COVID-19 Point-of-Care Diagnostics: Present and Future

Enrique Valera,* Aaron Jankelow, Jongwon Lim, Victoria Kindratenko, Anurup Ganguli, Karen White, James Kumar, and Rashid Bashir*

ABSTRACT: Point-of-care (POC) detection technologies that enable decentralized, rapid, sensitive, low-cost diagnostics of COVID-19 infection are urgently needed around the world. With many technologies approved for commercialization in the past 10 months, the field of COVID-19 POC diagnostics is rapidly evolving. In this Perspective, we analyze the current state of POC technologies for the diagnosis and monitoring of COVID-19 infection and discuss future challenges in COVID-19 diagnostics. As the COVID-19 pandemic becomes endemic, the advances gained during this past year will likely also be utilized for future prediction of emerging outbreaks and pandemics.

Since the severe acute respiratory syndrome (SARS-CoV-2) jumped from an animal reservoir to humans in December 2019, it has rapidly spread across the world, bringing critical challenges for public health, including being the number one cause of death in the United States in early 2021, disruption to daily life, and economic losses to businesses and individuals.1−3 The COVID-19 pandemic has highlighted the need to diagnose the disease rapidly and accurately at scales larger than ever before.4 Models have predicted that millions of tests per day are needed to remodelize the economy fully.4,5 However, many factors have contributed to a less-than-optimal availability of testing, including the shortage of laboratory supplies (which also impacts non-COVID testing) and test kits and the inability to scale the supply chain to meet demand.6,7 Although the current gold standard diagnostic method for the detection of COVID-19 is reverse transcription polymerase chain reaction (RT-PCR) for the RNA of SARS-CoV-2,8−10 loop-mediated isothermal amplification (LAMP) processes (e.g., reverse-transcription LAMP, RT-LAMP) are also gaining attention.11−15 Many specimens are approved for use in nucleic acid amplification tests, with the most common being nasopharyngeal (NP), nasal midturbinate, anterior nares, and saliva.16 It should be noted that swab-based samples are placed in a liquid transport medium, which is then subsequently analyzed.

The development of rapid, point-of-care (POC) molecular diagnostic tests that have sensitivity and specificity comparable to the current gold standard techniques can significantly aid testing expansion.6,17 Such POC devices could enable the convenient acquisition of information about both viral presence and host response (e.g., antibodies) in nonlaboratory settings with rapid turnaround times. The deployment of testing solutions out of centralized laboratories, for instance, at the primary or urgent care level, could be a key step for the rapid detection and identification of COVID-19 and prevention of transmission to the community.6 Point-of-care devices offer the possibility of (i) using more portable and cost-effective instrumentation; (ii) eliminating sample transport to a clinical laboratory for analysis; (iii) reducing sample processing; (iv) using samples, such as saliva or anterior nasal swabs, that do not require trained personnel for collection; and (v) measuring different entities (virus, antigen, antibodies) in symptomatic or asymptomatic patients that could contribute to precise determination of individuals who would benefit from clinical care or would require quarantine.

The type of diagnostic solutions needed depend on the throughput, portability, cost, and barriers to regulatory approvals. The testing solutions could be deployed out of

Published: May 13, 2021
The development of rapid, point-of-care molecular diagnostic tests that have sensitivity and specificity comparable to the current gold standard techniques can significantly aid testing expansion.

centralized laboratories and could offer throughputs ranging from thousands of tests per day to one test for personal use. Figure 1 shows the COVID-19 portable diagnostics options. The size of the diagnostics solution is typically inversely proportional to the portability and is directly proportional to the testing capacity. Whereas POC technologies are considered for self-use using hand-held devices, other factors for portable approaches such as a mobile laboratory, a self-contained benchtop system, or a suitcase that can be used for testing in large, medium, or small gatherings of people, respectively, should also be considered.

Point-of-care approaches are those that can provide results at the point of use, such as at home, or in hospitals, urgent care centers, elderly care centers, emergency rooms, or other settings, instead of samples being sent to a laboratory. These tests can still be used under the auspices of a Clinical Laboratory Improvement Amendments (CLIA) certified laboratory or used by an individual for self-testing. The tests may require a trained individual to collect the sample and to perform the analysis or they could also be used for self-testing by the patient themselves. Receiving a result should be as rapid as possible and not limited by the assay itself, but by the data and information management system used by the company or hospital providing the assay.

