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Review

A critical review of point-of-care diagnostic technologies to combat viral pandemics

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HIGHLIGHTS

- Despite decades of research, no products can meet the testing needs of a pandemic.
- We propose new guidelines for the design of point-of-care tests for pandemics.
- We summarize the literature on point-of-care diagnostics for pandemics.
- We find that sample preparation steps are the primary hindrance to deployment.
- We provide a list of recommended research topics to prepare for future pandemics.

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ABSTRACT

The COVID-19 global pandemic of 2019–2020 pointedly revealed the lack of diagnostic solutions that are able to keep pace with the rapid spread of the virus. Despite the promise of decades of lab-on-a-chip research, no commercial products were available to deliver rapid results or enable testing in the field at the onset of the pandemic. In this critical review, we assess the current state of progress on the development of point-of-care technologies for the diagnosis of viral diseases that cause pandemics. While many previous reviews have reported on progress in various lab-on-a-chip technologies, here we address the literature from the perspective of the testing needs of a rapidly expanding pandemic. First, we recommend a set of requirements to heed when designing point-of-care diagnostic technologies to address the testing needs of a pandemic. We then review the current state of assay technologies with a focus on isothermal amplification and lateral-flow immunoassays. Though there is much progress on assay development, we argue that the largest roadblock to deployment exists in sample preparation. We summarize current approaches to automate sample preparation and discuss both the progress and shortcomings of these developments. Finally, we provide our recommendations to the field of specific challenges to address in order to prepare for the next pandemic.

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1. Introduction

Despite the tremendous advances in medical technology in the last 100 years, our world is still vulnerable to pandemics. To mitigate the spread of a viral outbreak, epidemic, or pandemic, contact tracing (identifying individuals who have recently come into contact with infected individuals) is critical to facilitate self-quarantine and accurate epidemiological records as well as other preventative or diagnostic measures [1,2]. However, contact tracing only hinders viral transmission if sufficient testing is available and accessible [3,4]. Densely populated regions present a particular challenge, not only because of the close living and working conditions of inhabitants and the low number of hospital beds per capita, but also because of the difficulty of rapidly deploying diagnostic tests for a significant fraction of the population [5]. The rampant spread of COVID-19 disease (caused by the SARS-CoV-2 virus) in the United States in 2020 illustrates this observation. During the rapid expansion of the pandemic across the US, testing availability was far below the demand, often causing delays in testing results of up to several weeks as well as leaving too many patients untested. As a result, the virus spread rapidly, largely unchecked. To brace against future pandemics, it is clear that the world needs better testing capabilities [6].

Today, the vast majority of diagnostic tests for viral pathogens are performed in reference labs or large commercial labs. While the test itself is brief, the need to ship the sample to the centralized facility, where it may wait in a long queue for testing (due to the low number of testing labs per capita), implies that results are generally delivered to patients and public health officials within days instead of minutes. Over the course of 2020 a number of instruments and corresponding tests received waivers from the United States Food and Drug Administration (US FDA) to operate outside of a central lab. However, individual tests are prohibitively expensive, and each instrument is only able to process one test at a time, thus keeping throughput inadequate for fighting a widespread pandemic.

Despite decades of R&D on point-of-care (PoC) diagnostics prior to the COVID-19 pandemic, including broad efforts by the microfluidics community, a solution capable of large-scale deployment and rapid results has not been achieved. We believe that this is partially due to incorrect perceptions of the key requirements for PoC testing, and in particular the requirements for addressing a pandemic. Many research efforts have operated under the assumption that microfluidics implies PoC or that the employment of low-cost materials, such as paper, are sufficient to lead to PoC implementation and large-scale deployment. However, the vast research in these areas has not led to a viable solution to address testing needs during a pandemic. Instead of following these technological dogmas, we instead propose the following requirements for diagnostic tests in order to combat pandemics:

- **Sample-To-Answer**: single step, such that the tester only needs to insert the raw sample to receive the result.
- **Rapid** (assay time to answer, assay development time): result can be delivered to the patient before they leave the test site; new tests can be developed rapidly in response to emerging infectious agents.
- **Local**: performed at the patient testing site.
- **Inexpensive**: low per-test cost such that densely populated or urban regions can afford to test a significant fraction of their population.
- **Throughput**: high throughput, enabling tests to be conducted simultaneously and asynchronously on a high number of individuals with modest Equipment requirements.

Our STARLITE recommendations apply specifically to a pandemic response. More generalized guidelines for PoC infectious disease diagnostics have been put forth by the World Health Organization (WHO) using the acronym ASSURED—affordable,
sensitive, specific, user friendly, rapid and robust (no refrigeration required), equipment-free, and deliverable to those in need (Table 1) [7]. Since the advent of ASSURED, there have been suggested augments to keep the requirements up to date with current technology. For instance, Land et al., using the acronym REASSURED, added the need for real-time connectivity to increase the transmission of test data, increasing the ease of sample collection, and increasing the environmental friendliness [8]. The US FDA has established a similar set of guidelines that must be met for a diagnostic to be used outside of a certified central lab (i.e., the Clinical Laboratory Improvement Amendment – CLIA (Table 2)) [9]. On top of the guidelines in ASSURED, the FDA expands upon the “user friendly” term to include minimal required training, no precise measurements, no user interpretations, and no user intervention steps. While many research groups have been inspired by the WHO guidelines, the “user friendly” guideline is often under-appreciated, and thus sample-to-answer methods are rarely reported in the literature. At the same time, commercial solutions have not properly addressed the affordable and equipment-free aspects of the guidelines.

Consider how our proposed STARLITE criteria are aligned with the needs of massive testing in urban areas, and how the current commercial solutions are not aligned with these needs. One model of massive-scale testing in an urban region is illustrated clearly by the deployment of drive-through screening [10], in which patients stay in their cars, become eligible for testing via a questionnaire, and are then swabbed as they drive by technicians. Because of today’s limited testing capabilities, swabs are rationed to eligible patients despite evidence of asymptomatic transmission [11]. For those who are tested, swabs are compiled and shipped to large commercial or reference labs, where they wait in a queue. Thus, instead of delivering results immediately on-site to the patient and health care officials, results are not returned for days or weeks. This delay is significant for contact tracing, as it allows an extended period for those who may have been infected by the patient to further spread the virus. In addition, as observed directly by one of the authors (AT) who worked at a testing site, many of those tested are difficult to contact after they leave the drive-through testing due to lack of email address or reliable phone number. Moreover, patients without an email address must return to the testing site to retrieve a hard copy of the results, thus breaking quarantine and delaying the case fatality ratio [20,23]. Most recently, in 2019–2020, the World Health Organization declared COVID-19 a pandemic of global concern, and it is suspected to have caused at least one million deaths worldwide, significantly higher than the more recent, H1N1pdm09 Flu Pandemic (of swine origin) in 2009, which took the lives of an estimated 151,700 to 575,400 people worldwide [16].

