for a DNA polymerase to initiate DNA polymerization, normally at *ca.* 48–52 °C. In a PCR test, the primers' sequence determines the selectivity and specificity of the assay. The primers should be fully complementary to the target DNA and have as little as possible cross-complementarity to other DNA (or cDNA) sequences from other organisms.

**2.1.3. Extension.** A DNA polymerase synthesizes two new complete DNA strands. This step is usually set for the optimal enzyme temperature, which in conventional PCR reactions is around 68–72 °C. A new cycle of PCR is, then, ready to begin with double the amount of DNA of the previous cycle. The cycles continue until the requisite quantity of DNA is obtained.

Since PCR requires a set of specific primers that are complementary to the target DNA sequence, the extension step will not proceed if the reaction's mixture lacks the desired target DNA. This led to the development of PCR tests. A “closed” question is asked (for example, if the DNA of a certain pathogen exists in a sample) and by using appropriate primers and other PCR components (*e.g.* DNA polymerase, dNTPs and salts), one can follow the DNA amplification process in real time or by endpoint analysis, and decide whether the pathogen's target nucleic acid existed in a sample. This led to quantitative PCR tests (qPCR) that can quantitatively determine the number of target (pathogen) nucleic acid copies in a sample.



**Fig. 1** The PCR reaction mechanism. A set of two primers anneal to the target DNA. A DNA polymerase extends the newly synthesized strand, and a new cycle begins. Each cycle doubles the number of DNA strands.

Most qPCR systems rely on the fluorescence of intercalating dyes that indicate the amplification of DNA, or on that of fluorescent probes that fluoresce only when the PCR takes place (TaqMan- Thermo Fisher Scientific Inc.). Prior to any amplification technique, nucleic acid molecules must be extracted from the tested sample. One of the drawbacks of PCR is the high temperatures required for strand separation, which led to the use of DNA polymerases from thermophilic bacteria.<sup>24</sup> However, this also requires relatively heavy instrumentation (thermocyclers), a fact that restricts its use at the POC. To develop PCR testing devices that can function at the POC, scientists and engineers have developed solutions that do not require thermocyclers. One solution, continuous flow PCR,<sup>25</sup> involves running the sample between different compartments with constant temperatures (one for denaturation, one for annealing, and another for extension) that allow PCR to proceed. Another way is to develop amplification techniques that operate at a constant temperature, *i.e.*, isothermal amplification. Several isothermal amplification techniques have become common practice and are found in several POC nucleic acid diagnostic systems. Important to mention that, still, PCR is superior to all isothermal amplification methods in terms of accuracy and sensitivity.

## 2.2 Isothermal amplification techniques

Here, we will discuss several isothermal amplification methods that can replace PCR amplification for use in POC devices.

**2.2.1 Loop-mediated isothermal amplification (LAMP).** Invented by Notomi and co-workers in 2000,<sup>26</sup> this reaction uses two or three sets of primers (which increase selectivity) that recognize six or eight distinct sequences on the DNA target, as well as a DNA polymerase with a high strand displacement activity (peeling off the complementary to the template strand while synthesizing a new strand). The amplification process normally occurs at 60–65 °C. Typically, 4 different primers are used to amplify 6 distinct regions on the target gene, which increases the specificity, Fig. 2. The amplification product can be visualized either by optical systems, which usually detect intercalating dyes or fluorescent probes or by naked eye, for example, by measuring the turbidity caused by magnesium pyrophosphate precipitation. Both approaches can lead to real-time measurements. The reaction can amplify 10<sup>9</sup> copies of the target DNA in less than an hour. Designing appropriate primers for LAMP is often a challenge and usually, specially designed software is used. Another issue with LAMP is multiplexing. Whereas in PCR the choice of primers for different pathogens is straightforward, the large number of primers required for LAMP adds complexity to the design and increases the likelihood of primer–primer dimers, which might result in false positives. Importantly, LAMP generates a series of concatemers of the target region, giving rise to a ladder pattern in the gel electrophoresis analysis rather than a single band generated by PCR. Most POC devices, presented here, that use isothermal amplification techniques, are LAMP-based. Although LAMP has shown potential for POC diagnostics thanks to recent developments with speed and electricity-

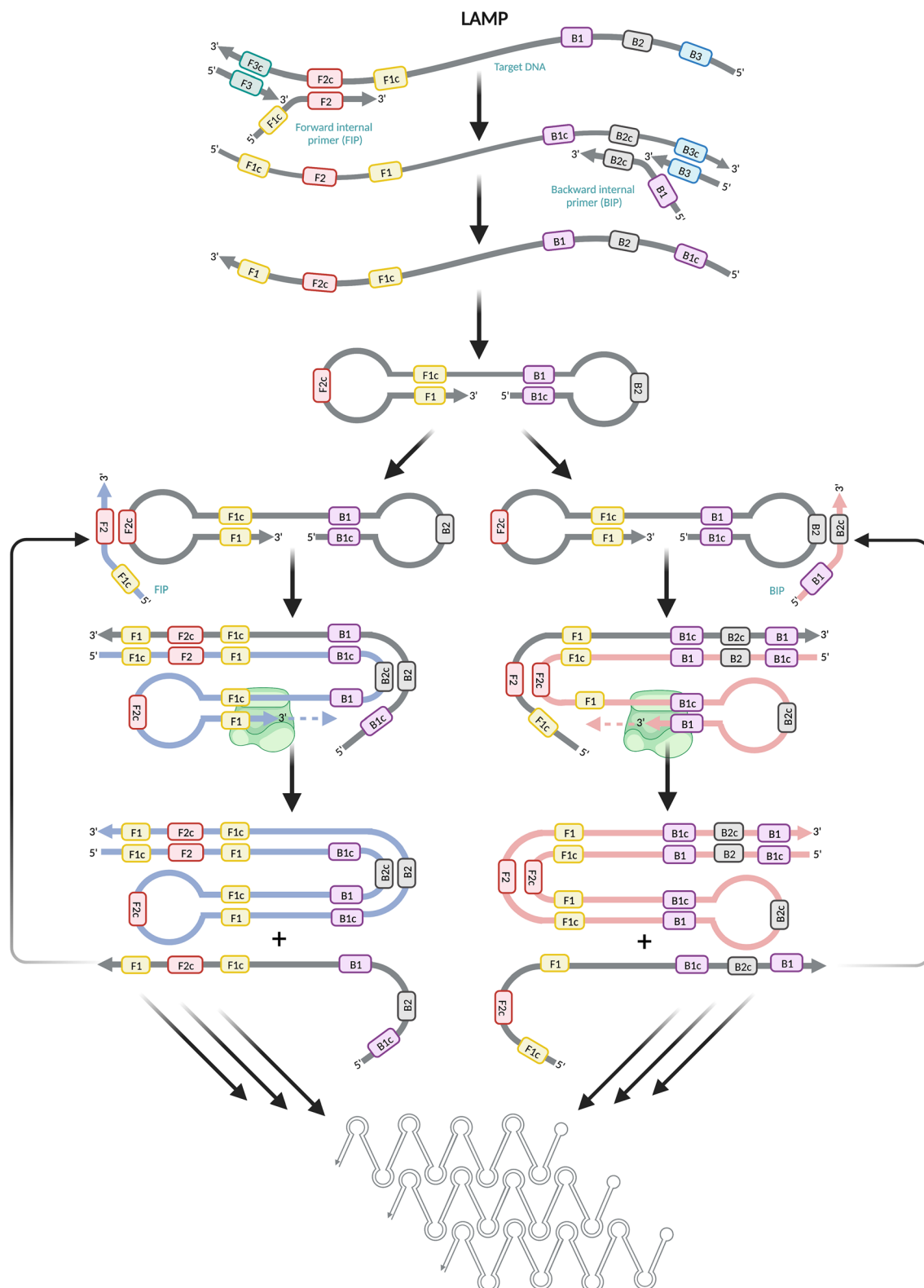
free systems, there remains some room for improvement when it comes to sample integration for microfluidics and POC friendly signal read-outs (unlike PCR which uses Taqman probes for real-time analysis, LAMP lacks this ability). Another disadvantage of LAMP is that increasing the number of primers raises the false positive rate. Sample integration for microfluidics and POC-friendly signal read-outs is also a challenge.<sup>27,28</sup> In 2017, Oloniniyi *et al.* used six primers to improve LAMP assay times (less than 20 min) for five different ebolaviruses using RT-LAMP<sup>29</sup> later, Nanayakkara *et al.*, demonstrated the ability to reduce a LAMP assay for the detection of MRSA by 15 min, by increasing the number of primers from four to six.<sup>30</sup> Recently, an RT-LAMP assay for the detection of SARS\_CoV\_2 from Yan *et al.*, that also utilized a six-primer system, was reported.<sup>31</sup>

