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COMMENTARY

The COVID-19 Diagnostic Dilemma: a Clinician's Perspective

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ABSTRACT In this commentary, we provide a broad overview of how the rapidly evolving coronavirus disease 2019 (COVID-19) diagnostic landscape has impacted clinical care during the COVID-19 pandemic. We review aspects of both molecular and serologic testing and discuss the logistical challenges faced with each. We also highlight the progress that has been made in the development and implementation of these assays as well as the need for ongoing improvement in diagnostic testing capabilities.

KEYWORDS COVID-19, SARS-CoV-2, molecular diagnostics, serologic testing

The pandemic due to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a newly described human coronavirus causing coronavirus disease 2019 (COVID-19), has reached catastrophic levels in many parts of the world. In the United States, hospitals in highly impacted areas have struggled to manage the volume of patients presenting for care, many of whom require admission to the intensive care unit and ventilatory support. Accurate and timely (and accessible) diagnostic assays are a cornerstone of managing infectious diseases in the acute care setting. When a patient presents with a clinical syndrome compatible with COVID-19 (or some other acute respiratory infection), an assay that can reliably detect or rule out SARS-CoV-2 infection allows for immediate management decisions at the time of admission. Importantly, this includes decisions regarding the use of appropriate personal protective equipment (PPE) and isolation procedures to decrease the transmission of SARS-CoV-2 to health care workers and among other hospitalized patients.

Confirming a diagnosis of COVID-19 triggers a cascade of infection control and public health measures, including isolation or quarantine of the individual as well as contact tracing to aid in further case finding, if resources are available to do so. Once patients are hospitalized, confirming a COVID-19 diagnosis is also important since these patients are often grouped together on hospital floors or in intensive care units, creating "warm zones," in which all providers and other staff must remain in PPE. This helps to facilitate care and also helps to decrease the rate of consumption of PPE (gowns, gloves, surgical masks or N95 respirators, face and/or eye protection) by allowing providers to reuse some PPE components (masks, respirators, eye protection) while moving between patients, rather than donning and doffing PPE for each individual patient. There have been local shortages of various PPE components, such as N95 respirators, especially in hard-hit areas, such as New York City, mandating changes to local infection control practices, such as using N95 respirators only for patients undergoing aerosol-generating procedures or reusing these single-use respirators for days or weeks at a time.

Additionally, given the lack of currently available therapeutic agents which have demonstrated efficacy in treating SARS-CoV-2 infection, many patients with COVID-19 may also be evaluated for the eligibility for participation in clinical trials or to receive other experimental therapies (e.g., remdesivir, convalescent-phase plasma, immunomodulatory agents). In general, a confirmed positive result for SARS-CoV-2 by a molecular diagnostic assay is necessary to confirm eligibility for experimental treat-

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ments through a clinical trial or some other means, and such treatments typically cannot be administered to a patient without a confirmed diagnosis of COVID-19. Finally, confirmation of a COVID-19 diagnosis can aid in clinical decision making regarding optimal supportive care measures as well as in discussions regarding an individual patient's prognosis.

The limited availability of diagnostic assays for COVID-19 has plagued the initial response to the pandemic in the United States. This has led to confusion among health care providers, patients, and hospital administrators and has significantly hampered our ability to care for patients and protect those around us from infection. Herein, we provide our perspective on the use of diagnostic assays for SARS-CoV-2 during the first several months of the pandemic and reflect on the need for ongoing adaptation to the rapidly evolving landscape of SARS-CoV-2 diagnostic testing.