The United States used Emergency Use Authorization (EUA) to enable emergency use of in vitro diagnostics for detection of SARS-CoV-2 or diagnosis of COVID-19 to expedite the process of such devices entering the commercial market. After EUA authorization, the test is categorized and can be performed in a particular setting under CLIA (e.g., moderate complexity or POC). This policy does not apply to at-home testing. In addition, EUA requests for COVID-19 diagnostic tests that can be performed entirely at home or in other settings outside a lab have their own recommendations concerning what data and information should be submitted with the request. For instance, due to the greater potential for error in specimen collection at home, the FDA recommends that the device has an internal control to indicate that an adequate human sample was collected and placed into the test for analysis. Likewise, the use of anterior nares (nasal) swabs, midturbinate swabs, or saliva as sample types is recommended to avoid the use of incorrect techniques that could result in patient harm.

When a subject exhibits signs of COVID-19, physicians need to test for the presence of COVID-19 and to quantify the severity of the disease, which can range from mild to critical. Symptomatic patients are isolated while awaiting test results. Highly suspect cases may remain isolated based on clinician judgment until follow up confirmatory testing through either repeat molecular methods or serology. Based on the illness severity and comorbidity conditions, physicians need to decide if the subject will require guideline-directed therapeutic management in the appropriate setting. Early and accurate testing is necessary to help guide the decision as to which clinical path the patient might follow. Consequences of these delays in hospitals include poor patient flow and possible nosocomial transmission. Therefore, rapid and accurate POC tests that can detect acute or past SARS-CoV-2 infections and that do not rely on centralized laboratories are urgently needed to lighten the demand for tests in hospitals and to ensure faster results to the population.

WHAT SHOULD BE DETECTED?

Currently, there are three types of COVID-19 tests: molecular diagnostics, antigen tests, and antibody tests (Table 1). Molecular diagnostics tests indicate the presence of the SARS-CoV-2 RNA, antigen tests detect specific proteins from the virus, and antibody tests determine whether the individual has developed antibodies to the virus. For molecular tests, the available targets are different regions of the RNA genome, whereas for antigen tests, the targets are the available structural proteins (antigens) that are anchored on or inside the viral envelope (Figure 2). SARS-CoV-2 virus contains a single-stranded RNA that includes target genes such as ORF1b, ORF8, N-protein, S-protein, RNA-dependent RNA polymerase, and envelope genes. The four major structural proteins are the spike surface glycoprotein (S), small envelope protein (E), matrix protein (M), and nucleocapsid protein (N).

MOLECULAR, ANTIGEN, AND ANTIBODY TEST POINT-OF-CARE DEVICES

The high specificity of RT-PCR and its ability to make billions of copies of a specific RNA or DNA sample rapidly make this amplification method the current gold standard for the detection of the SARS-CoV-2 virus. However, the reliance on thermal
cycling makes it difficult to translate this technology to a portable device due to the variance and accuracy in temperatures needed to amplify the genetic material in the sample. Likewise, the standard RT-PCR protocol utilizes an RNA extraction and purification step using commercially available kits. The RNA extraction kit not only extracts the RNA from the virus but also purifies the RNA and may also concentrate it depending on the volume of fluid used after purification, hence contributing to improving assay sensitivity.

Successful examples of EUA-approved RT-PCR-based POC devices are the Xpert Xpress SARS-CoV-2, Xpert Xpress SARS-CoV-2/Flu/RSV, Xpert Xpress SARS-CoV-2 DoD (all three from Cepheid), Accula SARS-CoV-2 Test (Mesa Biotech Inc.), cobas SARS-CoV-2 and Influenza A/B Nucleic Acid Test (Roche Molecular Systems, Inc.), BioFire Respiratory Panel 2.1-EZ (BioFire Diagnostics, LLC), and Visby Medical COVID-19 Point-of-Care Test (Visby Medical, Inc.). One example, the Visby Medical test, is a single-use (disposable), fully integrated test, where anterior nasal or midturbinate swabs samples can be self-collected by individuals 18 years of age or older, under the supervision of a health care provider. All of these EUA-approved tests are authorized for use at the POC (i.e., in patient care settings operating under a CLIA Certificate of Waiver, Certificate of Compliance, or Certificate of Accreditation).