**2.2.2 Helicase-dependent amplification (HDA).** First presented in 2009 by Jeong *et al.*, the helicase-dependent amplification utilizes the separation activity of helicase (and single-stranded binding proteins (SSBs)) to separate two hybridized strands of DNA, followed by primer annealing and amplification of the DNA template by a DNA polymerase.<sup>32</sup> The helicase unwinding activity replaces the heat-dependent dsDNA unwinding that is used in PCR. This system requires two primers for exponential amplification. The reaction temperature is commonly 65 °C. HDA assays for *Chlamydia trachomatis*, *Neisseria gonorrhoeae* and *Trichomonas vaginalis* were developed by the group of Klapperich.<sup>33–35</sup>

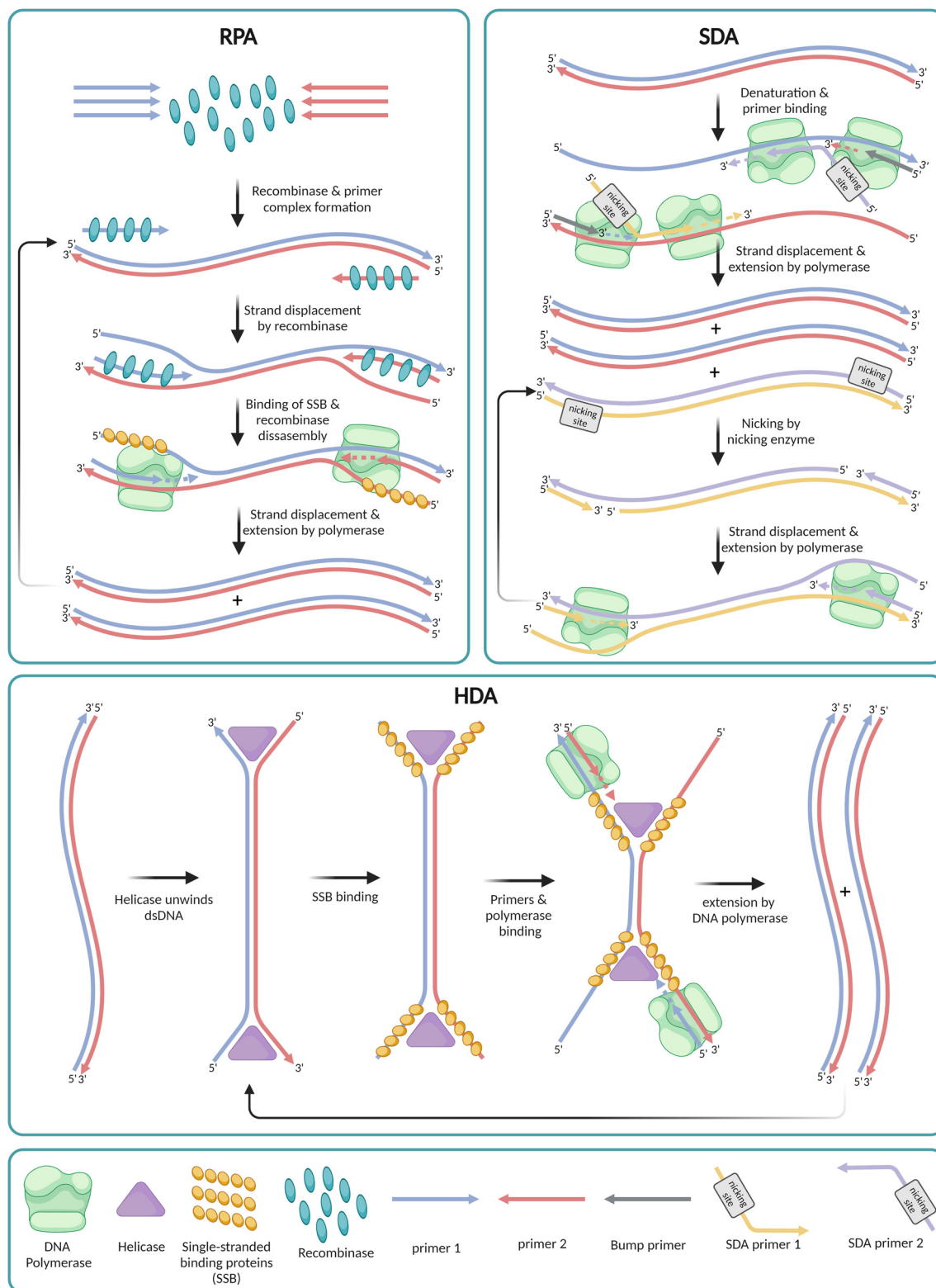
The application of two nucleic acid isothermal amplification methods—reverse transcription helicase dependent amplification (RT-HDA) and reverse transcription loop-mediated amplification (RT-LAMP), combined with a lateral flow assay, for the rapid detection of SARS\_CoV\_2, was presented by Zasada *et al.*<sup>36</sup> (Fig. 3).

