

The Successes and Challenges of SARS-CoV-2 Molecular Testing in the United States



Jennifer Dien Bard, PhD^{a,b,*}, N. Esther Babady, PhD^{c,d}

KEYWORDS

• Challenges • COVID-19 • Molecular • SARS-CoV-2 • Successes

KEY POINTS

- Molecular technological advances throughout the SARS-CoV-2 pandemic are far superior to what was witnessed during the SARS pandemic.
- There have been many successes of SARS-CoV-2 molecular testing in the United States, including the development of rapid and novel molecular diagnostic assays; the leveraging of the high adaptability of molecular tests; and the integration of SARS-CoV-2 genotyping into public health, clinical, and research laboratories.
- The challenges related to SARS-CoV-2 molecular testing, such as regulatory hurdles, supply chain constraints, and laboratory preparation, should also be recognized and addressed to ensure that we are well prepared for future pandemics.

INTRODUCTION

On December 31, 2019, the world was alerted to the possibility of an emerging viral pathogen in Wuhan, China, causing a pneumonia syndrome reminiscent of the disease caused by the severe acute respiratory syndrome coronavirus (SARS-CoV). The first SARS pandemic, which similarly emerged in China, started in November 2002 and spread to 26 countries, causing 774 deaths over 11 months. SARS was defined in March 2003 after several months of investigation, and the whole genome of SARS-CoV was available approximately 1 month later.¹ As such, the majority of SARS diagnoses made over the duration of the pandemic were primarily based on

^a Department of Pathology and Laboratory Medicine, Children's Hospital Los Angeles, 4650 Sunset Blvd, MS#32, Los Angeles, CA 90027, USA; ^b Department of Pathology, Keck School of Medicine of the University of Southern California, Los Angeles, CA, USA; ^c Clinical Microbiology Service, Department of Pathology and Laboratory Medicine, Memorial Sloan Kettering Cancer Center, 327 East 64th Street, CLM-522, NY 10065, USA; ^d Infectious Disease Service, Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY, USA

* Corresponding author. Department of Pathology and Laboratory Medicine, Children's Hospital Los Angeles, 4650 Sunset Blvd, MS#32, Los Angeles, CA 90027, USA
E-mail address: jdienbard@chla.usc.edu

the clinical case definition established by the World Health Organization (WHO), which had a sensitivity of 26% and specificity of 96%.² Fast forward 17 years and scientists in China reported the identification of a novel coronavirus, eventually called SARS-CoV-2, on January 9, 2020, as the cause of the coronavirus disease 2019 (COVID-19) syndrome. The identification of SARS-CoV-2 was less than 2 weeks from the initial report made to the WHO, and the whole genome of the virus was made publicly available the next day on January 10, 2020. By January 13, 2020, the first protocol for a reverse-transcriptase polymerase chain reaction (RT-PCR) for laboratory diagnosis was published.³ The incredible speed with which SARS-CoV-2 was identified and diagnostic methods developed was due in great part to the wider use of molecular methods in 2019 compared with 2002 during the SARS pandemic. From rapid, point-of-care RT-PCR tests to next-generation sequencing (NGS) assays, molecular methods have played a critical role in this pandemic. The goal of this review is to highlight the successes of molecular testing in the United States over the course of the pandemic and to also discuss the many challenges encountered and how the lessons learned during this pandemic, which is ongoing as of the time of writing, should allow for improved preparation of the next.

SUCCESSSES

Rapid Development of Molecular Tests

The initial discovery and identification of SARS-CoV-2 as a novel virus relied heavily on the use of molecular diagnostic assays⁴. In their study, Zhu and colleagues used a commercial multiplexed respiratory viral panel (RespiFinderSmart22kit, PathoFinder BV) that targeted 18 viruses and 4 bacteria. The lack of detection on the multiplexed panel prompted further investigations of the underlying cause of this pneumonia syndrome using unbiased whole-genome sequencing on both clinical samples and viruses grown in human respiratory epithelial cell cultures. The assembled genomes closely matched those of known beta coronaviruses, allowing development of a targeted, pan-beta coronavirus real-time PCR that further confirmed the presence of this novel virus in clinical samples. This initial study foreshadowed the vital role that molecular diagnostic methods would have in the management of this disease.