Isothermal amplification-based approaches have recently generated significant attention for detection of SARS-CoV-2 virus due to the simplicity of this technology (typically one-step) and the ease of translation to a point-of-use device, as these technologies eliminate the need for precise thermal cycles to achieve RNA amplification. Additional advantages are the possible elimination of the viral purification step, and the simplification of the instrumentation complexity. Shortly after the pandemic started, Abbott Diagnostics Scarborough, Inc. released the ID NOW COVID-19 test which uses RT-LAMP. This was the first isothermal technology to receive EUA authorization for COVID-19 testing. Cue COVID-19 Test (Cue Health, EUA approved) utilizes isothermal amplification (20 min) in a single-use cartridge that detects the virus from direct nasal swabs with a limit of detection of 20 genome copies per sample using an electrochemical detection method. The reader, which is not included in the test cartridge pack, can run thousands of tests before it needs to be replaced. Similar to the EUA-approved PCR devices, ID NOW COVID-19 and Cue COVID-19 Test are authorized for use at the POC (i.e., in patient care settings operating under a CLIA Certificate of Waiver, Certificate of Compliance, or Certificate of Accreditation). On March 5, 2021, Cue Health took an important step when the Cue COVID-19 Test for home and over the counter (OTC) use was approved for nonprescription home use, thus becoming the nation’s first molecular diagnostic test available without a prescription to consumers for home use and to enterprise users and healthcare professionals without CLIA certification. Another example of a single-use test is the Lucira COVID-19 All-in-One Test Kit (Lucira Health, Inc.). This rapid
(30 min) RT-LAMP-based hand-held battery-powered device, which received the first FDA authorization for COVID-19 self-testing at home, enables individuals 14 years and older to test themselves using self-collected nasal swabs.\textsuperscript{15}

**Isothermal amplification-based approaches** have recently generated significant attention for detection of the SARS-CoV-2 virus due to the simplicity of this technology (typically one-step) and the ease of translation to a point-of-use device.

Antigen tests rely on specific monoclonal antibodies to detect the SARS-CoV-2 structural proteins. These tests have been highlighted as a potentially important tool in an overall community testing strategy to reduce transmission.\textsuperscript{29} Although most of the currently available antigen tests target the N-protein, the use of the S-protein may be more specific because this protein has less sequence homology with the previous SARS-CoV and MERS viruses.\textsuperscript{30} Antigen tests are faster than PCR techniques (providing results in a few minutes); however, they are inherently less sensitive as no amplification of the target is involved. Likewise, these tests provide qualitative results only (they do not quantify the viral load in the sample). As seen in Table 1, antigen devices also have high false-negative rates. Thus, a negative test result may occur if the level of antigen in a sample is below the detection limit of the test.

Successful examples of antigen EUA-approved POC devices are the LumiraDx SARS-CoV-2 Ag Test (LumiraDx UK Ltd.), CareStart COVID-19 Antigen test (Access Bio, Inc.), BinaxNOW COVID-19 Ag Card (Abbott Diagnostics Scarborough, Inc.), BD Veritor System for Rapid Detection of SARS-CoV-2 (Becton, Dickinson and Company, LLC), Clip COVID Rapid Antigen Test (Luminostics, Inc.), QuickVue SARS Antigen Test, Sofia 2 SARS Antigen FIA, Sofia 2 Flu + SARS Antigen FIA (all three from Quidel Corporation), and Status COVID-19/Flu (Princeton BioMeditech Corp.). All of these technologies provide qualitative detection of the nucleocapsid protein antigen from SARS-CoV-2. Therefore, all of them include an extraction buffer to disrupt the virus particles present in the specimen and expose the internal viral nucleoproteins.\textsuperscript{26} Likewise, all of these EUA-approved technologies are authorized for use at the POC (i.e., in patient care settings operating under a CLIA Certificate of Waiver, Certificate of Compliance, or Certificate of Accreditation) and require trained operators.

In December 2020, the Ellume COVID-19 Home Test (Ellume Limited, EUA approved) became the first antigen test to be authorized for nonprescription, OTC home use.\textsuperscript{31} The
Ellume test is not yet available for purchase, however the estimated cost is $30. Another antigen home test is the BinaxNOW COVID-19 Ag Card Home Test (Abbott Diagnostics Scarborough, Inc., EUA approved). Unlike the Ellume device, this test requires a prescription and is to be performed only under the supervision of a telehealth proctor. On March 31, 2021, tests from the BinaxNOW family were authorized for nonprescription home use with self-collected samples from individuals aged 15 years and older or adult-collected anterior nasal swab samples from individuals aged 2 plus years old (BinaxNOW COVID-19 Antigen Self-Test14 and BinaxNOW COVID-19 Ag Card 2 Home Test).34 The options of at-home tests have also been expanded with the new members of the QuickVue family, the QuickVue At-Home OTC COVID-19 Test35 and the QuickVue At-Home COVID-19 Test.36 These devices clearly indicate a trend in the antigen testing market focusing on at-home testing.