**2.2.3 Strand displacement amplification (SDA).** SDA was first introduced in 1992 by Walker *et al.*<sup>37</sup> SDA utilizes a DNA polymerase with strand displacement activity and a nicking enzyme. A DNA primer is used as initiator for the polymerase amplification of complementary DNA strands. The primer includes a nicking site for the nicking enzyme that is used in the assay. DNA polymerase identifies the nicking site and starts a polymerization process while peeling off the hybridized strand (it polymerizes towards the 3' end of the newly synthesized strand, from the nicking site). The nick site is regenerated with any cycle of polymerization (after annealing of a new primer). This technology can result in exponential amplification, by designing two primers that hybridize to two complementary DNA strands. The enzymes that are used in this assay determine the appropriate temperature for it to proceed, normally at 37 °C. Usually, a pre-heating step for denaturation of the dsDNA is required (Fig. 3).

**2.2.4 Recombinase polymerase amplification (RPA).** In 2006, Armes and co-workers introduced an interesting solution to avoid temperature changes in order to separate two DNA strands and to allow primer annealing.<sup>38</sup> The recombination activity of a recombinase enzyme (and SSBs binding to the DNA) was used to anneal DNA primers. Recombinases can



**Fig. 2** Schematic representation of LAMP. The reaction mixture consists of dNTPs mix, a DNA polymerase, a fluorescent dye, primers and a DNA template. Six different regions on the dsDNA template are targeted by several primers. The reaction begins upon binding of the FIP to the F2c region and followed by polymerization of DNA by a DNA polymerase. F3 binds to its complementary region and replaces the newly synthesized DNA strand. Similarly, BIP and B3 operate as reverse primers. Displaced strands form dumbbell-shaped structures. FIP and BIP, then, bind to the dumbbell-shaped regions structures and form loops. Double-stem DNA loops are formed by elongation that is formed by a DNA polymerase and displace the original strand, which in turn, reform the dumbbell-shaped structures and undergo another round of amplification. FIP and BIP, then, initiate another strand displacement on the double-stem DNA loops. DNA structures of different lengths are amplified by repeated rounds of the reaction.



**Fig. 3** Isothermal amplification techniques. Top left, schematics of the RPA process. Primers and recombinases bind to the dsDNA template, recruit DNA polymerase with SSBs, and initiate DNA amplification. Top right, the SDA mechanism. DNA primers, nicking enzymes, and strand displacement polymerases amplify dsDNA. Bottom, the HDA reaction. Helicase unwinds dsDNA, followed by SSB binding, primer annealing, and DNA polymerization.

**Table 1** Summary of the isothermal amplification techniques

Amplification method	Single/multiple amplification products	Reaction temp. (°C)	Detection limit (copies)	Reaction time (minutes)	Advantages	Drawbacks
PCR	Single	95/48–52/ 68–72	1(Optimally, PCR identifies a single target copy)	20–60	<ul style="list-style-type: none"> <li>• Sensitivity</li> <li>• Accuracy</li> <li>• Exponential amplification</li> </ul>	<ul style="list-style-type: none"> <li>• Heavy equipment (thermocyclers)</li> </ul>
LAMP	Multiple	60–65	~10	30–60	<ul style="list-style-type: none"> <li>• Does not require thermocyclers</li> </ul>	<ul style="list-style-type: none"> <li>• Primer's design</li> <li>• Difficulty in multiplex amplification</li> </ul>
HDA	Single	65	~10	60–120	<ul style="list-style-type: none"> <li>• Does not require thermocyclers</li> <li>• Simple primer design</li> <li>• No initial heating steps</li> </ul>	<ul style="list-style-type: none"> <li>• High error rate</li> <li>• Expensive proteins and enzymes</li> </ul>
SDA	Single	37 (Usually requires initial heating 95)	~20	10–60	<ul style="list-style-type: none"> <li>• Does not require thermocyclers</li> <li>• Power saving (operates at a single low temperature)</li> </ul>	<ul style="list-style-type: none"> <li>• Nuclease selection is complex</li> </ul>
RPA	Single	37–42	~20	30–60	<ul style="list-style-type: none"> <li>• Does not require thermocyclers</li> <li>• Simple primer design</li> <li>• Power saving</li> </ul>	<ul style="list-style-type: none"> <li>• Inefficient in long sequences</li> <li>• Usually Requires initial heating</li> <li>• Expensive proteins and enzymes</li> </ul>

pair oligonucleotide primers with a homologous sequence in duplex DNA, which allows forward and reverse primers to anneal to the ends of the DNA template strands.<sup>39</sup> RPA can produce amplicons of 1 kb and typically it is performed at 37–42 °C. In recent years, RPA has become a promising technique for pathogen detection. Ma *et al.* developed a porcine delta coronavirus and an RPA detection system for the influenza A virus that required a short (20 minutes) amplification step,<sup>40,41</sup> (Fig. 3).

Table 1 summarizes the different isothermal amplification techniques. A few other isothermal amplification methods, such as Rolling Circle Amplification (RCA), Whole Genome Amplification (WGA), and nucleic acid sequence-based amplification (NASBA), also exist.<sup>42–44</sup>

### 3. Nucleic acid POC from sample to result diagnostic systems

Here, we will present a few state-of-the-art POC nucleic acid testing devices. All the devices presented here have been developed in the last couple of decades. We will discuss the biochemical reactions used for DNA amplification in each system, as well as the sensing element in each device. In addition, we will try to evaluate the advantages and drawbacks of each system. Some of the information presented here has been collected from commercial websites, some from scientific papers, and other information from published patents. All prices are as of October 2022.