The rapid availability of the first SARS-CoV-2 genomes, which were sequenced and deposited in the China National Microbiological Data Center in early January, was crucial for laboratories across the world to start designing primers and probes to detect unique viral genome regions of this novel virus.⁵ In the United States, the first published RT-PCR assay was designed by the Centers for Disease Control and Prevention (CDC) and used to confirm the first case of COVID-19 from a returning traveler from Wuhan, China.⁶ This assay initially targeted three distinct sequences of the gene encoding the nucleocapsid (N) protein of SARS-CoV-2, with a positive result requiring the detection of at least 2 of the 3 targets.⁷ On February 4, the CDC became the first institution to receive emergency use authorization (EUA) only regulatory status from the US Food and Drug Administration (FDA) for their COVID-19 RT-PCR. Initial rollout of the CDC tests to public health laboratories was met with several challenges secondary to inconsistent assay performance, which required the test to be modified and optimized to focus on the detection of 2 instead of 3 sequences of the N gene, with results reported either positive (two targets detected) or presumptive (only one target detected). Results were invalid if the internal control, human RNase P, was not detected.⁸ Despite the delays in the rollout of the CDC assay, the publicly available information on the primers and probes of the CDC COVID-19 test offered many high-complexity laboratories in the United States the option to adopt or modify the CDC

test to support case ascertainment of COVID-19 at the local level. In the early days of the pandemic in the United States, this was a major success of molecular method. The CDC COVID-19 test was eventually removed from the list of FDA EUA tests in the summer of 2021, at a point when other commercial assays had become readily available.

Development of Novel Molecular Tests

In addition to well-established nucleic acid amplification tests (NAATs), emerging molecular technologies including clustered regularly interspaced short palindromic repeats (CRISPR)-based detection⁹ and digital PCRs were approved for in vitro diagnostic (IVD) use. Before the pandemic, CRISPR-based assays for infectious diseases were just beginning to show promise for use as a point-of-care diagnostic tool for Zika viruses and dengue viruses.^{10,11} During the pandemic, 2 assays, the Sherlock CRISPR SARS-CoV-2 test (Sherlock Biosciences, Inc) and the SARS-CoV-2 DETECTR (Mammoth Biosciences, Inc.) received FDA EUA status for SARS-CoV-2 detection. Both methods used a combination of reverse transcriptase-loop-mediated isothermal amplification (RT-LAMP) and CRISPR-based detection of the target RNA sequences. Although ultimately the goal of these CRISPR-based methods is detection at the point of care, the current EUA assays still require instruments to measure fluorescence (e.g., ABI 7500 Dx platforms or BioTek Plater reader). Unlike CRISPR-based methods, digital droplet PCR (ddPCR) has been in use for several years in the research space but had yet to achieve IVD status for the diagnosis of any infectious disease¹². Two assays, the Bio-Rad SARS-CoV-2 ddPCR test (Bio-Rad Laboratories, Inc.) and FastPlex Triplex SARS-CoV-2 (PreciGenome LLC) have now received FDA EUA status. Although there are currently no peer-reviewed publications on these specific assays, several studies have reported increased sensitivity of ddPCR assays compared with quantitative PCR (qPCR), showing its potential as a valid alternative.^{13,14} Furthermore, the ability of ddPCR to provide absolute quantification could fill an existing gap related to the inability to perform viral load measurement for disease monitoring and infection control purposes. For the time being, currently authorized ddPCR platforms remain qualitative only and turnaround time to results are longer than for most qPCR instruments. Digital PCR instruments are also not as widely available in clinical microbiology laboratories as PCR thermocyclers.