REVIEW OF MOLECULAR TESTING FOR SARS-CoV-2

Even before the first case of COVID-19 was identified in the United States, there were concerns regarding how to diagnose this infection among persons presenting with a compatible clinical syndrome who did not have any known sick contacts or other epidemiologic risk factors for infection. Given the successful implementation of molecular assays to detect SARS-CoV-2 RNA from a nasopharyngeal swab specimen in many other countries that were affected by the pandemic before the United States was and the excellent performance characteristics and availability of this type of assay for many other respiratory infections, from a clinician's perspective, it was unclear that there was much of a diagnostic challenge to surmount. However, after the pandemic began to spread in the United States, it immediately became clear that the availability of this diagnostic testing needed to be rapidly developed and scaled up. SARS-CoV-2 molecular testing was initially performed only at the CDC, with local state health departments collecting, processing, and forwarding samples to the CDC. This labor-intensive process generally resulted in a turnaround time for test results of at least several days. Further, access to CDC testing was initially strictly limited to patients who had a clear epidemiologic risk factor (e.g., travel to Wuhan, China) or who had a known close contact with another individual with confirmed COVID-19 or with COVID-19 testing already in process. In our experience, these early testing limitations prevented us from testing a patient with a compatible clinical syndrome whose husband had recently recovered from a similar viral illness soon after returning from a business trip to China (though not to Wuhan). Fortunately, this patient convalesced uneventfully at home, though anecdotal reports of similar scenarios in much sicker patients abound. Although the capability to perform molecular testing was soon expanded to a larger network of public health laboratories beyond the CDC, difficulties with logistics and capacity at these labs as well as challenges with the reliability of the test itself revealed that this was not an efficient or sustainable approach to diagnostic testing in the midst of a rapidly expanding pandemic.

As the molecular testing capacity eventually increased in response to demand and expanded to clinical laboratories across the United States, new challenges and questions arose. First, the performance characteristics of these assays were not immediately evident, which created great concern among providers, given the need to make immediate clinical and logistical decisions based upon the test results. While the analytic performance (i.e., reproducibility, lower limit of detection, specificity) of these assays allowed some early confidence in positive results, the evolving understanding of the true diagnostic performance (in particular, the sensitivity and negative predictive value) of individual assays made interpreting a negative result in an individual with a high pretest probability of COVID-19 more challenging. Multiple testing platforms came online in a very short period of time, further adding to the confusion over test accuracy and interpretation of test results. At times, there were discrepant results when a single sample was run across different molecular platforms as part of subsequent validation efforts, which not only highlighted the variability of the performance characteristics among the available assays (and/or variability in the techniques used to perform some

of the earlier nonautomated testing) but also had a huge individual and system-wide impact on those few patients whose test results were amended from negative to positive and on the health care workers and other hospitalized patients with whom they may have been in contact.

Beyond issues with tests already in hand, the supply chain for obtaining additional diagnostic tests as well as testing supplies (e.g., nasopharyngeal swabs) and reagents (e.g., viral transport media) remained unpredictable at best. An unprecedented rapid and elevated global demand for these products paired with a limited number of manufacturers, many of whom had operations limited by the local effects of the pandemic, combined to create limitations in these supplies that persist even months later. Given these supply issues, access to multiple testing platforms, when possible, was essential to maintaining the ongoing availability of diagnostic testing in order to limit reliance on any one assay. At our institution, after extensive efforts by our clinical microbiology leadership, we incrementally gained access to seven different molecular assays, which, although costly and logistically challenging, allowed for maximum flexibility in dealing with the limited availability of individual assays. The reality of limited test availability led to the rationing of molecular testing in the early phases of the pandemic and the need to prioritize testing for patients who might benefit the most from a clinical trial or experimental therapy (and who needed a confirmed diagnosis to do so), as well as for the myriad infection control decisions that needed to be made to keep hospitalized patients and health care workers safe. At our center, daily briefing calls were held which included leaders from clinical microbiology, infection control, multiple clinical services, and the hospital administration, who collectively reviewed the number of available tests (and the number of tests that may or may not arrive in the coming days), which molecular platforms (i.e., rapid versus nonrapid) were thus available, and how these tests would best be allocated across the hospital system. These conversations would then inform testing at various patient care locations and would, for that day, inform clinical decision making, the logistics of patient flow, and infection control practices. Ongoing uncertainty with respect to the supply of tests led to a need for ongoing flexibility and adaptability from all to maximize the use of these limited testing resources on a day-to-day basis.