#### 3.1 Lucira health

This Californian enterprise has developed the first US Food and Drug Administration (FDA) – approved “at home” end-

point SARS\_CoV\_2 nucleic acid test on a battery-operated device.<sup>45</sup> The Lucira SARS\_CoV\_2 kit utilizes reverse transcriptase (RT)-LAMP isothermal amplification and is highly accurate. For example, the SARS\_CoV\_2 test obtained 100% positive accuracy and 96.7% accuracy for negative results.<sup>45</sup> The heater of the system is also used for membranes rupture and release of nucleic acids. The SARS\_CoV\_2 test uses two internal controls: one to check the validity of the device and one to check for a human gene; the other 3 chambers check for SARS\_CoV\_2. The amplification is followed by color changes of halochromic agents, due to pH changes that result from nucleic acid amplification (DNA polymerization releases protons that change the pH). The device is an endpoint system and the results are qualitative. For the SARS\_CoV\_2 test, the results are expected within thirty minutes. As of October 2022, the price of a SARS\_CoV\_2 Case of 24 Single-Use Test Kits is \$1800. However, this device suffers from the drawbacks of LAMP (the amplification mechanism), such as lower sensitivity and specificity, compared with PCR. Moreover, the results are not obtained in real time.

#### 3.2 Cue health

Located in San Diego, USA, Cue has developed a hand-held POC self-test, nucleic acid diagnostic device.<sup>46</sup> Cue's technology involves an isothermal amplification (the isothermal technique is unspecified). According to a published patent,<sup>47</sup> it can be assumed that the amplification process includes biotin – and Horse Radish Peroxidase (HRP) – labeled primers. The biotin moiety is probably used to bind the amplicons to streptavidin-modified surfaces, and the HRP enzyme is used for colorimetric detection. The system is an endpoint qualitative (YES/NO) reporting system (it does not report in real time), and it is cartridge based (extraction, amplification, and detec-



tion); for the SARS\_CoV\_2 assay, the results are expected within *ca.* twenty minutes. Studies at the Mayo clinic, using the COVID-19 test, reported 97.8% accuracy *vs.* conventional PCR tests.<sup>48</sup> The system is supported by a mobile phone app and up to six devices can be connected simultaneously. The COVID-19 test is FDA approved and sold over the counter with no need for a prescription. Currently, the price of the reader and three COVID-19 test cartridges is \$444. The test is easy to perform with minimal training or previous laboratory testing experience; therefore, it can be considered as a feasible solution to implement at sites requiring a POC solution.<sup>48</sup>

### 3.3 Genedrive

Genedrive is a Manchester (UK)-based company that has developed a simple-to-use, portable, and robust POC nucleic acid diagnostic device for genotyping, pathogen detection, and other clinical applications.<sup>49</sup> The Genedrive system utilizes RT-LAMP isothermal amplification, and the output is qualitative (endpoint). The device is claimed to report positive results for SARS\_CoV\_2 within 7.5 minutes and negative results within 17 minutes. In a clinical study, the Genedrive COVID-19 RT-PCR test obtained a sensitivity of 98.2% and a specificity of 98.9%.<sup>50</sup> Several tests have been developed for this POC system; these include MTFRRN1 (Mitochondrially encoded 12S ribosomal RNA), COVID-19, and HCV (hepatitis C virus). Similar to the Lucira Health system, Genedrive's device uses LAMP, which is not as accurate and sensitive as PCR-based devices.<sup>51</sup>

### 3.4 Thermo Fisher (Mesa Biotech) Acculia

This portable endpoint automatic device was developed by Mesa Biotech, which was later sold to Thermo Fisher Scientific.<sup>52</sup> The system uses a single cassette for cell lysis, extraction, reverse transcription (for RNA viruses), and amplification of DNA by an oscillating amplification reaction (OSCAR), which was invented at Mesa Biotech.<sup>53</sup> This amplification methodology involves temperature oscillations of only ~15–20 °C around the primers' melting temperature, compared with conventional PCR systems that cycle between 55 and 95 °C. Thus, OSCAR is dramatically faster than common PCR amplification techniques and does not require as heavy an instrumentation suite. The total time for the SARS\_CoV\_2 qualitative assay is *ca.* thirty minutes. The detection involves a lateral flow strip (dye-labeled amplicons bind to surface-bound complementary strands through Watson & Crick base pairing). Acculia assays for Influenza A/B, respiratory syncytial virus (RSV), and Streptococcus A (Strep A) have already received section 510(k) clearance and a CLIA waiver from the FDA. The Acculia SARS\_CoV\_2 Test has been authorized for emergency use by the FDA in the USA only. According to the US FDA, the Acculia SARS\_CoV\_2 Test can sense as few as 475 nucleic acid detectable units (NDU) per ml, whereas most other FDA-approved sample-to-result tests have a detection limit of thousands of NDU per ml. Although the FDA SARS\_CoV\_2 Acculia's measured detection limit is extremely low, and it reached 100% positive and negative agreement with the EUA-authorized RT-PCR SARS\_CoV\_2 tests, there have been several SARS\_CoV\_2 false positive reports; conse-

quently, the FDA issued a recall.<sup>54</sup> As of October 2022, the price of the Acculia dock is \$371.5 and 9 cassettes for SARS\_CoV\_2 cost \$69.6. The Acculia system includes a line for the internal positive control, one for the internal negative process control, and one for the actual SARS\_CoV\_2 test. The system is miniature and fully portable.

In 2016 Abbott acquired the diagnostic giant – Alere for *ca.* \$5.8 billion and established Abbott Rapid Diagnostics.<sup>55</sup> Here we will discuss Abbot's ID NOW and m-PIMA, which were developed by Alere.

### 3.5 Abbott ID NOW

Formerly, Alere i, this is a rapid and portable (6.6 pounds) isothermal system for the qualitative detection of infectious diseases in 30 minutes or less.<sup>56</sup> This device includes a disposable cartridge that performs cell lysis, nucleic acid extraction, isothermal amplification, and qualitative (endpoint) detection of fluorescently labeled probes. The cartridge-based LAMP assay was approved by the FDA for emergency use authorization for rapid POC testing. Results are expected within 30 minutes. A study that tested the ID NOW COVID-19 assay *vs.* conventional RT-PCR test found that in both nucleic acid-based detection assays, ID NOW detected with a sensitivity and specificity of 87% and 98%, respectively.<sup>57</sup> Although the device showed comparable results to common RT-PCR systems, on May 14, 2020, the US FDA issued a public warning about potentially inaccurate results from using the ID NOW COVID-19 assay.<sup>58</sup> The inaccuracy of the ID NOW system was also detected in a study that compared it to Chepeid's Xpert system and found an accuracy (negative agreement) of 73.9% only, and a positive agreement of 34.3%, when the viral load is very low (Ct value <30).<sup>59</sup> As of October 2022, a pack of 24 COVID-19 tests costs \$2080.