One of the major successes of molecular testing during the pandemic, was the availability of rapid, point-of-care molecular tests. The ID Now COVID-19 test (Abbott Diagnostics Inc., Scarborough, ME) is an isothermal NAAT designed for testing on the ID Now platform, which is cleared for use at the point of care and provides results in as little as 5 minutes. Early studies comparing the performance of the ID Now COVID-19 test to other PCR tests showed sensitivity ranging from 55% to 98% when compared with SARS-CoV-2 PCRs, highlighting the lower sensitivity of RT-LAMP methods compared with PCR.¹⁵⁻¹⁸ The pandemic also gave rise to the first-ever FDA EUAs conferred for Clinical Laboratory Improvement Amendment (CLIA)-waived IVD molecular assays that can be performed at home. These assays combine the speed of antigen testing with the sensitivity expected from conventional laboratory molecular tests.¹⁹ Four platforms, the Lucira COVID-19 All-in-One test, the Lucira CHECK-IT COVID-19 test, the Cue COVID-19 test, and the Detect COVID-19 test are currently on the market. All 4 tests use RT-LAMP to amplify and detect SARS-CoV-2 RNA in less than 30 minutes from self-collected nasal swabs. Whereas these assays provide an opportunity for individuals to self-test at home, they are still not readily available and are relatively expensive compared with rapid antigen tests. There are also currently no available peer-reviewed data on their clinical and analytical performance compared with real-time RT-PCR tests, but given that amplification is isothermal, it is

expected that sensitivity would be lower (though higher than for rapid antigen tests). Data from the manufacturer report the limit of detection (LOD) as 800 to 1300 copies/mL. Therefore, a negative test in a symptomatic patient should be confirmed with a PCR test, which is similar to algorithms that rely on antigen testing.

High Adaptability of Molecular Testing

Not only was molecular testing critical for diagnosing symptomatic individuals with COVID-19, it played a key role in keeping places such as health care settings and schools as safe as possible through surveillance testing of asymptomatic individuals. The ability to maintain highly sensitive and accurate testing at a large scale was largely due to the inherent adaptability of molecular testing. The ability to modify nearly any aspect of the preanalytical and analytical components of a PCR testing protocol, from collection devices to amplification techniques, allows it to be tailored to the specific needs of the setting in which it is deployed while optimizing its robustness to shortages in supplies and labor.

Preanalytical accommodations

Alternate Sample Collection Devices. Shortages of collection devices, such as swabs, prompted commercial companies and laboratories to pursue alternative methods for specimen collection. A lack of viral transport media (VTM) prompted the exploration of VTM-free protocols, such as collection in phosphate-buffered saline (PBS), Hanks' balanced salt solution (HBSS) or use of "dry" swabs that would be eluted in a small amount of media upon arrival at the testing laboratory. The performance of PCR in the detection of SARS-CoV-2 from these alternate buffers have been demonstrated to be comparable to VTM.^{20,21} Detection of SARS-CoV-2 RNA by PCR appears to be robust at various temperatures and after exposure to multiple freeze thaw cycles. The logarithmic increase in testing needs early in the pandemic resulted in a sudden and dramatic shortage in swabs that was mitigated either by the foresight to hoard tens of thousands of swabs for internal use, the acquisition of swabs from less well-known sources, or to explore the use of 3D-printed swabs. The rapid adoption of 3D printing is a prime example of the creativity and the perseverance of laboratorians to dodge interruptions in clinical service. The performance of these swabs was found to be equivalent to flocked swabs, paving the way for a stable supply of this critical resource.²²