These limitations with molecular testing forced many providers to rethink their approach to providing clinical care. Many decisions had to be made in the absence of clinical data that would typically be available for other diseases. In particular, given the need to prevent the spread of infection within the health care system, if testing was not available to confirm or refute a COVID-19 diagnosis in a patient with a compatible clinical syndrome, the patient would often receive a presumptive diagnosis, with the appropriate infection control measures instituted in order to ensure the safety of hospital staff as well as other patients. This led to an increase in the use of PPE and negative-pressure rooms, both of which were already in short supply and being rationed. Further, even in patients who had a negative COVID-19 test result, there was the potential for a false-negative result due to multiple factors, including the specimen collection technique, the sampling site (oropharynx versus nasopharynx, upper versus lower respiratory tract), the timing of testing with respect to the disease course, other unknown individual or viral factors impacting test sensitivity, or limitations of the assay itself. Some patients were still presumed to have SARS-CoV-2 infection based on their presenting clinical syndrome, despite their negative test result. If this presumption was wrong, a provider may have been anchored to a diagnosis of COVID-19 while missing an alternate and potentially treatable process, and further PPE and other hospital resources may have been misallocated to this patient. If the negative test result was implicitly trusted but later found out to be inaccurate, the patient may not have received appropriate clinical care in the interim and many other individuals may have been unnecessarily exposed to SARS-CoV-2. These uncertainties, coupled with the potential multiplicative impacts of a misdiagnosis, have had a major impact on providers and hospital staff, given their desire to provide optimal care to all of their patients while also worrying about their own personal safety as well as that of their

coworkers and families. The imperfect sensitivity of molecular assays has also led clinicians to develop various unvalidated diagnostic algorithms (e.g., serial molecular testing with the same or different assays, molecular testing following by serologic testing, molecular testing with specimens from different sites) which attempt to improve the performance of this "gold standard" test to confirm a diagnosis in patients with a high pretest probability for COVID-19 due to their epidemiologic risk factors, clinical presentation, and other laboratory and radiographic findings.

Several months into the pandemic, many of these earlier challenges with SARS-CoV-2 molecular diagnostics have been somewhat alleviated. Multiple tests and testing platforms are now available, which has helped to ease the pressure on individual supply chains, though access to rapid testing remains somewhat limited. Further, in centers with access to multiple diagnostic platforms, rapid platforms (which provide results within minutes to several hours) can be prioritized for use in the emergency department or other settings where the results impact the logistics of patient flow or triage, while nonrapid platforms can be used for nonurgent testing, such as for preoperative screening or for the repeat testing necessary for many patients with COVID-19 who are being discharged to a sub-acute care nursing facility. Further challenges remain. Questions regarding the diagnostic performance of these assays continue to be examined, including investigations of the factors contributing to false-negative test results as well as persistently positive test results. In some cases, these tests can remain positive for weeks or even months (presumably due to the detection of prolonged viral shedding and/or RNA presence), despite the lack of any ongoing clinical disease or evidence thus far that these patients remain infectious to others. These persistently positive results can complicate subsequent care decisions, such as when convalescent patients can return to work or to a group living facility, and can delay otherwise indicated medical or surgical therapies or procedures. Finally, while most acute care hospitals have or are rapidly approaching adequate molecular testing capabilities, these centers represent only one very small part of the diagnostic testing landscape necessary to effectively combat the pandemic. Increased accessibility of diagnostic testing in underserved communities, in outbreak settings, and at the point of care will be key to avoiding a loss of the progress that we have made thus far.

REVIEW OF SEROLOGIC TESTING FOR SARS-CoV-2

Serologic assays have several intrinsic limitations, in that they rely on the host to react to a pathogen and develop a measurable antibody response, which may not occur early in disease or in immunocompromised hosts. Serologic assays are often unable to distinguish a current infection from a prior infection, and the sensitivity and specificity of individual assays may vary dramatically. Reduced specificity may arise from the inappropriate detection of closely related pathogens or interfering substances causing a false-positive result, and factors contributing to reduced sensitivity are most often intrinsic to the assay design (i.e., the particular test format, viral epitope, antibody class, and specimen type selected). Interest in serologic testing for SARS-CoV-2 grew rapidly with the emergence of the pandemic as the limitations of molecular testing became apparent and in response to demands for additional diagnostic capabilities.