Unlike influenza viruses whose mortality typically fall under 0.1% of all infected cases, Ebola virus disease (EBVD) has mortality rates upwards of 50% in the case of the Zaire ebolavirus (one of four ebolaviruses transmissible to humans) [17,18]. Generally limited to sub-Saharan Africa, outbreaks of EBVD have been a periodic occurrence since its discovery in 1976, often spiking at a few hundred cases. In 2014, however, a large epidemic of Zaire ebolavirus spread rapidly from Guinea to the surrounding region [18,19]. A vaccine against Zaire ebolavirus and several antiviral treatments were developed to curb the spread, which eventually infected over 28,600 and killed 11,325 people [17,18].

Over the last two decades, several varieties of betacoronaviruses have become of increasing potential pandemic concern. Beginning in 2003, a short outbreak of a Severe Acute Respiratory Syndrome (SARS) virus (of suspected horseshoe bat origin) emerged in China and quickly spread to 31 countries [20–22]. SARS infected 8098 and killed 774 worldwide and was determined to have an 11% global case fatality ratio [20,23]. Most recently, in 2019–2020, the world has been assailed by a new coronavirus, SARS-CoV-2 (of suspected bat origin) first identified in Wuhan, China [24]. At the time of

Table 2
FDA CLIA-waiver criteria.

|   | Knowledge | minimal technical knowledge |
|---|-----------|-----------------------------|
| 1 | Training  | minimal training required   |
| 2 | Reagent preparation | stable reagents and minimal preparation |
| 3 | Operational steps | no manual steps required   |
| 4 | Calibration materials | stable calibration materials |
| 5 | Troubleshooting | troubleshooting is automatic or requires minimal judgement |
| 6 | Interpretation | minimal interpretation of results |
While responses to the virus have varied across countries, the scientific endeavours to end the crisis have been relentless and informed by oversights in past pandemics [26]. Perhaps most notably, the genome of SARS-CoV–2, which was quickly sequenced and compared with that of SARS-CoV, has increased our understanding of the viral mechanics and has sped up production of diagnostics, proposed treatments, and vaccines [27].

While this review focuses on pandemics, the lessons learned from diagnostics of influenza, EBVD, and SARS/COVID can be applied to many other dangerous viruses, including HIV, Hepatitis C, and tropical viruses, such as Zika virus. Given the prevalence of the diseases associated with these viruses in low-middle income countries, there is a clear need for a field-deployable and low-cost solution, though the requirements may differ from the STARLITE recommendations that we are proposing for pandemics. However, the benefit to providing a diagnostic result upon first patient contact is consistent across all these viruses.

3. Today’s commercial tools do not combat pandemics

3.1. Nucleic acid amplification tests

The current gold-standard for diagnosing a viral infection is reverse-transcriptase polymerase chain reaction (RT-PCR), which directly detects the presence of the virus’s RNA genome in the sample taken from the patient. The steps of the process are diagrammed in Fig. 1. In this method, the sample is added to a lysis buffer, which releases the RNA from the viral capsid. The RNA is then isolated using a multistep process in which it is solid substrate or magnetic capture beads, washed, and then eluted into the RT-PCR reaction mixture. In the RT-PCR reaction, reverse transcriptase copies all RNA into complementary DNA (cDNA) (for viruses with DNA genomes, this reverse transcriptase step is skipped). Finally, quantitative PCR is run on the cDNA. In this reaction, DNA primers bind to the specifically targeted unique sequence that is used to identify the particular virus, which enables DNA polymerase to make a copy by extending the primers; this reaction is cycled repeatedly, resulting in expansion of the targeted sequence at an exponential rate. Reaction cycling is driven by temperature cycles for annealing of DNA primers, extension of primers, melting of DNA strands, annealing, etc. For quantification, a real-time fluorescence signal is used to determine a time point when a signal threshold is crossed; this time point is directly correlated to the number of cDNA copies in the initial reaction.

Progress in instrumentation and reagents have made this procedure relatively automated and fast. Instruments have been developed that perform automatic RNA extraction from multiple samples. Reagent kits are available that combine the multi-step RT-PCR assay into a single step through the “hot-start” procedure, which sequesters the PCR reagents until the RT reaction is completed. Combined with high-throughput robotic sample handling, thousands of samples can be processed each day by one set of instruments. However, because of the size, sophisticated user steps, and cost of this instrumentation, these capabilities are limited to large reference and commercial clinical labs, implying that results are returned days after the initial specimen collection, i.e., after the initial contact between patient and health-care official.

Challenges exist in transitioning RT-PCR to a PoC platform that meets our proposed STARLITE criteria, as well as the ASSURED criteria and/or can receive a CLIA waiver. One challenge is...
eliminating the multiple precise steps of the RNA isolation and addition to the reaction. A second challenge is eliminating the costly instrumentation requirements for thermocycling that is required by PCR. Currently there are three platforms that have received a CLIA waiver from the US FDA to diagnose various respiratory infections in the setting of a clinician’s office. In addition, these three systems have received Emergency Use Authorization (EUA) from the US FDA for the diagnosis of COVID-19.

The Cepheid GeneXpert is a modular platform that enables asynchronous testing in individual samples and has received a CLIA waiver for both influenza and RV. The system is cartridge-based such that the cartridge contains the materials and reagents for nucleic acid purification and amplification. The sample (typically in viral transport media) is loaded into the cartridge without the need for precise pipetting. Within the cartridge, virus particles are lysed, RNA is purified, and then the targeted RNA sequences are amplified with RT-PCR, all without the need for precise reagent transfers or interventions from the user. Cepheid states that the hands-on time is less than 60 s and the time to result is less than 30 min. Several reports in the literature have assessed the performance of the Cepheid system [26–31], all confirming the brief hands-on time. The literature shows a trend of decreasing assay time, with recent publications valuing an approximate 30-min sample-to-answer claim [31]. In this work, Valentin et al. tested over 300 nasopharyngeal swabs from patients in an emergency department and measured a sensitivity of 98.2% and a specificity of 97% for influenza A/B. Cohen et al. tested swabs for influenza A/B, as well as RV in emergency departments, outpatient clinics, and urgent care clinics, thus confirming its use in settings outside of the central lab [30]. Sensitivities ranged from 97% to 100% for the three viruses, while specificity ranged from 95% to 100%.