Alternative Sample Types. Molecular testing is technically agnostic to sample type. However, in order for results to be meaningful for a provider, the body site must be considered clinically relevant and the sample type must have undergone sufficient validation. Although the collection of nasopharyngeal swabs (NPS) is considered the gold standard specimen collection method for SARS-CoV-2, several challenges have prompted commercial partners and CLIA-certified laboratories to explore alternate specimen sources that does not require specialized collection devices (e.g., NPSs and VTM). These challenges include the often painful or uncomfortable collection process, the need for personal protective equipment (PPE) for health care workers performing testing and, as mentioned above, supply chain shortages.²³ Thus, considerable efforts were made to explore and implement alternate clinical specimen types for the molecular detection of SARS-CoV-2. Currently, the Infectious Diseases Society of America (IDSA) and the Centers for Disease Control and Prevention (CDC) have approved other specimen types for SARS-CoV-2 testing including oropharyngeal swabs (OPS), midturbinate swabs (MTS), anterior nares swabs (ANS), saliva, and lower respiratory specimens. Other biological specimens including stool, blood,

CSF, and urine have also been explored with limited success.²³ Saliva samples, which had not routinely been used to diagnose respiratory tract infections before the pandemic, became central to community testing programs in schools and universities. Much attention has been given to saliva because of the ease of collection, and the lack of need for a swab, buffer solution, and even PPE.²⁴ The sensitivity of molecular tests on saliva samples varied widely depending on the assays but performance as high as 95% has been reported,^{24–26} when compared with PCR testing on nasopharyngeal swabs and several assays have received FDA EUA for detection of SARS-CoV-2.¹⁹ As data emerged on the utility of alternative samples for SARS-CoV-2 detection, saliva samples as well as nasal and MTS samples were preferred over the use of the more established OPS²⁷. The success of molecular testing on saliva and other non-nasopharyngeal sample types will likely have a major impact on the diagnosis and management of many other infectious diseases post this pandemic.

Analytical Accommodations

Molecular testing approaches for SARS-CoV-2 have the flexibility to improve the speed and throughput of testing by bypassing external extraction steps and through specimen pooling algorithms, respectively. Neither approach is considered novel and they have been an integral part of laboratory medicine for many years before the COVID-19 pandemic. For example, many multiplexed syndromic testing panels for respiratory infections and gastroenteritis are built as sample-to-answer instruments that integrate nucleic acid extraction with amplification and signal detection. Results are typically available within 1 h.²⁸ The ability to modify or eliminate steps is an important feature of any molecular test, and particularly for PCR-based testing, as it can significantly reduce the time to result without affecting analytical sensitivity.^{18,29}

With regard to specimen pooling, combining multiple specimens before nucleic acid extraction can be easily accommodated from the analytical perspective as the extraction process itself remains the same. Specimen pooling algorithms have been successfully deployed for other infectious diseases such as HIV,³⁰ with minimal effects on sensitivity. Pooling studies for SARS-CoV-2 using PCR-based methods and other molecular approaches like CRISPR have found that sensitivity can be maintained when up to 5 specimens are pooled, with decreases thereafter as the number of specimens included in the pool increases.³¹ Pooling can be an effective approach for asymptomatic surveillance in the community to facilitate early detection of COVID-19, provided that disease prevalence remains low.³²

Application of Molecular Testing for SARS-CoV-2 Genomic Surveillance

The availability of NGS to investigate the initial Wuhan clusters and produce the first complete genome of SARS-CoV-2 within days was crucial to diagnostic assay development and to the molecular characterization of this novel virus.⁴ As a public health tool, NGS has had a transformative role in replacing older typing methods to investigate outbreaks and for surveillance of known and emerging pathogens.^{33–35} Genomic surveillance of SARS-CoV-2 by NGS is without a doubt one of the biggest successes of molecular testing during the pandemic and highlights the benefits of global collaboration and open data sharing.