### 3.6 Abbott m-PIMA

Formerly, Alere q, is another Abbott nucleic acid amplification diagnostic device.<sup>60</sup> This fully automated RT-PCR device for HIV testing is a benchtop system that delivers results within *ca.* 70 minutes. The m-PIMA™ HIV-1/2 VL test is now commercially available in selected countries and has received CE-IVD marking and WHO prequalification. However, the product is not available in the United States.<sup>60</sup> As of October 2022, 50 HIV tests cost \$1250. Abbott's m-PIMA™ performs well in a POC setting (compared to other POC devices), but it is typically more costly than the other systems and only comes as a single module instrument.<sup>61</sup>

### 3.7 DxNA Genestat.MDx

The POC GeneSTAT System (Utah, USA) is a testing device that focuses on infectious disease agents and antibiotic resistance.<sup>62</sup> This technology uses RT-PCR for amplifying the target nucleic acid. It is an automated, cartridge-based system. For low throughput needs (up to 3 targets), numerous Genestat devices can be operated in parallel by one laptop. The test kit includes all reagents for nucleic acid extraction and amplification. On March 2018, the Genestat system obtained FDA clear-



ance for SARS\_CoV\_2 and for Valley fever tests.<sup>63</sup> Being a PCR-based device, this is a very accurate system with 100% reproducibility. Further FDA clearance of additional clinical specimens, at a later date, would enhance the diagnostic capabilities of the assay.<sup>64</sup>

### 3.8 Visby Medical

Visby Medical (California, USA) developed a hand-held portable POC testing device.<sup>65</sup> Nucleic acids are PCR amplified. The SARS\_CoV\_2 test and the sexually transmitted diseases panel tests are FDA approved.<sup>66</sup> Results for the COVID-19 test are obtained within thirty minutes. The device accommodates a single disposable test cartridge that includes the reagents needed for cell lysis and nucleic acid extraction as well as for DNA amplification and detection. The detection is of an insoluble colored product. One disadvantage of this device is the generation of waste, which is an issue that should be addressed. The company is exploring recycling components to reduce the generated waste.<sup>67</sup>

### 3.9 Genomadix cube

This Canadian enterprise (formerly Spartan Bioscience)<sup>68</sup> developed a portable, PCR testing device for POC settings. The system is designed for SARS\_CoV\_2, Legionella, and the genetic CYP2C19<sup>69</sup> testings. The sensing technology is optical. The cube genetic testing assay revealed a sensitivity of ~99–100%.<sup>70</sup> The results are qualitative (endpoint). The need for thermocyclers for the PCR reaction limits further miniaturization of the device.

Table 2 summarizes the central parameters (the amplification technique, detection technology, time of the assay, accu-

racy of the test, device targets, and price) of the different devices mentioned above. All the devices are targeted to a test time of less than thirty minutes: five systems are PCR based, and three or four amplify the target nucleic acid by LAMP. An optical detection method is used in all systems described here.

## 4. Discussion

In this article, we discussed in detail 9 POC diagnostic devices that we found most interesting. There are, however, numerous other sample-to-result nucleic acid testing POC systems and other diagnostic systems for laboratory use that are worth mentioning. These systems include Cepheid Genexpert systems, Biomerieux's Biofire, Roche's cobas Liat, Qiagen's QIAstat Dx, DNAE's LiDia-seq platform, Oxford Nanopore Technologies' LAMPOR, HOLOGIC's Panther fusion, MolBio Truelab, NeuMoDx, and Quidel's Solana. Unlike heavy benchtop instruments that can analyze tens of samples in parallel, the POC devices, presented here, are designed to test only single or few samples at a time. The limiting factor regarding the number of tests per device depends mostly on the detection technology, which in all POC devices, presented here, is optical sensing; this is difficult to miniaturize more than has already been done in the existing systems. By using different detection technologies, such as nanopores or nanowires, further miniaturization can take place and hundreds or thousands of different PCR/isothermal tests could be performed simultaneously, using a hand-held device. In recent years, several scientific reports on portable POC nucleic acid assays have been published. An automated device that can sense 300 RNA

**Table 2** A summary of the main features of the different POC systems discussed in the manuscript

Device	Amplification	Detection method	Time (min)	Accuracy (and detection limit if published)	Tests	Price (\$) (as of October 2022)
Lucira	RT-LAMP	Color change due to pH change	≤30	96–98% Detection limit-1 copy per μl	SARS_CoV_2	Case of 24 single use test kits – 1800
Cue	Isothermal (not specified)	Horse Radish Peroxidase coloring	20	97.8%	SARS_CoV_2	Reader + 3 cartridges – 444
Genedrive	RT-LAMP	Fluorescent dye	≤30	98%	MT-RNRI; SARS_CoV_2; HCV	Unpublished
Accula (TFS)	OSCAR	Dye-labeled amplicons bind to a lateral flow strip	≤30	100%	Influenza B; RSV; Strep A	Dock – 371.5
ID NOW (Abott)	LAMP	Fluorescently labeled probes	13	Detection limit – 475 copies per ml 87–98%	SARS_CoV_2; Influenza A & B; RSV; Strep A 2	9 cassettes – 69.6
m-PIMA (Abott)	PCR/RT-PCR	Fluorescently labeled probes	70	95%	HIV (not in the USA)	Pack of 24 tests – 2080
DxNA Genestat	PCR/RT-PCR	Fluorescence	Less than 1.5 hours for the Coccidioides test	99.6–100% Detection limit – 10 genomic equivalent quantity (GEq) per ml	SARS_CoV_2; Valley fever; coccidioidomycosis	50 tests – 1250
Viby Med	PCR/RT-PCR	Insoluble colored product	≤30	95–100%	SARS_CoV_2	Unpublished
Genomadix cube	PCR/RT-PCR	Optical detection	~60	99–100%	SARS_CoV_2; CYP2C19; Legionella	Unpublished

copies per reaction of the SARS\_CoV\_2 virus, using the LAMP reaction in a single cartridge, and that can accommodate 104 saliva samples simultaneously, in 35 minutes, was reported by Deng *et al.*<sup>71</sup> Elsewhere, a SARS\_CoV\_2 sensing system that utilizes the LAMP reaction for accurate and sensitive detection (0.44–1.09 copies per  $\mu\text{l}$ ) was presented.<sup>72</sup> Another portable, rapid, on-cartridge device that uses magneto-fluidic purification and PCR testing for detecting *Neisseria gonorrhoeae* (a sexually transmitted disease) was developed.<sup>73</sup> The CRISPR technology, which has revolutionized molecular biology, has gained considerable attention in the development effort of diagnostic assays. In 2020, a rapid (<40 min), easy-to-implement, and accurate CRISPR-Cas12-based lateral flow assay for detecting SARS\_CoV\_2 from respiratory swab RNA extracts was developed by Broughton *et al.*<sup>74</sup>