The Roche Cobas Lab-in-a-tube (LiAt) PCR system has received a CLIA waiver, CE-IVD mark, and is FDA 510(k) cleared for influenza A/ B (without virus-A subtypes) and RV. Its use requires that individual nasopharyngeal swab samples be submersed in 2 mL virus transport medium and 200 μL of specimen be pipetted into a single-use assay tube. Once the tube is inserted into the system, the processing of the sample is fully automated — including nucleic acid extraction and purification followed by PCR amplification and detection. Roche states that the overall time required to process a sample is 20 min (5 min of handling and 15 min of processing). Several studies [30,32–38] have assessed and compared the reliability of the Roche Cobas LiAt against similar competing devices and concur on its fast and easy-to-use aspects. Adults tested in a “real-world” clinical setting [37] yielded the lowest values of sensitivity (influenza A: 83.0%, influenza B: 84.6%, RV: 77.8%) in the literature. Other reports in more classical diagnostic settings generally reported high sensitivity (influenza A: 96.2%–100%, influenza B: 94.4%–100%, and RV: 96.8%–100%). Specificities for all diseases were consistently high, averaging as follows: influenza A: 99.3%, influenza B: 99.6%, RV: 99.3%. Regarding studies on SARS-CoV-2 testing [39,40], results were limited by small sample sets, lack of gold-standard comparison, and limited understanding of the virus and COVID-19 infection, among other hurdles. Nevertheless, these studies showed an average sensitivity of 94.6% and specificity of 99.8%.

Abbott Laboratories’ ID NOW is a CLIA-waived tabletop device designed as a rapid detection sample-to-answer assay for the detection of certain viruses, including influenza A/B and more recently, SARS-CoV-2. As opposed to the Liat and GenExpert, the ID NOW uses an isothermal amplification method based on strand displacement amplification (SDA) [41]. After two sample containment units are loaded into the ID NOW machine and heated, a nasal, throat, or nasopharyngeal swab sample is stirred in the pre-warmed elution/lysis buffer for 10 s, after which the unit is sealed, and the nucleic acid amplification begins. The ID NOW for influenza A/B has been shown to be effective at diagnosing both influenza A and B, offering a quick turnaround of fewer than 13 min, or 5 min in the slightly less specific “early callout” mode. It is able to consistently achieve sensitivities at and above 95% and is overall considered to be one of the most effective tests for influenza A and B [42–44]. When adapted for SARS-CoV-2 detection, the ID NOW test continues to require less than 13 min, but only achieves overall sensitivity of 73.9%, which drops to only 34.3% for low viral load cases [45]. These low sensitivities likely result from incompatibility between the ID NOW system and the typical workflow for COVID-19 diagnostics, where viral transport media serves as the sample [46,47].

These three systems represent excellent progress towards bringing diagnostics closer to the patient. They can confirm influenza, RV, and other common infections in the doctor’s office or walk-in clinic. However, these systems do not have the low per-test cost or throughput needed to deliver massive testing in the field to respond to a pandemic. Each instrument only processes one test at a time, requiring a high number of instruments to be deployed at testing sites such as drive-through locations. Further progress is needed to optimize testing in response to a pandemic, in particular, the elimination of costly equipment and a significant increase in testing throughput while maintaining sample-to-answer capabilities.

3.2. Antigen tests

An alternative to nucleic acid amplification tests is detecting the presence of the virus through specific proteins on or in the virus, i.e., antigens. Although this appeals to PoC settings as they can operate at ambient temperatures and require no RNA purification step, the lack of exponential amplification can hinder detection limits. However, viruses do contain many copies of some antigen biomarkers, which can somewhat make up for this lack of amplification.

Quidel has developed a fluorescent immunoassay based on lateral-flow technology called Sofia to detect a variety of viruses including RV, influenza A, influenza B, and most recently, SARS-CoV-2. Sun et al. discussed the performance of the Sofia against another rapid antigen test, BinaxNOW [48], and determined Quidel’s assay outperformed its competitor in terms of detection limit when detecting RV. Sofia also provides rapid results (15 min) and a long shelf life (2 years) with no refrigeration required, appealing to PoC settings in particular. As compared to conventional lateral flow immunoassays (LFIA s), the Sofia uses a fluorescence readout with a powerful camera to enable its limit of detection to be comparable with nucleic acid amplification.

Since the release of the Sofia, a few additional antigen tests have received an EUA from the US FDA. Because they have been released recently, these tests do not yet have independent and peer-reviewed assessments in the literature. Thus, we describe them here but do not provide quantitative performance values.

Similar to the Sofia, BD’s Veritor is a LFA, providing rapid results in about 15 min, while reporting competitive detection limits. However, like the Sofia, this antigen test uses a fluorescence reader, which does not align with testing during a pandemic as it requires an expensive piece of equipment and has a limited throughput as each device can only perform one test at a time. The LumiraDx SARS-CoV-2 antigen test is a microfluidic system that can be performed in about 12 min, with detection limits comparable to the aforementioned LFIA s. Again, in order to get these competitive detection limits, LumiraDx requires the use of a fluorescence reader, which only reads one chip at a time and thus does not meet the proposed STARLITE criteria for pandemics due to the high cost and low throughput.
An additional antigen test for COVID-19, from Abbott, has received an EUA from the FDA. Similar to the assays described above, this LFIA requires the patient to use a nasal swab. The user drops extraction buffer into a hole on the well of the device before inserting the swab. After closing the device, the results are available in 15 min, a comparable assay time. However, uniquely, this test does not require the use of an instrument to reach a competitive detection limit. Because this test does not require an instrument to receive the diagnosis, a high number of tests can be processed quickly (e.g., simply by taking photos with a tablet); thus, there is a potential to integrate it into the workflow of a large-scale testing center, such as a drive-through test site, while providing the patients with their results before leaving the facility.

Although these new antigen tests are promising, particularly the instrument-free solution, about 30 million people were infected and about 900,000 people died before the Abbott system became available, illustrating the drawback of simply relying on antigen tests. Contrastingly, RT-PCR tests can be developed within about a week of the sequencing of the pathogen, suggesting that nucleic acid amplification tests should continue to be considered as the first line of defense for future pandemics.