The initial investment in whole genome sequencing of SARS-CoV-2 was made by research and public health laboratories interested in monitoring and investigating SARS-CoV-2 genome evolution.³⁶ However, when genomic changes, including single nucleotide polymorphisms (SNPs) and deletions, were observed to impact the virus transmission, infectivity and detection by diagnostic tests, the interest and need for genomic surveillance expanded significantly.^{37,38} In November 2020, Public Health

England reported an increased in COVID-19 cases associated a variant of SARS-CoV-2, now referred to as the Alpha variant, that was characterized by key changes (N501Y and P618H) and deletions (del69–70) in the spike gene³⁹. These changes affected the performance of assays targeting the S gene and were easily identified as S gene target failures (SGTFs) when using the affected PCR tests. The impact of emerging mutations in the SARS-CoV-2 genome over the course of the pandemic underscored the importance of the approach taken early in the pandemic to design molecular tests with multiple genomic targets. With the continual accrual of global data on the impact of emerging variants on the analytical performance of molecular diagnostic tests, the FDA issued guidance for all manufacturers of SARS-CoV-2 diagnostics tests to monitor and confirmed continued performance of their EUA tests.⁴⁰ The approval of SARS-CoV-2 vaccines in the fall of 2020 provided further incentive to use NGS to monitor genome evolution and the potential for emerging variants to reduce neutralization from antibodies acquired through natural infection, vaccination, or through monoclonal antibody therapeutics.

As genomic surveillance of SARS-CoV-2 expanded, the WHO in collaboration with other public health networks including the US SARS-CoV-2 Interagency Group (SIG), developed a naming scheme to classify variants based on their potential impact on the pandemic, including variants of interest (VOI) and variants of concerns (VOC). SARS-CoV-2 variants with mutations that are predicted or known to affect transmissibility, disease severity, immunity, diagnostic accuracy, or therapeutic success were considered VOIs. VOCs were VOIs for which there was actual evidence of increased transmissibility or increased virulence, decreased protection from vaccination or previous infection, diagnostic failures or reduced effectiveness of therapeutics.^{41,42} As VOIs and VOCs started to emerge in many countries, a naming scheme that used the Greek alphabet was developed by WHO experts to facilitate discussions by nonscientific audiences and to prevent the stigma associated with naming VOIs/VOCs after the countries in which they were first identified. Beyond global surveillance testing to monitor emerging SARS-CoV-2 lineages, NGS performed in clinical laboratories provided data that supported local hospital outbreak investigations and transmission events or local efforts to monitor and establish links between emerging variants and vaccines breakthroughs.^{43–46}

Implementation of NGS in clinical laboratories for genomic surveillance was challenging, particularly as traditional NAAT-based diagnostic testing required constant human, material, and financial support throughout the pandemic. Furthermore, for most clinical virology and microbiology laboratories, NGS was not a technique used routinely and thus to perform surveillance in-house required a significant amount of new investment not only in NGS instrumentation but also in technologists with strong skillsets for molecular techniques as well as in staff with the ability to use bioinformatic tools for data analysis of SARS-CoV-2 sequences. The complexity of NGS for routine surveillance of SARS-CoV-2 prompted the development of targeted PCR to identify specific VOC/VOI through detection of known key mutations^{47,48}. This approach took advantages of existing skills and infrastructures in clinical laboratories to rapidly identify VOC/VOI. However, as successive waves of the pandemic increased and decreased and the frequencies of VOC/VOI changed frequently, the utility of targeted approaches became limited.

NGS has become the method of choice for surveillance of SARS-CoV-2 as it is agnostic to variants. However, the use of NGS comes with its own set of challenges that include the introduction of artifacts into output datasets by the methods (tilled amplicons vs metagenomics), and platforms (e.g., long reads vs short reads) used. It is essential that the chosen bioinformatic pipelines are validated to reduce the risk of bias in data interpretation and standardization⁴⁹. Despite the increased complexity

of NGS, there is increasing interest and a potential role in offering SARS-CoV-2 genotyping as a clinical test as it may contribute to the selection or avoidance of some monoclonal antibody or antiviral therapies.⁵⁰