Another CRISPR system, the Cas-12a-based system, which utilizes DNA functionalized with gold nanoparticles (AuNPs) for the amplification-free detection of cell-free DNA with high sensitivity and specificity in 30 minutes, was developed.<sup>75</sup> Elsewhere, a Cas12a naked-eye colorimetric detection system for SARS\_CoV\_2 was developed by Xie *et al.*<sup>76</sup> Cas 13 was also used for detecting bacterial nucleic acids in droplet microarrays.<sup>77</sup>

We foresee a dominant role for POC nucleic acid testing devices in the future. However, due to the lack of massive parallelism in the above-mentioned devices and due to the skilled expertise required for appropriate sample collection, there will still be a need for central diagnostic facilities that include heavy laboratory equipment. Agriculture is another field in which it might be easier to incorporate these POC testing devices. The POC devices mentioned above can be relatively easily transformed to sense plants and animals' pathogens as well as those of domestic pets. Among the variety of startups in this field, Sensona, Inc. (co-founded by one of the authors of this manuscript (Y. W.)) is especially interesting; it has been developing a POC PCR testing device that uses propriety gold nanoparticle technology for rapid heating and cooling of the sample. Importantly, it achieves a full PCR analysis in *ca.* 90 seconds, as opposed to tens of minutes in commonly used PCR devices. This dramatic shortening of the reaction time might enable a significant increase in the number of tests per instrument per day. We anticipate that in the near future, nucleic-acid POC diagnostic devices will become prevalent in private homes, clinics, and rural places.

## Conflicts of interest

There are no conflicts to declare.

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## References

- 1 Y.-W. Tang, G. W. Procop and D. H. Persing, *Clin. Chem.*, 1997, **43**, 2021–2038.
- 2 V. C. C. Cheng, W. W. Yew and K. Y. Yuen, *Eur. J. Clin. Microbiol. Infect. Dis.*, 2005, **24**, 711–720.
- 3 A. M. Caliendo, *Clin. Infect. Dis.*, 2011, **52**, S326–S330.
- 4 P. Khan, L. M. Aufdembrink and A. E. Engelhart, *ACS Synth. Biol.*, 2020, **9**, 2861–2880.
- 5 E. Giakoumaki, M. Minunni, S. Tombelli, I. E. Tothill, M. Mascini, P. Bogani and M. Buiatti, *Biosens. Bioelectron.*, 2003, **19**, 337–344.
- 6 T. Goda, M. Tabata and Y. Miyahara, *Front. Bioeng. Biotechnol.*, 2015, **3**, 29.
- 7 S. A. Bustin and R. Mueller, *Clin. Sci.*, 2005, **109**, 365–379.
- 8 S. C. Tan and B. C. Yiap, *J. Biomed. Biotechnol.*, 2009, **2009**, 574398.
- 9 W. K. Roth, *Transfus. Med. hemotherapy*, 2019, **46**, 67–75.
- 10 A. Manz, N. Graber and H. M. Widmer, *Sens. Actuators, B*, 1990, **1**, 244–248.
- 11 A. Niemi, T. M. Ferguson and D. S. Boyle, *Trends Biotechnol.*, 2011, **29**, 240–250.
- 12 E. van Pelt-Verkuil, A. van Belkum and J. P. Hays, *Principles and Technical Aspects of PCR Amplification*, Springer, Dordrecht, 1st edn, 2008.
- 13 O. G. Brakstad, K. Aasbakk and J. A. Maeland, *J. Clin. Microbiol.*, 1992, **30**, 1654–1660.
- 14 L. Yan, J. Zhou, Y. Zheng, A. S. Gamson, B. T. Roembke, S. Nakayama and H. O. Sintim, *Mol. Biosyst.*, 2014, **10**, 970–1003.
- 15 P. J. Asiello and A. J. Baeumner, *Lab Chip*, 2011, **11**, 1420–1430.
- 16 L. M. Zanolini and G. Spoto, *Biosensors*, 2013, **3**, 18–43.
- 17 M. L. Everitt, A. Tillery, M. G. David, N. Singh, A. Borison and I. M. White, *Anal. Chim. Acta*, 2021, **1146**, 184–199.
- 18 S. G. Beal, N. Assarzagdegan and K. H. Rand, *Expert Rev. Mol. Diagn.*, 2016, **16**, 323–341.
- 19 W. Y. Au and P. P. H. Cheung, *Lancet Microbe*, 2021, **2**, e704–e714.
- 20 S. A. El-Kafrawy, M. M. El-Daly, A. M. Hassan, S. M. Harakeh, T. A. Alandijany and E. I. Azhar, *Diagnostics*, 2022, **12**, 828.
- 21 Z. Tang, N. Kong, X. Zhang, Y. Liu, P. Hu, S. Mou, P. Liljeström, J. Shi, W. Tan, J. S. Kim, Y. Cao, R. Langer, K. W. Leong, O. C. Farokhzad and W. Tao, *Nat. Rev. Mater.*, 2020, **5**, 847–860.
- 22 Z. Luo, M. J. Y. Ang, S. Y. Chan, Z. Yi, Y. Y. Goh, S. Yan, J. Tao, K. Liu, X. Li, H. Zhang, W. Huang and X. Liu, *Research*, 2020, **2020**, 6925296.
- 23 R. K. Saiki, S. Scharf, F. Faloona, K. B. Mullis, G. T. Horn, H. A. Erlich and N. Arnheim, *Science*, 1985, **230**, 1350–1354.
- 24 NEB, Thermophilic DNA Polymerases, <https://international.neb.com/tools-and-resources/selection-charts/thermophilic-dna-polymerases>. (accessed October 2022).
- 25 M. U. Kopp, A. J. de Mello and A. Manz, *Science*, 1998, **280**, 1046–1048.