4. Assay methodologies under investigation

4.1. Isothermal nucleic acid amplification

Due to the strict thermal requirements of traditional RT-PCR, newer isothermal amplification methods have been developed to both reduce the complexity and cost associated with the rapid cycling temperatures, while also speeding up the assay. However, thermal cycling is challenging to eliminate. In particular, the melt step in PCR enables the newly polymerized double strand to separate, allowing the primers to bind during the annealing step. Isothermal amplification methods inherently eliminate high temperatures for denaturation and thus instead must rely on alternative strategies for primer binding. This section illustrates the progress made regarding these isothermal amplification strategies, while also highlighting the limitations, particularly in the context of pandemics. The primer sequences used in the amplification schemes discussed in this review can be found in the Electronic Supplementary Materials.

4.1.1. Reverse transcription loop-mediated isothermal amplification (RT-LAMP)

LAMP is the most commonly investigated isothermal amplification scheme for PoC devices. As opposed to PCR, LAMP uses four or six primers that hybridize to the complement target region of DNA without the need of cycling the temperature (Fig. 2) [49]. LAMP operates at approximately 65 °C, an optimal temperature for enzyme activity and primer binding. The inner primers contain a portion identical to a downstream target region, which is used to form a hairpin. The inner primer initiates DNA polymerization and the strand is extended along the target region. This extended strand is displaced by the polymerase starting from the outer primer, which forms a hairpin loop at the end. The other set of primers bind to the displaced strand and extend in a similar manner, resulting in a double-stranded target sequence, one strand containing a dumbbell DNA structure. The loop region is key, as it enables primers to continue to bind without the need to melt the polymerized DNA. Primers bind to the loop regions and expedite amplification to produce an identifiable elongated sequence. If the target sequence is RNA, as are many viral genomes, a reverse transcription step is required before LAMP proceeds, referred to specifically as RT-LAMP. Fluorescent probes can also be incorporated in order to detect multiple targets in real time [50–52].

LAMP has several advantages that make it a competitive candidate for PoC nucleic acid diagnostics. LAMP is particularly known for its speed, a priority for PoC settings where results need to be delivered to the patient immediately on-site. In 2019, 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 [52]. Also using six primers, Oloniniyi et al. were able to show similar assay times (less than 20 min) for five different ebolaviruses using RT-LAMP [53]. Most recently, there is a reported RT-LAMP assay for the detection of SARS-CoV-2 from Yan et al., that demonstrates detection in 20 min utilizing a six-primer system [54].

In addition to speed, multiple groups have optimized LAMP systems to be equipment-free and more deliverable by eliminating instrumentation via electricity-free LAMP and microfluidics, respectively. In order to maintain the temperature required of a LAMP assay without electricity, Curtis et al. and Song et al. utilized MgFe salts for rapid heating and a phase-change material to hold the temperature steady inside an incubated container [55,56]. Removing the need for electricity allows these diagnostics to leave the clinic and appeal to a greater variety of PoC settings. Microfluidics have the potential to increase portability of these viral diagnostics. For instance, Lin et al. successfully incorporated centrifugation, amplification, detection, and real-time display into a portable device with a built-in fluorescence detector [57]. Similarly, Kaarj et al. developed a paper microfluidic device for Zika virus detection [58]. However, despite this progress in isothermal assays for PoC applications, this work does not offer a complete solution to address the testing needs of a pandemic.

Another consideration when developing a PoC LAMP assay is the signal read-out. Traditionally, RT-PCR can display a real-time signal by employing a Taqman approach. However, an analogous method
in LAMP has not been developed. Additionally, intercalators like SYBR measure the amount of double-stranded DNA present in the sample, but are not able to differentiate between specific sequences, preventing the ability to detect multiple targets in one reaction. Probe-based systems have been developed for LAMP but have been shown to inhibit amplification while increasing the complexity of assay design [50,51]. Some have developed read-out methods that have been optimized for PoC settings, but those often require extra steps and add complexity to the overall assay. Lateral-flow assays are a common PoC readout method. Typically, this involves using biotin or FITC-labeled primers such that the amplification products are fluorescent. As the amplification products flow along a strip, the biotin or FITC bind to anti-biotin antibodies (or streptavidin) or anti-FITC antibodies respectively at known locations along the strip to allow for visualization within minutes [59,60]. Unfortunately, much like sample preparation, these read-outs are often not incorporated into the amplification and require additional steps to transfer the amplification products onto a lateral flow strip. Although this forgoes the need for equipment to visualize the diagnosis, the extra steps decrease user-friendliness due to the increased complexity. Colorimetric readouts tend to be possible without needing an extra transfer step, as in many lateral-flow readout assays. However, colorimetric readouts are often designed to detect a non-specific amplification product, like double-stranded DNA. For example, Ma et al. developed a LAMP system that utilizes hydroxy naphthol blue (HNB) dye to provide visual detection of the amplified DNA, independent of sequence [61]. Colorimetric methods, although less complex than non-integrated lateral-flow readout systems, are often limited by the lack of ability to detect multiple targets in one reaction.

Although LAMP has shown potential for PoC diagnostics due 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. An additional disadvantage of LAMP is that increasing the amount of primers increases the false positive rate [62,63]. Thus, further investigation into the assay is required before primer design can be optimized. Another major downside of the LAMP assay specifically is its intricate design scheme, especially when six primers are required to increase the speed of the assay. This complexity makes LAMP assays difficult to optimize. Due to the nature of a pandemic, rapid assay development is crucial in mitigating the impact of the virus.

### 4.1.2. Reverse transcription recombinase polymerase amplification (RT-RPA)

RPA is another isothermal amplification scheme with potential for PoC diagnostics. Primer-recombinase complexes form and exchange with the target DNA (Fig. 3). The primer is inserted with loop stabilization from single-stranded binding proteins, avoiding the need for thermal melting and high temperatures. A DNA polymerase then extends the primer along the target sequence, producing a new double-stranded DNA amplicon, ready for another cycle of duplication. RPA operates at approximately 37 °C, for optimal enzyme activity. Similar to RT-LAMP, a reverse transcription step can be performed before the RPA in order to detect RNA targets.

LAMP is well-known for being a rapid assay, especially compared to other isothermal amplification methods, but recently, RPA has become more competitive. One assay, designed for the detection of porcine delta coronavirus, required only a 20-min amplification step, comparable to LAMP [64]. More recently, Ma et al. developed an RPA assay for the detection of an influenza A virus (H7N9) that also only required a 20-min amplification step [65]. However, as with LAMP, these assay times do not include sample preparation (extraction and isolation) or transfer to a lateral flow assay for visual read-out.