The detection of antibodies to the SARS-CoV-2 virus cannot be considered an “immunity passport” or “risk-free certificate”. It is currently unknown if people who have recovered from COVID-19 and have antibodies are protected from being infected again, because some confirmed and suspected cases of reinfection have been reported.37,38 Likewise, depending on the timing of infection and sampling for serologic testing, recently infected individuals may be antibody positive while still shedding the virus.39 However, important roles such as determining the true prevalence of this virus and monitoring the temporal immune responses in vaccine recipients are expected to be accomplished by serologic testing.39

Although more than 100 serology tests have been EUA approved (including EUA submission pending) in recent months, only a few of them have been approved as POC devices: Assure COVID-19 IgG/IgM Rapid Test Device (Assure Tech.,), RightSign COVID-19 IgG/IgM Rapid Test Cassette (Hangzhou Biotest Biotech), RapCov Rapid COVID-19 Test (Advait, Inc.), MidaSpot COVID-19 Antibody Combo Detection Kit (Nirmidas Biotech, Inc.), and Sienna-Clarity COVIBLOCK COVID-19 IgG/IgM Rapid Test Cassette (Salofa Oy).26 Similar to the EUA-approved molecular and antigen devices, EUA-approved antibody tests are authorized for use at the POC (i.e., in patient care settings operating under a CLIA Certificate of Waiver, Certificate of Compliance, or Certificate of Accreditation). Most of these devices provide qualitative detection and differentiation of IgM and IgG antibodies to SARS-CoV-2 in human venous whole blood, serum, and plasma, or finger-stick whole blood. All these tests are rapid (10–20 min) and include control lines. Important because even vaccinated patients, while asymptomatic, may still carry and transmit live virus from the upper airway. Some specific points are expanded below.

- Molecular diagnostics tests such as RT-PCR are the gold standard for detecting the presence of SARS-CoV-2 virus due to high sensitivity and reliability. However, these processes still take longer than desired. RT-PCR tests can take 60–90 min minimum plus the time for data analysis and reporting. Although isothermal amplification techniques such as RT-LAMP can reduce this time, the time for the assay is 30–45 min plus data reporting to provide the final results to the patient. On the other hand, antigen tests are rapid and can provide results in as little as 5 min but are ~100x less sensitive. Therefore, one of the main goals for POC devices is the development of a molecular amplification-based test that can provide results in 5 min at the cost of an antigen test. Such a device would replace antigen tests while offering high sensitivity.
- It is expected that COVID-19 will become an endemic disease. Therefore, multiplexed POC devices that are able to test to differentiate between different coronavirus variants and other seasonal respiratory illnesses, such as the flu, will be needed. The performance of these devices should be tested across all known variants at the time of validation while taking into account the potential impact of future variants, as recommended by the FDA.45
- Routes to reduce the overall cost of sample collection, testing, and analysis are necessary. In this direction, the use of saliva as a specimen could be a satisfactory solution if the sensitivity of the assay is not affected. Saliva has demonstrated to be an alternative upper respiratory tract specimen type for SARS-CoV-2 detection.44 Furthermore, saliva offers a number of advantages over nasopharyngeal swabs when considering mass testing, as it can be self-administered. For instance, it is known that the use of NP swabs can cause discomfort or irritation and can increase the risk of exposure for the medical providers;38 variation in nasopharyngeal sampling may be an explanation for false negative results. In contrast, saliva collection does not require a certified swab, specific collection receptacle, or transport media and does not have to be obtained by a skilled healthcare provider.12 Importantly, RNA purification-free RT-PCR and RT-LAMP assays have been developed for detection of SARS-CoV-2 from saliva clinical samples.12
- Importantly, it should also be noted that clinical testing practices are migrating to nasal swabs from anterior nostrils, which can also be self-administered. We believe that the use of either nasal swabs of the anterior nostrils or saliva will facilitate the scaling of diagnostics. The sensitivity and specificity of the tests from these sources can be different and saliva can be expected to be a more sensitive indicator of the state of the respiratory system.
- Increasing testing capacity and increasing antigen and molecular testing manufacturing in the United States are part of President Biden’s National Strategy for the COVID-19 Response and Pandemic Preparedness. In particular, there is a major interest in OTC, at-home testing, and we think this is one of the most attractive directions. For instance, Ellume USA was recently awarded $231.8 million to produce the Ellume COVID-
Many reports have demonstrated that COVID-19 The duration for which immunity lasts after infection or receives the vaccination will be crucial. Gingras et al. recently reported that anti-SARS-CoV-2 antibodies were detected in serum and saliva, with peak IgG levels attained by 16–30 days postsymptom onset. Their study revealed that anti-SARS-CoV-2 IgA and IgM antibodies rapidly decayed, whereas IgG antibodies remained stable up to 105 days in both biofluids. Studies have reported that IgG can suggest immunity; however, in some cases, the sensitivity can be as low as 70.5% for the lateral flow devices used to detect antibodies.