- 26 T. Notomi, H. Okayama, H. Masubuchi, T. Yonekawa, K. Watanabe, N. Amino and T. Hase, *Nucleic Acids Res.*, 2000, **28**(12), e63.
- 27 R. J. Meagher, A. Priye, Y. K. Light, C. Huang and E. Wang, *Analyst*, 2018, **143**, 1924–1933.
- 28 N. Kaur, N. Thota and B. J. Toley, *Comput. Struct. Biotechnol. J.*, 2020, **18**, 2336–2346.
- 29 O. K. Oloninyi, Y. Kurosaki, H. Miyamoto, A. Takada and J. Yasuda, *J. Virol. Methods*, 2017, **246**, 8–14.
- 30 I. A. Nanayakkara and I. M. White, *Analyst*, 2019, **144**, 3878–3885.
- 31 C. Yan, J. Cui, L. Huang, B. Du, L. Chen, G. Xue, S. Li, W. Zhang, L. Zhao, Y. Sun, H. Yao, N. Li, H. Zhao, Y. Feng, S. Liu, Q. Zhang, D. Liu and J. Yuan, *Clin. Microbiol. Infect.*, 2020, **26**, 773–779.
- 32 Y.-J. Jeong, K. Park and D.-E. Kim, *Cell. Mol. Life Sci.*, 2009, **66**, 3325–3336.
- 33 J. C. Linnes, A. Fan, N. M. Rodriguez, B. Lemieux, H. Kong and C. M. Klapperich, *RSC Adv.*, 2014, **4**, 42245–42251.
- 34 A. L. Horst, J. M. Rosenbohm, N. Kolluri, J. Hardick, C. A. Gaydos, M. Cabodi, C. M. Klapperich and J. C. Linnes, *Biomed. Microdevices*, 2018, **20**, 35.
- 35 J. M. Rosenbohm, J. M. Robson, R. Singh, R. Lee, J. Y. Zhang, C. M. Klapperich, N. R. Pollock and M. Cabodi, *Anal. Methods*, 2020, **12**, 1085–1093.
- 36 A. A. Zasada, E. Mosiej, M. Prygiel, M. Polak, K. Wdowiak, K. Formińska, R. Ziółkowski, K. Żukowski, K. Marchlewicz, A. Nowiński, J. Nowińska, W. Rastawicki and E. Malinowska, *Biomedicines*, 2022, **10**, 2329.
- 37 G. T. Walker, M. C. Little, J. G. Nadeau and D. D. Shank, *Proc. Natl. Acad. Sci. U. S. A.*, 1992, **89**, 392–396.
- 38 O. Piepenburg, C. H. Williams, D. L. Stemple and N. A. Armes, *PLoS Biol.*, 2006, **4**(7), e204.
- 39 H. Zaghoul and M. El-Shahat, *World J. Hepatol.*, 2014, **6**, 916–922.
- 40 L. Ma, F. Zeng, B. Huang, Y. Zhu, M. Wu, F. Xu, L. Xiao, R. Huang, J. Ma, F. Cong and P. Guo, *Transboundary Emerging Dis.*, 2019, **66**, 1324–1331.
- 41 S. Ma, X. Li, B. Peng, W. Wu, X. Wang, H. Liu, L. Yuan, S. Fang and J. Lu, *Biol. Pharm. Bull.*, 2018, **41**, 1804–1808.
- 42 M. M. Ali, F. Li, Z. Zhang, K. Zhang, D.-K. Kang, J. A. Ankrum, X. C. Le and W. Zhao, *Chem. Soc. Rev.*, 2014, **43**, 3324–3341.
- 43 NEB, Nucleic Acid Sequenced Based Amplification and Transcription Mediated Amplification, <https://international.neb.com/applications/dna-amplification-pcr-and-qpcr/isothermal-amplification/nucleic-acid-sequenced-based-amplification-and-transcription-mediated-amplification>. (accessed October 2022).
- 44 NEB, Whole Genome Amplification & Multiple Displacement Amplification, <https://international.neb.com/applications/dna-amplification-pcr-and-qpcr/isothermal-amplification/whole-genome-amplification-and-multiple-displacement-amplification>. (accessed October 2022).
- 45 Lucira Health, Lucira Health, <https://www.lucirahealth.com/>. (accessed October 2022).
- 46 Cue Health Inc., Cue Health, <https://cuehealth.com/>. (accessed October 2022).
- 47 A. Khattak, C. Sever, P. Nelson, R. Cooper, T. Congdon, J. Demartino, R. Shapiro and M. Duncan, *US pat*, US9962703B2, 2017.
- 48 L. J. Donato, V. A. Trivedi, A. M. Stransky, A. Misra, B. S. Pritt, M. J. Binnicker and B. S. Karon, *Diagn. Microbiol. Infect. Dis.*, 2021, **100**, 115307.
- 49 Genedrive, Genedrive, <https://www.genedriveplc.com/>. (accessed October 2022).
- 50 M. D. N. News, Genedrive's Covid-19 test kit obtains CE-IVD certification, [https://www.medicaldevice-network.com/news/genedrive-covid-19-test-kit-ce-ivd/?utm\\_source=ArmyTechnology&utm\\_medium=website&utm\\_campaign=MustRead&utm\\_content=Image](https://www.medicaldevice-network.com/news/genedrive-covid-19-test-kit-ce-ivd/?utm_source=ArmyTechnology&utm_medium=website&utm_campaign=MustRead&utm_content=Image). (accessed October 2022).
- 51 M. W. McCarthy, *Expert Rev. Mol. Diagn.*, 2021, **21**, 1–2.
- 52 Thermo Fisher Scientific - Accula Rapid PCR Testing Success Stories, [https://www.thermofisher.com/il/en/home/clinical/diagnostic-testing/point-of-care/accula-rapid-pcr-system/accula-rapid-pcr-testing-successstories.html?cid=0se\\_gaw\\_06072022\\_CYFU2K&ef\\_id=Cj0KCQjwxb2XBhDBARiAOjDZ34ahNNXC9k6rE-JdhJyMgWsuDD7P9d2e7qV9QuGUZHOM](https://www.thermofisher.com/il/en/home/clinical/diagnostic-testing/point-of-care/accula-rapid-pcr-system/accula-rapid-pcr-testing-successstories.html?cid=0se_gaw_06072022_CYFU2K&ef_id=Cj0KCQjwxb2XBhDBARiAOjDZ34ahNNXC9k6rE-JdhJyMgWsuDD7P9d2e7qV9QuGUZHOM). (accessed October 2022).
- 53 H. Cai and N. J. Cobb, *US pat*, US9428781B2, 2012.
- 54 FDA, Mesa Biotech, Inc., Recalls Certain Accula SARS-CoV-2 Tests for Risk of False Positives Caused by Contamination, <https://www.fda.gov/medical-devices/medical-device-recalls/mesa-biotech-inc-recalls-certain-accula-sars-cov-2-tests-risk-false-positives-caused-contamination>. (accessed October 2022).
- 55 Abbott, Abbott to acquire Alere, becoming leader in point of care testing and significantly advancing global diagnostics presence, <https://abbott.mediaroom.com/2016-02-01-Abbott-to-Acquire-Alere-Becoming-Leader-in-Point-of-Care-Testing-and-Significantly-Advancing-Global-Diagnostics-Presence>. (accessed October 2022).
- 56 Abbott, ID NOW, <https://www.globalpointofcare.abbott/en/product-details/id-now.html>. (accessed October 2022).
- 57 S. L. Mitchell and K. S. George, *J. Clin. Virol.*, 2020, **128**, 104429.
- 58 FDA news release, Coronavirus COVID-19, *Update: FDA Informs Public About Possible Accuracy Concerns with Abbott ID NOW Point-of-Care Test*, <https://www.fda.gov/news-events/press-announcements/coronavirus-covid-19-update-fda-informs-public-about-possible-accuracy-concerns-abbott-id-now-point> (Accessed October 2022).
- 59 M. C. Smithgall, I. Scherberkova, S. Whittier and D. A. Green, *J. Clin. Virol.*, 2020, **128**, 104428.
- 60 Abbott, m-PIMA™ ANALYSER, <https://www.globalpointofcare.abbott/en/product-details/m-pima-analyser.html>. (accessed October 2022).
- 61 S. J. Girdwood, T. Crompton, M. Sharma, J. Dorward, N. Garrett, P. K. Drain, W. Stevens and B. E. Nichols, *EclinicalMedicine*, 2020, **28**, 100607.
- 62 GeneSTAT, <https://genestatdiagnostics.com/the-genestat-system/>. (accessed October 2022).