RPA operates between 37 °C and 42 °C, significantly lower than LAMP, which tends to be closer to 65 °C. Due to this low operating temperature, equipment-free systems like one from Crannell et al. have been developed that only utilize body heat to raise the reaction temperature [66]. Reducing the equipment required removes some of the overall assay complexity, appealing to PoC settings. This advancement also removes the time required for a pre-heat step that would normally be necessary with a traditional heat block or water bath.

Again, like LAMP, RPA assays for PoC settings require a method to read the signal either during or after the amplification. A similar concern regarding readout arises with RPA as it does with LAMP. Some groups have developed read-out methods that have been optimized for PoC settings, like lateral flow assays, but often require manual transfer of amplification product to flow strip [64,67,68]. Some isothermal assays have opted to use visual dye as a marker for amplification to avoid transferring to a lateral flow strip. Although more user-friendly, these dyes, like HNB dye [61] that detects Mg²⁺ as a product of DNA polymerization, do not allow for single pot multiplexing, limiting the device’s overall impact. Alternatively, numerous groups have leveraged CRISPR methods for post-amplification readouts [69]. For example, Huang et al. have developed a CRISPR/Cas12 system to read out the signal from an RPA reaction to detect SARS-CoV-2 [70]. After amplification Cas12 is led to specific regions of the amplified product via guide RNA. A signal is generated when Cas12 cleaves and separates a probe and quencher duo. A CRISPR system would allow for more specific recognition of targets and for multiple targets to be detected in a single reaction. Though these read-out methodologies are clever and simple, they require additional steps by the user, and thus do not meet the sample-to-answer requirement of the large-scale testing centers utilized in a pandemic.

As with LAMP, RPA requires an initial reverse transcriptase step to detect RNA-based viruses, which can sometimes double the

![Fig. 3. Schematic of RPA: (a) primer-recombinase complexes are inserted into complementary regions of the template sequence, stabilized by single-stranded binding proteins, (b) DNA polymerase extends the inserted primers, (c) producing a double-stranded product. New primer-recombinase complexes can then be inserted into this product for exponential amplification.](image-url)
overall assay time. There is a greater abundance of literature regarding LAMP compared to RPA as a PoC detection scheme for viral diagnostics. This suggests less resources to optimize RPA, which could slow down assay development. As previously mentioned, time to assay development is high priority when working amidst a pandemic. Due to this, RPA may not be an ideal choice for rapid isothermal amplification development.

In this section we have focused on LAMP and RPA, two well-studied isothermal amplification methods that have been popular both in the literature and for commercial development for PoC infectious disease diagnostics, especially COVID-19. However, there are several additional, less utilized isothermal amplification techniques that could circumvent some of the challenges presented by LAMP and RPA, particularly for viral RNA detection. For example, one understudied method is Exponential Amplification Reaction (EXPAR), which involves a symmetric template where sequences complementary to the target flank the recognition site of a nicking enzyme [71]. Upon binding and extension of the target, a target analog is created and released upon nicking. This newly released analog can then bind to another template and continue the amplification reaction. EXPAR is a simple technique that can be quickly designed, however it tends to have high nonspecific background signals [72]. Another technique, Nucleic Acid Sequence-Based Amplification (NASBA), mimics the in vivo retroviral replication of an RNA template by using three enzymes and two primers to achieve exponential product generation. NASBA detects RNA targets with high sensitivity [73], but it has been reported that for specific diseases, NASBA does not provide the necessary specificity [74]. A third amplification scheme, Hybridization Chain Reaction (HCR), operates by the target-triggered assembly of DNA monomers into a nicked double helix [75]. Its primary advantage is that it is enzyme-free, thus decreasing the cost and increasing the stability of the assay. However, the thermodynamics of the reaction are notoriously difficult to balance, thus leading to high false positive rates. There has been little effort in the literature to utilize some of these less explored isothermal amplification techniques for COVID-19, but given the imperfections of LAMP and RPA, it is worthwhile for the research community to continue to push forward on alternatives.

### 4.2. Viral protein detection

Another subset of viral infection diagnostics is viral protein detection via immunoassay. As with molecular amplification, immunoassays present their own set of challenges when transitioning to PoC settings. Traditionally, immunoassays utilize a linear amplification scheme with no positive feedback to label the immuno-detection of a biomarker at a surface. Immunoassays appeal to PoC settings since they tend to operate at ambient temperatures and because there is no RNA purification step required as in molecular amplification. However, there are often multiple precise steps involved, including wash steps that add to both the complexity and duration of the assay. An amplification step is unnecessary as each enzyme, held at the surface due to the presence of a biomarker, can act upon a high number of substrate molecules. As a result, the signal amplification is linear, where for every bound viral protein, there is a directly proportional number of chromophores to generate a signal. However, because the amplification is not exponential, limits of detection usually fall in the picomolar range. Further steps have been taken to adopt traditional immunoassays to be better suited for PoC.

Enzyme-linked immunosorbent assays (ELISAs) are considered the gold-standard for protein detection in clinical diagnosis. However, their long assay times and hands-on interventions tend to keep ELISAs away from PoC settings. Recently, Kamilla et al. and Li et al. were able to engineer an abbreviated ELISA to detect SARS-CoV [76] and SARS-CoV-2 [77] respectively. Both developed systems to detect the nucleocapsid protein that encapsidates the genetic material of the coronavirus. The wash steps in both assays, generally the time-consuming steps in an ELISA, were shortened to less than 5 min each. This technique resulted in higher limits of detection than traditional ELISAs due to nonspecific binding.

