SARS-CoV-2 Testing

Trials and Tribulations

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Coronavirus disease 2019 (COVID-19), the disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), started in the Wuhan province of China and is now a pandemic that has caused a great number of deaths across the globe.[1,2] The number of cases in the United States is increasing steadily, and the epidemic curve mimics the start of the infection in both China and Italy. Due to challenges associated with ramping up testing capacity, reliable estimates of the number of infections in the United States are not available. Multiple people, including Anthony Fauci, MD, Director of the National Institute of Allergy and Infectious Diseases, have stated that testing for COVID-19 has been problematic,[3] with some dubbing the situation "testgate." Below we will explore the evolution of tests in the United States, alternative tests, the logistics of increasing testing, and issues regarding laboratory staffing in response to the increased demands of testing.

As data emerged from the epicenter of the outbreak in Wuhan, China, the Centers for Disease Control and Prevention (CDC) designed a nucleic acid amplification test that targeted three independent regions of the SARS-CoV-2 nucleocapsid gene (N1, N2, N3), in addition to the human ribonuclease P (RNase P) gene, which serves both as an internal amplification control and indicator of specimen adequacy. Importantly, detection of each target required a separate polymerase chain reaction (PCR), ie, four reactions were required for each specimen tested. This test received Emergency Use Authorization (EUA) from the Food and Drug Administration (FDA) on February 4, 2020, with the limitation that it could be used only at the CDC. Given the limited test capacity, the CDC implemented a triage system in which only symptomatic patients with positive travel history or epidemiologic contacts (persons under investigation [PUI]) were tested. As focal disease spread in the United States, state health laboratories were permitted to perform the CDC developed assay.[4] Unfortunately, manufacturing errors, along with cross-reactivity of the N3 primer with other coronaviruses, led to a new round of test validations, which took precious time as the pandemic was spreading. In addition, the definition of a PUI was retained, although it was evident that testing capabilities needed to be increased, as the number of people that required testing was exponentially growing. In particular, the slow turnaround time associated with sending out of the test led to the inability of providers to discontinue isolation precautions and creation of hospital backlogs. At the time, the main barrier to expansion of testing was the inability of hospital laboratories to develop in-house molecular tests due to restrictions placed on them by complex regulatory frameworks.

On February 29, 2020, the FDA issued guidance to laboratories specifying the requirements for an accelerated EUA pathway, which included the ability for Clinical Laboratory Improvement Amendments (CLIA) certified "high complexity" laboratories to design, manufacture, or obtain reagents (including those labeled as research use only) necessary to develop their own tests for the detection of SARS-CoV-2.[5] Many centers and referral laboratories rushed to develop the test, guided by the CDC, but the possibility to scale up the test was challenging, as it required 3 reverse transcription PCR wells (N1, N2, and RP) to test one patient sample. Although the guidance was certainly helpful, a source for reference material for the test verification was not provided, and laboratories

had to wait until positive patients were available after they had been tested by a state health laboratory. Once laboratories were running tests in-house, testing tripled or quadrupled the capacity that the CDC and state health laboratories could perform. However, for most persons that needed testing, including those that had symptoms, health care workers, others who had been exposed, and the worried, there still existed a shortage of tests. This bottleneck placed laboratories in the uncomfortable position of prioritizing samples: Should this be prioritized by severity of illness, bed flow process (emergency room patients over admitted patients), or staffing needs (health care workers over patients)? Also looming are the shortages of collection swabs, viral transport media, RNA extraction and other reagents, and consumables.

Another issue that arose, which has added yet another burden to laboratories, was the desire of primary care providers, hospital administration, and individual patients to be notified immediately of negative or positive test results, placing undue burden on laboratories. Clearly, if the result cannot be relayed electronically it is important that the result be relayed via phone call. However, this is not a critical value in the classical sense. A positive test can occur in a mildly symptomatic patient or in a severely ill one and the result does not necessarily dictate treatment options, as these decisions are guided by the clinical severity. In addition, the value is easily flagged by electronic health care systems, obviating the need for a call. On the other hand, a negative result may be of higher importance, as it is necessary to get the patient out of isolation and assist in the rationing of valuable hospital resources. However, negative results can occur in the inpatient or outpatient setting. Thus, a system of reporting the negative results to whoever is responsible for discontinuing isolation in inpatients is probably most useful for the health care system, and can be achieved by different means such as creating electronic patient lists that can be distributed swiftly to the correct authorities (such as hospital infection prevention personnel) or by creating automated reports that are sent to units where patients are housed.