The success of NGS during the SARS-CoV-2 pandemic was also due in part to the increase in data sharing globally, primarily through the Global Initiative on Sharing All Influenza Data (GISAID) initiative, which pivoted early in the pandemic to developing and providing tools that enabled validation and free data sharing of SARS-CoV-2 genomes as well as for visualization and real-time tracking.⁵¹ As of February 2022, more than 7,700,000 SARS-CoV-2 genomes have been submitted to GISAID. Although submissions are heavily biased toward laboratories from high and middle-income countries, the free access and the extent of sharing of genomic data have been unprecedented and has allowed for truly global surveillance of viral evolution. In addition to GISAID and other data repository tools (e.g., NCBI GenBank), open software platforms such as Nextstrain and Phylogenetic Assignment of Named Global Outbreak Lineages (PANGOLIN) have facilitated the assignments of SARS-CoV-2 lineages, particularly for laboratories with limited bioinformatics skills.^{52–55}

CHALLENGES AND TRIALS

Although the successes of molecular testing are worthy of celebration, we would be remiss to not discuss their failures as well. Among them are the bureaucratic failures that severely delayed the deployment of these vital tests at a critical moment early in the pandemic, global supply chain bottlenecks, the inability of molecular tests to assess contagiousness, the paucity of skilled laboratory scientists to meet testing demands, and the lack of coordination with state and federal public health laboratories.

US FDA Regulatory Hurdles

Although the CDC was able to achieve EUA status for their COVID-19 assay, most clinical laboratories were not equipped to undertake the challenges associated with the complexity and length of an FDA EUA submission. However, as cases of COVID-19 started to increase and local transmission became evident, clinical laboratories and commercial entities appropriately worked diligently to submit SARS-CoV-2 PCR tests for FDA review and approval. It became immediately apparent that a significant challenge was the lack of a readily available gold standard. Most laboratories at the beginning of the pandemic did not have access to samples positive for SARS-CoV-2 or to well-characterized viral isolates. Furthermore, guidance was issued early in 2020 advising against the performance of viral cultures on any respiratory samples, unless done in a Biosafety level 3 (BSL-3) laboratory, which is not available in many clinical laboratories. These hurdles made establishing the performance characteristics of an assay for this novel pathogen quite challenging. Ultimately, on February 28, the FDA issued a guidance for high-complexity laboratories and diagnostic manufacturers that provided a simplified path to developing and obtaining EUA clearance for molecular diagnostic tests.⁵⁶ This guidance allowed laboratories to perform validation using viral RNA transcripts in the absence of true clinical samples. Only after that point were both clinical laboratories and commercial vendors able to move forward with developing molecular tests for SARS-CoV-2 RNA detection. In a matter of a few months, hundreds of SARS-CoV-2 molecular tests became available.

The Supply Chain

One of the biggest challenges of the pandemic has undoubtedly been an inadequate supply chain. Global travel and economic output came to a standstill in spring 2020

because of the strict lockdowns occurring in many parts of the world simultaneously. At the same time, once molecular tests were implemented in clinical laboratories, cases surged and with it, an unexpected and unprecedented demand for rapid testing. This led to a significant mismatch between supplies and demand for one of the most critical operations during the pandemic. The challenges associated with limited supplies from sample collection devices (e.g., viral transport media, nasopharyngeal swabs) to PCR reagents and instruments, created the need for validating alternative molecular testing methods including RT-LAMP and transcription-mediated amplification (TMA), and alternative sample types to minimize the need for specialized collection kits or NAAT reagents. The first nonPCR NAATs to become commercially available were based on isothermal amplification (e.g., ID Now COVID-19 Test, Abbott Inc.; Solana SARS-CoV-2 Assay, Quidel Inc.), with some having the added benefit of being able to use dry nasal swabs.¹⁹ As described previously, the analytical sensitivity of various RT-LAMP assays was lower than PCR. However, the performance of SARS-CoV-2 TMA assays (e.g., Aptima SARS-CoV-2 Assay, Hologic Inc.) showed similar sensitivity and specificity compared with PCRs, allowing for further expansion of sensitive and accurate molecular tests during the pandemic.⁵⁷