The most common method for PoC viral protein detection is the LFIA because they are deliverable and rapid, which makes them easy to use in the field. LFIAs are generally developed in a dipstick system [Fig. 4] or housed in a cassette, both intended to increase portability. A sandwich assay forms at a specific location on the strip when the biomarker is present, which produces a visible line. Brangel et al. has developed a serological PoC diagnostic strip for the detection of ebolavirus antibodies [78]. The test was designed alongside a smartphone application to aid with tracking the spread of the virus during the Ebola epidemic in 2018. Hwang et al. developed a lateral flow biosensor for the detection of Tamiflu-resistant influenza virus, specifically the mutated neuraminidase on the surface of the virus [79]. Both assays reduce the time even further from the abbreviated ELISA. Additionally, as there are no washing steps required, the manual steps are also greatly reduced. An alternative to the LFIA is a pipetting-based immunoassay. Noh et al. developed a pipetting-based immunoassay for the detection of the nucleoprotein on the influenza A virus [80]. They engineered a removable magnetic pipette tip that allowed them to purify magnetic beads that had captured an antibody-targeted protein specific to influenza A. They were able to isolate this complex and use enzymatic color development to detect the presence of the virus. Noh suggests one benefit over the LFIA is that they were able to achieve lower limits of detection. However, a downside to this detection method is the multiple manual pipetting steps requiring a certain degree of expertise to consistently perform. This aspect decreases the user-friendliness and increases the complexity, suggesting a pipetting-based immunoassay may not be suitable for a PoC setting, especially when sample-to-answer capability is required.

![Fig. 4. LFIA dipstick system: The dipstick is dipped in sample containing viral particles. As the sample flows along the dipstick, the virus particles bind to labeled antibodies. As these virus-antibody complexes continue to flow along the dipstick, they form a sandwich at the test line when the virus particles bind to a capturing antibody. Labeling antibodies without a bound virus will bind to antibodies at a different location, thus a negative sample will result in a visible line at the control line, whereas a positive sample will result in visible lines at both the control line and the test line.](image-url)
Without the implementation of amplification into these immunoassays for viral protein detection, it is difficult to reach the necessary limits of detection for early diagnosis. The gold standard for nucleic acid detection, RT-PCR, is known to be capable of detecting down to one copy of viral RNA [81]. Several of the aforementioned isothermal amplification methods have shown limits of detection in the tens of copies [61,82]. The gold standard for viral protein detection is an ELISA, which tends to reach detection limits in the picomolar range [83,84]. Assuming average protein size and average ELISA volumes, this suggests a detection limit on the order of $10^7$ protein molecules. Of course, there is some degree of amplification as there are generally many copies of a protein per virus, which could bring the detection limit closer to $10^4$–$10^6$ viruses.

4.3. Serological testing

Alternatively, another subset of viral diagnostic development is in serological tests for the detection of antibodies. These tests differ from the methods previously discussed as the presence of antibodies indicates a past infection rather than an ongoing one. These tests identify patients who have been infected for about two weeks, which is too late to limit the spread of the virus through contact tracing. However, tracking exposure and infection rates is still useful to slowing the spread of the disease during a pandemic. Similar to the nucleic acid and protein tests described above, there remains a challenge to transition serological tests to a PoC setting. As with viral protein detection, ELISAs are the gold standard for detecting antibodies, but long assay times and precise hands-on steps remain an obstacle for evolving to a PoC method. Fatima et al. has designed an assay to detect SARS-CoV-2 seroconversion, following the traditional steps of an ELISA [85]. This protocol was a major component of the first serological test to get an EUA from the US FDA. The authorization was issued to Mount Sinai Laboratory in April 2020. Although establishing this gold standard to be performed in clinical laboratories is important, there remains a need for a PoC option.

Serological tests designed for PoC settings have been developed for other viruses. The work of Chin et al. in 2010 stands out because of its emphasis on usability and mass production, both of which had been rare in the PoC literature at the time. The authors demonstrated the integration of the multistep assay into a sample-to-answer device by pre-loading all reagents into a tube connected to the plastic microfluidic device, which was used to test patient samples in a PoC setting [86]. With their “mChip,” they showed high sensitivity and specificity for HIV testing in remote settings when they evaluated their immunoassay in Rwanda with 70 local samples.

While the mChip had been the standard bearer in the literature for some time, the urgency of the COVID-19 pandemic has spurred a number of new serological tests. Dozens of serological tests have received an EUA since the original ELISA from Mount Sinai Laboratory. One of these tests is from Abbott Laboratories, which has developed a chemiluminescent microparticle immunoassay for quantitatively determining the presence of IgG antibodies against the SARS-CoV-2 nucleocapsid protein [87]. Although this system has sensitivity comparable to an ELISA, the assay only works with serum. Thus, extra sample preparation of the whole blood sample is required before the test can be performed. Roche Diagnostics has developed an electrochemiluminescence immunoassay, receiving an EUA due to its competitive sensitivity (99%) and not requiring serum to perform the assay [88]. This assay operates at high sensitivity with a whole blood sample. DiaSorin Inc. has also developed a chemiluminescent assay receiving an EUA for COVID-19 [89]. Additionally, this system is semi-quantitative, providing ranges of possible antibody concentrations, even prompting a sample to be re-assessed if the antibody concentration is measured near the cut-off for positive diagnosis. Although these assays have achieved the sensitivity of a traditional ELISA, none have received the CLIA-distinction for being appropriate for a PoC setting. This is largely due to the current necessity for additional equipment to read the fluorescence or luminescence of the samples.

In order to circumvent the requirement of expensive equipment, many assays that received an EUA from the US FDA opted to develop LFIs. Most of these LFIs operate similarly. For example, Xiamen Biotime has developed a traditional LFA, able to detect both IgG and IgM against SARS-CoV-2 in a single assay, with results available in less than 20 min [90]. Even though there is currently an excess of LFIs receiving EUs, only one assay, from Assure Tech, has received the distinction of a CLIA-waiver, authorizing its use in PoC settings. This distinction appears to be somewhat due to the integration of sample collection with the workflow of the assay [91]. Several of the LFIs receiving EUs are lacking the PoC authorization for this specific reason. This concept brings up a final, crucial downside of the use of a serological test. The use of blood as the medium for serological tests can increase the complexity of sample collection, sometimes even to the point of requiring a technician. In fact, at the time of submission, about 50 serological tests have received EUA from the FDA; the vast majority use serum (not whole blood) as the sample, while those that use blood cannot be used with a finger prick [92].

5. Sample preparation is the key challenge

Much of the literature discussed in Section 4 focuses on transitioning from traditional thermal-cycled RT-PCR to isothermal approaches, under the assumption that isothermal methods may enable simple instrumentation and/or devices, speeding up time to result and enabling PoC use. In reality, even with a successful isothermal method, the need for precise intervention steps during sample preparation prevents most diagnostic methods from qualifying as PoC. For nucleic acid amplification tests, the preparation steps include the lysis of the virus capsid (often with chemical agents that inhibit PCR), the immobilization of the genome, the removal of PCR inhibitors (generally with alcohol to rinse away inhibitors but not the nucleic acids), and the elution of the purified genome into the assay (Fig. 5a). Commercially available kits (including Qiagen’s QIAamp DNA/RNA purification kit, NucliSens isolation kit, and the Puregene DNA isolation kit [93]) enable relatively rapid sample preparation, but are not useable at the PoC. There has been work to utilize sophisticated microfluidic chips that include all of the steps, including bead- or membrane-based RNA capture, alcohol rinses, and elution into an on-chip reaction chamber [94], but replacing the manual reagent exchanges by microvalves and external pumps results in cost-prohibitive chips and bulky setups.