Demand for testing continues to increase exponentially as the pandemic grows, and commercial vendors continue to develop tests in their platforms and seek FDA authorization (a continually updated list of all available EUA tests is available at https://www.fda.gov/medical-devices/emergency-situations-medical-devices/emergency-use-authorizations). Commercial vendors are using a plethora of platforms including high throughput or cartridge-based instruments that promise shorter testing turnaround times. Despite these efforts, we may still find ourselves behind the curve as case numbers continue to rise. Our laboratories have to continue to work under the CLIA regulatory framework, and verification of these commercial assays does not happen overnight. In short, even with all the efforts, being able to scale up testing of molecular technologies, the current reference standard, is a significant hurdle and may not be enough to keep up with demand.

Serologic tests, either detecting antibodies or antigens, have not yet risen to the top during this pandemic; however, they could be very useful, as they were during the 2002 SARS outbreak.[6] In general, serologic tests are cheaper, require a shorter analytical time, and throughput can be greater compared to molecular testing. Even though generation of IgM antibodies could occur as rapidly as genetic viral material can be detected in respiratory specimens, the timeframe for production ranges from less than 5 days after symptom onset to 10 days or longer, limiting the applicability of serologic tests for diagnosis of acute infections.[7] Because of the lag in availability of PCR testing and depending on the timeframe in which the patient presents, these tests may be helpful to fill the testing gap. In addition, if there is a shortage of swabs, tubes to draw blood are usually plentiful. From a public health perspective, testing for presence of IgG anti-SARS-CoV-2 could determine who has been exposed and better define the possibility of asymptomatic infections and give us truer estimates of case counts and mortality. In addition, determination of serostatus may be useful for identifying individuals who have been infected but suffered only minor symptoms and did not seek medical attention. If these seroconverted individuals are health care workers, they could potentially safely care for patients if personal protective equipment is limited. Tests that detect SARS-CoV-2 antigens in respiratory samples or blood would likely not be as sensitive as molecular tests; however, similar to serologic tests, they may have the advantage of being cheaper, having shorter turnaround analytical time, and having higher throughput. Serologic tests could be an alternative to increasing testing capabilities, particularly when used as part of an algorithmic approach combined with molecular testing.

As hospitals start seeing more COVID-19 infections, several measures are being taken, eg, cancelation of elective surgeries or of screening procedures such as colonoscopies or mammograms. Thus, our laboratories will have areas with minimal need of staffing, while in others there will be a surge in need to perform different tasks. The surge will likely be happening in sample processing, sample referral (even when the test is performed in-house), molecular testing, result reporting of the new tests, and microbiology/molecular testing for other respiratory viruses and bacteria, to name a few areas. Here the question is how fast can medical laboratory scientists be trained from areas that have a decrease in testing, as well

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as pathology residents and faculty, to do different tasks needed in times of crisis. We will also face staffing shortages as personnel become infected or if they are required to stay home with their children due to school closures. Having a staffing plan defining which tests your laboratory can perform based on the number of personnel present is imperative in this crisis.

The COVID-19 pandemic is happening as this editorial is being written, and the circumstances are rapidly changing day to day. An analogous situation is currently being faced by physicians in Italy, with escalating numbers of critically ill patients and dwindling capacity for ventilatory support. Calls to frontline staff have been made for a "soft utilitarian" approach in the face of resource scarcity. We hope to have highlighted the challenges of testing capabilities in the midst of a pandemic outbreak of a novel pathogen. As laboratorians we need to stay informed of the hospital situation and policies, as they will be changing continually. We need to disseminate information to our staff, be nimble in our response, and relay information to the hospital administration about the situation in the laboratory. In other words, manage the flow of information simultaneously from both above and below.

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