The challenges associated with the supply chain and the costs of molecular tests led to increasing delays in resulting turn-around times early in the pandemic. In response, nonmolecular tests, primarily antigen-based, were developed to further expand testing and also fill the gap for rapid, point-of-care testing. To date, over 25 antigen tests have received the FDA EUA status.⁵⁸ The lower sensitivity of antigen assays, which do not employ amplification methods, compared with molecular tests, was an anticipated challenge based on prior experience with rapid influenza diagnostics tests (RIDTs). Numerous studies have now shown rapid antigen tests for SARS-CoV-2 to have sensitivities as low as 35% in asymptomatic patients compared with PCRs, though this improves to over 90% for samples with high viral loads (e.g., Ct values < 30).^{59–62} Results of studies varied widely depending on the patient population tested (e.g., symptomatic vs asymptomatic patients), the type of tests (e.g., lateral-flow assays vs high-throughput chemiluminescence assays) and the timing of testing (e.g., early vs late infection). As such, recommendations from the IDSA guidelines still support the use of molecular over antigen testing whenever possible.⁶³ The CDC guidelines similarly suggest an approach that takes into consideration the patient population and the goal of testing to optimize the use of antigen testing with recommendations to follow up negative antigen tests with molecular testing in cases of high suspicion of infection.⁶⁴

Dead or Alive?

A major challenge for all types of diagnostic testing for SARS-CoV-2 is the inability to discriminate between actively replicating virus and viral RNA fragments. This is particularly the case for highly sensitive molecular testing assays. Early in the pandemic, the CDC recommended performing a NAAT test for “clearance” of infection. However, it became rapidly evident that it was not uncommon to detect viral RNA fragments for weeks or months after an individual has completely recovered from COVID-19.⁶⁵ As such, the CDC modified their previous guidance to remove testing for clearance and shift to a time-based strategy for safely returning back to work or society.⁶⁶

Some have championed the use of viral cultures to determine active infection or “infectivity,”⁶⁷ which is unrealistic in practice as performing viral cultures is a time consuming process that requires the availability of a BSL-3 laboratory. Due to low sensitivity, viral cultures are also considered a suboptimal reference method compared with NAAT and the reason for why the majority of clinical laboratories have abandoned its use for the detection of most viral pathogens.⁶⁸

The use of rapid antigen tests (RAT) has also been proposed as a scalable and relatively cheap means to detect “infectious” cases. The association between a positive antigen test and contagiousness has been attributed to its lower sensitivity—meaning it should in theory only detect samples with high viral load or low cycle threshold (Ct) values seen by PCR.⁶⁹ Unfortunately, data correlating Ct values and infectivity are lacking and the widespread use of RAT has its own unique challenges. Additional discussion on the strengths and weaknesses of RAT for SARS-CoV-2 is covered in a separate article in this issue.

Lastly, a molecular approach that has been proposed to identify actively replicating virus compared with “dead” virus is the detection of minus-strand SARS-CoV-2 RNA⁷⁰ or subgenomic RNA.⁷¹ These molecular testing approaches are tools that may assist in the determination of infectiousness in certain clinical contexts, such as in immunocompromised patients with prolonged shedding,⁷² and could inform isolation strategies in a hospital setting.⁷⁰ However, conflicting data have been reported for the role of subgenomic RNA as a suitable indicator of actively replicating virus.⁷³ Clinical laboratories that wish to explore either minus-strand SARS-CoV RNA or subgenomic RNA RT-PCR should do so in the context of clinical and epidemiologic findings.