There have been many efforts to reduce the number of steps required in viral sample preparation (e.g., Fig. 5b). For instance, Zhang et al., were able to integrate the lysis step by using ultrasound to rapidly (<1 min) extract nucleic acids on chip without using PCR inhibitors [95]. Likewise, Heiniger et al. showed that by using Achromopeptidase, a proteolytic enzyme for the lysis step, the clean-up step before amplification could be eliminated (but this method requires a high temperature heat-kill, and thus is best matched with RT-PCR, not isothermal methods) [86]. The nucleic acid isolation step has also been automated several different ways to appeal to PoC settings. Neto et al. used magnetic beads to bind and subsequently pull viral RNA through an immiscible phase filter (thus eliminating alcohol rinse steps), using an automated magnet [97]. Similarly, Cui et al. were able to drag capture beads through oil
to an aqueous phase, where the beads could be removed, free of the lysis components [98].

Alternatively, Hagan et al. used a charge-switching method to eliminate the need for the alcohol rinses [99]. In this approach, chitosan — a biopolymer that is positively charged below pH 6.5 and neutral above pH 6.5 — is grafted onto a microchannel surface. The lysate passes through the channel in low-pH buffer, causing the negatively charged DNA to be captured by the positively charged chitosan. Then the elution buffer (at a higher pH) is passed through the channel; the neutralization of the chitosan causes the DNA to be released into the RT-PCR reaction.

In another clever approach, Ferguson et al. developed an on-chip method that bypasses the challenge of extracting nucleic acids altogether [100]. In their approach, following the lysis step, the viral nucleocapsid protein is captured via immunomagnetic beads; the RNA is bound to the nucleocapsid protein, and thus it is pulled out of lysis buffer by the beads [100]. The protein and RNA, now bound to magnetic beads, are then loaded into a chip and amplified via RT-PCR.

Given that microfluidic chips can be costly and can require bulky instrumentation, some have aimed to eliminate chips altogether by performing all sample preparation on paper or similar membranes. Rodriguez et al. were able to remove the need for instrumentation by using a paper-based method of sample preparation, thus suggesting a lower cost solution [60]. A lysed virus sample is pipetted onto the paper membrane before two wash steps on the surface. The nucleic acid amplification also occurs directly on the paper. Although this method eliminates the need for a chip, several precise pipetting steps are required, implying that it may not be useable for PoC. Similarly, Ye et al. were also able to show that sample preparation could be done on paper, with only 5 min required for sample preparation, and without the need for alcohol rinses [101]. However, the prepared sample then had to be cut from the membrane and inserted into a standard well plate or tube array for RT-PCR amplification.

While the need for lysis and RNA purification is the standard, some have experimented with skipping RNA purification and using heat-based lysis, which does not introduce inhibitors to the amplification reaction (Fig. 5c) [102,103]. In a report by Priye et al., the authors claim that the temperature for the LAMP reaction is sufficient to lyse virus particles and that RT-LAMP can proceed without any purification. However, this work used cultures instead of clinical samples and did not confirm the absence of free RNA in the samples. Yang et al. did not include specified steps to extract the nucleic acid but did place the sample in an undisclosed “sample solution” at high heat to lyse the virus particles, then purified the sample through centrifugation by adding only the supernatant to an amplification reaction. If in fact RT-LAMP can be carried out directly on clinical samples with no chemical lysis or DNA purification, this would provide a much easier pathway to develop low-cost solutions for sample-to-answer PoC viral diagnostics. If heat is not sufficient alone, perhaps ultrasonic lysis can aid in releasing RNA without the need for binding/washing. Regardless, it is clear that more research is necessary to confirm that a lyse-to-amplify design is possible for clinical samples.

Much of the work summarized in this section has demonstrated a reduction in the overall number of sample preparation steps.

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**Fig. 5.** Sample preparation steps: In conventional sample preparation, (A) a viral sample is prepared by first chemically lysing the virus to expose the RNA. The RNA is then captured and isolated before being added to an amplification reaction. This traditional process is lengthy and requires several manual steps. Alternatively, (B) clever approaches have used alternative nucleic acid capture methods to eliminate rinse steps. (C) Some have experimented with non-chemical lysis to avoid adding amplification inhibitors that require clean-up. However, these methods still need to be further proven.
However, all of these methods still required either hands-on interventions or instruments. Thus, while intriguing, they have not completely addressed the necessary requirements to combat pandemics. To truly meet the requirements, the approaches should be inexpensive (and thus mass-producible) while also not requiring any user intervention to get from the initial clinical sample to the amplification result (i.e., sample-to-answer).

6. Fully integrated systems are necessary for sample-to-answer

As previously discussed, in order to be most suitable for a PoC setting, diagnostics need to integrate sample preparation with amplification and signal readout. In moving toward a fully integrated system, Ferguson et al. and Cao et al. combined target capture, concentration, purification, and RT-PCR all on-chip to detect H1N1 [106,104]. Ferguson et al. implemented a device in which reagents flow in and out automatically, while the target remains attached to magnetic beads, decreasing the amount of manual operational steps. Cao et al. used solid phase extraction to concentrate and purify the samples, using pumps to move the sample to different locations on the chip. Alternatively, Hagan et al. leveraged integrating the charge switching method with on-chip RT-PCR to demonstrate the detection of influenza A [99]. Finally, Shen et al. were able to produce a true sample-to-answer system with full integration on a PDMS chip to detect influenza A [94]. However, these methods do not completely satisfy the needs of STARLITE or even typical PoC settings. Some still required manual intervention, such as off-chip lysis and reagent transfer [100,104]. Others required pumps with either manual tubing exchanges or costly microfluidic valves [94,99,104].