Many reports have demonstrated that COVID-19 disproportionately impacted people of color and under-resourced regions. Access to testing was limited in these communities and exacerbated the impact of the pandemic. Use of POC devices can significantly aid in bridging the social divide in COVID-19 and other pandemics in the future.

- New approaches are needed to capture virus particles in their aerosolized forms. Self-contained systems that can reliably sample and capture particles from air, and subsequently identify the viruses using RNA amplification or detection of antigens, would have widespread use.

- Finally, we note that POC device technologies should be ready for any future outbreaks, from new strains or novel viruses. The response to this pandemic when it comes to drug development has been extremely fast. However, the need for a diagnostic upscale was not met. The POC technology platforms developed this year should quickly be translated to the detection of new pathogens, once the sequence of the new pathogens is known.

**AUTHOR INFORMATION**

**Corresponding Authors**

**Enrique Valera** — Department of Bioengineering and Nick Holonyak Jr. Micro and Nanotechnology Laboratory, University of Illinois at Urbana—Champaign, Urbana, Illinois 61801, United States; orcid.org/0000-0003-1359-6619; Email: evalerac@illinois.edu

**Rashid Bashir** — Department of Bioengineering and Nick Holonyak Jr. Micro and Nanotechnology Laboratory, University of Illinois at Urbana—Champaign, Urbana, Illinois 61801, United States; Department of Biomedical and Translational Science, Carle Illinois College of Medicine, Urbana, Illinois 61801, United States; orcid.org/0000-0002-7225-9180; Email: rbashir@illinois.edu

**Authors**

**Aaron Jankelow** — Department of Bioengineering and Nick Holonyak Jr. Micro and Nanotechnology Laboratory, University of Illinois at Urbana—Champaign, Urbana, Illinois 61801, United States

**Jongwon Lim** — Department of Bioengineering and Nick Holonyak Jr. Micro and Nanotechnology Laboratory, University of Illinois at Urbana—Champaign, Urbana, Illinois 61801, United States

**Victoria Kindratenko** — Department of Bioengineering and Nick Holonyak Jr. Micro and Nanotechnology Laboratory, University of Illinois at Urbana—Champaign, Urbana, Illinois 61801, United States

**Anurup Ganguli** — Department of Bioengineering and Nick Holonyak Jr. Micro and Nanotechnology Laboratory, University of Illinois at Urbana—Champaign, Urbana, Illinois 61801, United States

**Karen White** — Department of Biomedical and Translational Science, Carle Illinois College of Medicine, Urbana, Illinois 61801, United States; Carle Foundation Hospital, Urbana, Illinois 61801, United States

**James Kumar** — Department of Biomedical and Translational Science, Carle Illinois College of Medicine, Urbana, Illinois 61801, United States; Carle Foundation Hospital, Urbana, Illinois 61801, United States

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.nanolett.1c02981

**Notes**

The authors declare no competing financial interest.
ACKNOWLEDGMENTS

R.B. and E.V. acknowledge support from the Foxconn Interconnect Technology sponsored Center for Networked Intelligent Components and Environments (C-NICE) and the Jump Applied Research through Community Health through Engineering and Simulation (ARCHES) endowment through the Health Care Engineering Systems Center at the University of Illinois at Urbana–Champaign. We also thank the National Science Foundation for a Rapid Response Research (RAPID) grant (award 2028431) to R.B. and E.V.

REFERENCES