Limited Molecular Testing Capability and Shortage of Highly Skilled Molecular Technologists

The lack of molecular testing capability in many clinical laboratories, particularly nonacademic medical centers, was and remains a significant challenge. Early in the pandemic the only tests available had to be performed in laboratory spaces approved for high complexity testing. Unfortunately, many clinical laboratories lack that level of infrastructure and expertise. This shifted the testing demand to reference laboratories, which quickly became overwhelmed themselves. It was not uncommon to hear about testing delays of up to 10 days from time of collection. As commercial kits were granted EUA by the FDA, the dependency on reference laboratories shifted to dependency on diagnostic companies that offer moderately complexed testing kits (i.e., sample-to-answer molecular tests) that do not require external extraction step and molecular expertise.

The availability of rapid, point-of-care and at-home testing also highlighted one of the major challenges of the pandemic, namely the staffing of high-complexity laboratories with skilled technologists to run molecular testing. The shortage of qualified staff preceded the pandemic but the problem was greatly exacerbated by the stresses placed upon the laboratory by the massive testing demand. Many clinical laboratories resorted to collaboration with their research counterparts to find the necessary people with appropriate skills for performing molecular clinical testing. This also required that licensing be suspended in many places for the duration of the pandemic. While the COVID-19 pandemic is still ongoing, efforts to anticipate future challenges will need to consider how solutions implemented during this pandemic to manage staffing and supply shortages can be readily reactivated when the next pandemic hits.

In order to learn from this challenge, steps must be put in place before the next pandemic. These include significant investments in clinical laboratories including (1) establishment of molecular testing infrastructure in clinical laboratories; (2) increase in the training of medical laboratory scientists with a focus on development of molecular skills; and (3) requirement of doctoral level trained clinical microbiologists to oversee clinical microbiology laboratories. Unless improvements are made it is very likely that the laboratory community will encounter the same issues as with the COVID-19 pandemic. An opinion editorial in the *New York Times* by Dr. Robin Patel (Mayo Clinic) and Dr. Stefano Bertuzzi (CEO, America Society for Microbiology) suggested the need

for a “biomedical scientists version of the national guard” that would be activated in times of need to prevent the staffing challenges experienced during the COVID-19 pandemic.⁶⁴

Clinical Versus Public Health Laboratories

The dependency of SARS-CoV-2 molecular testing, particularly early on in the pandemic, brought to light the disconnect between clinical laboratories and their public health laboratory partners. As per the WHO, a strong national infectious disease diagnostic and surveillance testing strategy should have a robust public health laboratory network as well as clinical laboratories and emphasizes the importance of the interconnectedness of both.⁷⁴ For clinical laboratories, collaboration with public health laboratories is critical as it may be the only source for clinical specimens required for validation of molecular testing platforms. In hindsight, the lack of standardized assistance and services offered to clinical laboratories in the US may have contributed to delays in implementation of SARS-CoV-2 molecular testing in laboratories capable of performing high complexity testing.

Early recognition of the critical role of the clinical laboratories in combating a pandemic is needed. An approach akin to the Laboratory Response Network for detection of biological terrorism that includes clinical laboratories as sentinel laboratories for early detection of the pathogen of interests may be considered. Standardization from the CDC level to all public health laboratories regardless of states or regions to offer early support to clinical laboratories can also help mitigate some of the issues encountered during this pandemic. This would include providing specimens or standards to accelerate the validation and implementation of the molecular test. The COVID-19 pandemic has taught us that there is no success in being siloed.

SUMMARY

Since the start of the pandemic, we continue to witness significant innovation in the laboratory and at the point of care. The pandemic also expedited the advancement of infectious diseases genomic surveillance in both public health and clinical laboratories for variant detection and outbreak investigation. As such, molecular development has grown by leaps and bounds over the past 2 years and it behooves us to (i) take advantage of this innovation wave and explore opportunities for other infectious diseases and (ii) recognize and learn from the challenges encountered to ensure that we are not victims to it when the next pandemic arrives on our shores.

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