An ideal sample-to-answer system uses a low-cost chip, requires no interventions by the user, and utilizes a small and portable instrument. One method that appears to satisfy this list of requirements is the spinning disk technology in which reagent release and manipulation is carried out using centrifugal forces within a disk-shaped microfluidic device. The device is generally made with plastic using injection molding or similar low-cost high throughput methods. One example of sample-to-answer virus identification has been reported in the literature using this approach. Stumpf et al. were able to demonstrate sample-to-answer capability with their PoC diagnostic for the detection of influenza A [105]. They used a spinning disk to move reagents and beads around the chip and were able to perform lysis, extraction, and PCR all on the disk without need for intervention. Jung et al. also used the spinning disk technology to automate most steps but performed lysis off chip [106]. The spinning disk technology appears to be promising, as it satisfies the sample-to-answer requirement with a low-cost chip, but it is not yet clear that the instrument (essentially a disk player that can operate at multiple speeds and has integrated optics) can process a high number of samples asynchronously, which we believe is a requirement to address the high-testing-volume needs of a pandemic. Sample preparation methods discussed in Sections 5 and 6 are summarized in Table 5.

7. Outlook and recommendations

In response to the ongoing COVID-19 pandemic, researchers worldwide have worked to adapt existing technologies for viral disease diagnostics to detect SARS-CoV-2. With regards to the nucleic acid amplification test, it is apparent that the research community prefers LAMP and RPA over RT-PCR. However, LAMP and RPA are known to be less robust than PCR, and are more vulnerable to false positives, making the design of new assays for emerging pathogens challenging. In particular, if multiple targets need to be detected in a single reaction, the use of LAMP and RPA present challenges due to the variability of optimal reaction conditions for different targets. It is noteworthy that although the literature does not favor the SDA reaction, the Abbott ID NOW system has utilized a form of this reaction [41] to deliver rapid results (although it has been demonstrated that the ID NOW had a high false negative rate for COVID-19 [45–47], this was likely related to the mismatch of the sample interface with established sample handling procedures of testing labs). Researchers may benefit by investigating the use of SDA in their integrated PoC prototypes.

While continuing to investigate isothermal methods is of value, the largest gap to address the testing needs of a pandemic exists in sample preparation. We summarized the progress in reducing the number of necessary steps to transition from a clinical sample to the amplification reaction. For the most part, however, results in the literature feature cost-prohibitive microfluidic technology or user interventions that are incompatible with use in the field at high-volume patient test sites. Nonetheless, several valuable insights can be drawn. First, avoiding chemical lysis and traditional solid phase extraction, which require the use of nucleic acid amplification inhibitors, can lead to simplified chip or cartridge designs. Further investigation of heat and ultrasonic lysis is thus recommended. Coupled with this is the need to investigate the inhibition of nucleic acid amplification in clinical samples. For example, it is known that Taq is inhibited by heme [107] while Bst is not [108–116], and thus LAMP (which uses Bst) is more compatible with blood samples than PCR.

It is conceivable that lysis and DNA purification will continue to be a challenge for researchers. On the other hand, as immunoassays directed toward the outer surface of the virus do not require lysis or purification, it may be tempting to pursue amplified immunoassays, such as immunoPCR [117]. However, traditional immunoassays require several washes, and immunoPCR increases this burden further, suggesting that deployment at the PoC may not be realistic. Alternatively, LFAs have shown promise for performing immunoassays with minimal interventions. One potential method to increase the detection performance for LFAs for viral detection would be to employ gold-nanoparticle-labeled detection antibodies with silver amplification, as has been demonstrated in other formats [118,119].

Additionally, further work on instrumentation and signal readout methods is recommended. As discussed above, while there are commercially available PoC tests for respiratory diseases, the costly instruments only test one sample at a time, and thus are not appropriate for high-volume patient testing in the field. Likewise, while the spinning disk microfluidic technology is promising, an instrument that can process multiple disks at the same time with random access to the instrument is needed. In addition to considering how the instrument interfaces with the chip or cartridge, we also recommend considering how the instrument connects with the user. An ideal solution would include a wireless connection with mobile devices used by the tester, such that information collected from the patient (e.g., scan of identification card and contact information) can be electronically tied to the sample result and immediately delivered to public health officials for contact tracing purposes.

Finally, we recommend that researchers consider designing their chips or cartridges to interface directly with either swabs or saliva. While swabs have been considered the standard for respiratory viruses, saliva has advantages. It avoids the risk of supply shortages, which have been persistent during the COVID-19 pandemic. Even more importantly, using saliva enables a simple and robust sample collection method that can be performed...
independently by the user and dramatically reduces the probability of infecting the tester. To date, encouraging results have been achieved in detecting the presence of SARS-CoV-2 in patient saliva samples [120,121]. However, while some PCR tests have been developed for saliva, all of the available antigen tests utilize swabs, and the available serological tests use blood or serum.

8. Conclusions

After reviewing our response to the last 100 years of pandemics, including the ongoing COVID-19 pandemic, we can conclude that we remain unprepared to address the testing needs of the next global pandemic. Although central laboratory tests have improved, attempts to bring RT-PCR to a PoC setting have been slow. Current approved systems have shown progress in bringing diagnostics closer to the patient, but these systems do not have the low per-test cost or throughput needed to deliver massive testing in the field to respond to a pandemic. Research on isothermal amplification methods, especially LAMP and RPA, has eliminated the need for equipment typically required for temperature control in PCR. However, these methods are imperfect, as they tend to involve months of assay development and are challenging to multiplex. Even with the ideal isothermal assay methodology, sample preparation remains a substantial hurdle due to the number of required steps. While clever and sophisticated methods have emerged, it is vital to ensure that in integrating sample preparation with virus detection, the diagnostic does not become unsuitable for PoC by increasing in cost or by failing to eliminate manual intervention steps. Detecting the viral protein in a LFIA, rather than the viral genome, eliminates many of the sample preparation steps required for RNA detection; however, the development of antigen tests is orders of magnitude slower than the development of nucleic acid tests. In addition to the guidelines already required by the WHO and FDA, we propose the emphasis of development of nucleic acid tests. In addition to the guidelines independently required by the WHO and FDA, we propose the emphasis of rapid, local, inexpensive, throughput, and equipment-free. The recent worldwide outbreak of the SARS-CoV-2 virus demonstrated that, despite progress regarding central laboratory diagnostics, a PoC solution capable of large-scale deployment and rapid results has not been achieved. As we prepare to combat the next pandemic, we believe that incorporating our recommendations into the development of rapidly mobilizable PoC testing infrastructure will be crucial to protecting our world.

Declaration of competing interest

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

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Appendix A. Supplementary data

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