- 63 FDA Clears First Molecular Test for Valley Fever, <https://www.aacc.org/clin/articles/2018/march/fda-clears-first-molecular-test-for-valley-fever>. (accessed October 2022).
- 64 M. A. Saubolle, B. R. Wojack, A. M. Wertheimer, A. Z. Fuayagem, S. Young and B. A. Koeneman, *J. Clin. Microbiol.*, 2018, **56**(2), DOI: [10.1128/JCM.01277-17](https://doi.org/10.1128/JCM.01277-17).
- 65 Visbymeical, <https://www.visbymeical.com/>. (accessed October 2022).
- 66 V. Medical, Visby Medical™ Receives FDA Clearance and CLIA Waiver at the Point of Care for PCR Sexual Health Test, <https://www.visbymeical.com/news/visby-medical-receives-fda-clearance-and-clia-waiver-at-the-point-of-care-for-pcr-sexual-health-test/>. (accessed October 2022).
- 67 A. Renzoni, F. Perez, M. T. Ngo Nsoga, S. Yerly, E. Boehm, A. Gayet-Ageron, L. Kaiser and M. Schibler, *Diagnostics*, 2021, **11**, 813.
- 68 Genomadix, <https://genomadix.com/the-genomadix-cube/>. (accessed October 2022).
- 69 N. L. Pereira, M. E. Farkouh, D. So, R. Lennon, N. Geller, V. Mathew, M. Bell, J.-H. Bae, M. H. Jeong, I. Chavez, P. Gordon, J. D. Abbott, C. Cagin, L. Baudhuin, Y.-P. Fu, S. G. Goodman, A. Hasan, E. Iturriaga, A. Lerman, M. Sidhu, J.-F. Tanguay, L. Wang, R. Weinshilboum, R. Welsh, Y. Rosenberg, K. Bailey and C. Rihal, *J. Am. Med. Assoc.*, 2020, **324**, 761–771.
- 70 J. D. Roberts, G. A. Wells, M. R. Le May, M. Labinaz, C. Glover, M. Froeschl, A. Dick, J.-F. Marquis, E. O'Brien, S. Goncalves, I. Druce, A. Stewart, M. H. Gollob and D. Y. F. So, *Lancet*, 2012, **379**, 1705–1711.
- 71 H. Deng, A. Jayawardena, J. Chan, S. M. Tan, T. Alan and P. Kwan, *Sci. Rep.*, 2021, **11**, 15176.
- 72 G. Xun, S. T. Lane, V. A. Petrov, B. E. Pepa and H. Zhao, *Nat. Commun.*, 2021, **12**, 2905.
- 73 A. Y. Trick, J. H. Melendez, F.-E. Chen, L. Chen, A. Onzia, A. Zawedde, E. Nakku-Joloba, P. Kyambadde, E. Mande, J. Matovu, M. Atuheirwe, R. Kwizera, E. A. Gilliams, Y.-H. Hsieh, C. A. Gaydos, Y. C. Manabe, M. M. Hamill and T.-H. Wang, *Sci. Transl. Med.*, 2021, **13**, 593.
- 74 J. P. Broughton, X. Deng, G. Yu, C. L. Fasching, V. Servellita, J. Singh, X. Miao, J. A. Streithorst, A. Granados, A. Sotomayor-Gonzalez, K. Zorn, A. Gopez, E. Hsu, W. Gu, S. Miller, C.-Y. Pan, H. Guevara, D. A. Wadford, J. S. Chen and C. Y. Chiu, *Nat. Biotechnol.*, 2020, **38**, 870–874.
- 75 J.-H. Choi, J. Lim, M. Shin, S.-H. Paek and J.-W. Choi, *Nano Lett.*, 2021, **21**, 693–699.
- 76 S. Xie, D. Tao, Y. Fu, B. Xu, Y. Tang, L. Steinaa, J. D. Hemmink, W. Pan, X. Huang, X. Nie, C. Zhao, J. Ruan, Y. Zhang, J. Han, L. Fu, Y. Ma, X. Li, X. Liu and S. Zhao, *ACS Synth. Biol.*, 2022, **11**, 383–396.
- 77 S. G. Thakku, C. M. Ackerman, C. Myhrvold, R. P. Bhattacharyya, J. Livny, P. Ma, G. I. Gomez, P. C. Sabeti, P. C. Blainey and D. T. Hung, *PNAS Nexus*, 2022, **1**(1), DOI: [10.1093/pnasnexus/pgac021](https://doi.org/10.1093/pnasnexus/pgac021).