Early challenges with SARS-CoV-2 serologic testing have been largely centered around accessing a reliable assay. The FDA initially took a rather hands-off approach to these serologic assays and did not require either formal approval or an emergency use authorization of a particular assay prior to it being used in a clinical setting. This has led to a flourishing marketplace which offers numerous assays of variable quality and widely varying price and availability. In most cases, the reliability of each of these assays has been preliminarily determined by small validation studies performed by the test manufacturer and at the site of implementation. In turn, this has made the appropriate use and interpretation of SARS-CoV-2 serologic tests challenging. The FDA has recently begun to exercise more strict oversight of these tests and is now requiring manufacturers to submit an emergency use authorization request within 10 days of beginning to market or distribute an assay.

The imperfect performance and limited supply of molecular testing led to early demand for a serologic test that could definitively confirm or rule out COVID-19 in a patient with a compatible clinical syndrome. Given the prolonged symptomatic period often associated with COVID-19, there are several clinical scenarios where a reliable SARS-CoV-2 serologic assay may be a useful adjunct for diagnosing an acute infection. When a patient has evidence for COVID-19 with a clear exposure history, compatible clinical syndrome, and supportive laboratory and radiographic findings but has negative results by molecular testing for SARS-CoV-2, a positive serologic assay can help to confirm the diagnosis and guide clinical management decisions. Ideally, this serologic testing would be performed at least 14 to 17 days after symptom onset, when test sensitivity is expected to be the highest. Conversely, a negative serologic test result (obtained at least 14 to 17 days after symptom onset) may help to rule out COVID-19 and lead to the consideration of other respiratory infections, though a thoughtful approach remains necessary, since the results need to be interpreted in the context of the time since symptom onset and multiple host factors, such as any underlying immunosuppression or the use of medications which may affect the humoral response to infection.

Despite these challenges, the demand for serologic testing persists. At the individual level, though it has not yet been conclusively demonstrated, a positive test may connote some level of immunity, which may influence decisions regarding risk-taking behaviors in the community and in the work environment for health care workers. At the population level, seroprevalence surveys may inform public health strategies for managing the pandemic. Both of these paths are fraught with uncertainty and the possibility of misinterpretation of test results. The efficacy and durability of postinfectious immunity are not known at this time. Given the recent emergence of the pathogen, we do not yet know if COVID-19 always induces a strong humoral response and whether a measured humoral response correlates with protection from reinfection. Given the relatively low anticipated prevalence of COVID-19 in many populations, the imperfect sensitivity and specificity and the window period associated with any serologic assay may give misleading results in seroprevalence studies. Careful selection of a well-validated assay, a specific population to test, and the appropriate timing of testing will be key to designing studies with interpretable results.

FINAL REMARKS

The COVID-19 pandemic has challenged the medical community across the globe in ways that could not have been predicted. In particular, the development and rollout of SARS-CoV-2 diagnostic assays in the United States have been fraught with missteps and proceeded at an inadequate pace to allow for optimal clinical care in many communities across the country. The reasons for these events are likely multifactorial, including both national and local factors related to pandemic preparedness and the public health laboratory infrastructure, which are at the mercy of local, state, and national budgets and political support. In the near future, a careful retrospective analysis of the failures of our public health system in responding to COVID-19 will need to be conducted in an effort to prevent such events in the future.

Somewhat surprisingly, access to diagnostic testing has been one of the main chokepoints in the execution of comprehensive public health strategies designed to combat this pandemic. Recent progress has been made with the increased availability of reliable molecular tests, though there are still shortages of some tests. Several serologic tests that have demonstrated promising initial diagnostic performance have been developed, though validation studies adequately powered to justify their widespread use remain to be seen. Undoubtedly, the lessons learned with the COVID-19 pandemic will better prepare clinicians and laboratories for future global pandemics.