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SARS-Cov2 Clinical Diagnostics: Academic Scientists Take on the COVID-19 Pandemic

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In this issue of *Med*, Vanuytsel and colleagues[1] demonstrate how academic institutions are stepping up to the forefront of SARS-CoV-2 testing by rapidly implementing a COVID-19 diagnostic test at a large safety net hospital serving an at-risk population, providing a regulatory and logistical roadmap to broaden testing capacity.

All molecular biologists are trained in the elementary skills of RNA isolation and qPCR that are used to identify SARS-CoV-2 in patient specimens, but the complexity required to apply these skills to the challenges of running diagnostic tests during a pandemic can come as a profound shock. That said, despite many challenges, several teams at major research universities—the University of Washington[2], UCSF (https://www.ucsf.edu/news/2020/03/416936/statement-ucsf-healths-drive-increase-testing-capacity-coronavirus), the Broad Institute (https://www.broadinstitute.org/news/broad-institute%E2%80%99s-clia-certified-testing-center-begins-processing-covid-19-patient-samples), and the Innovative Genomics Institute at UC Berkeley[3], among others—have in the past 2 months been able to set up, staff, and run diagnostic laboratories for SARS-CoV-2 that have addressed a major unmet need in public health. To this list we can now add a team of academic scientists and clinicians at Boston University and Boston Medical Center, whose effort and overall experience is both commendable and instructive.[1]

This need resulted from an unprecedented failure of both the government and private sector in designing, manufacturing, and distributing a robust diagnostic test (https://www.nytimes.com/2020/03/28/us/testing-coronavirus-pandemic.html; https://www.theatlantic.com/health/archive/2020/03/next-covid-19-testing-crisis/609193/). By mid-March 2020, the turnaround time for a qPCR-based SARS-CoV-2 diagnostic test was around 7 days across the US, whether in Boston or Berkeley. This meant not only that ER physicians at major hospitals could not make prompt decisions about care for incoming patients with COVID-19-like symptoms but also that many individuals were unnecessarily exposed to others who were infected but undiagnosed.

On March 16, a team from George Murphy's laboratory at Boston Medical Center stepped up to the 96-well plate and began running tests in 7 days; 3 weeks later they had analyzed and reported to physicians the results from their 1,000th patient specimen. This is a truly formidable achievement. A key dataset in the paper is that 44% of the patients were virus positive, as contrasted with an overall rate of 18% in the state of Massachusetts. The disparity is a socioeconomic one: Boston Medical Center is a safety-net hospital that serves vulnerable populations. In the San Francisco Bay area, as gauged by UCSF and UC Berkeley data, the rate of virus-positive individuals in the University community is strikingly lower than among vulnerable populations in the city[3] (https://www.ucsf.edu/news/2020/05/417356/initial-results-mission-district-covid-19-testing-announced). This emphatically highlights the urgent and persistent need to widely extend such testing to typically underserved groups and communities[4].

How did they do it? One might think of this as a short saga with a happy end: 7 days from project inception to first diagnostic test run! This impression is erroneous, because it is difficult for a group of people with a specific area of expertise alone to succeed in the real-world terrain of clinical-grade diagnostics. Beyond the fundamental molecular biology, the three major and separate obstacle courses to traverse for any academic group that wishes to follow in the footsteps of Murphy and colleagues, or any of the other groups listed above, are (1) meeting both state and federal level regulatory requirements for performing such testing in a CLIA (Clinical Laboratory Improvement Amendments Act, 1988)-compliant fashion; (2) establishing a process to interface the testing lab with a healthcare provider such that tests can be requisitioned by physicians and results can be returned in a HIPAA- (Health Insurance Portability and Accountability Act, 1996) and CLIA-compliant fashion with due protection of PHI (personal health information), as well as reporting test results to public health authorities; and (3) stockpiling adequate supplies to ensure that testing can be performed at scale.

Scientists and clinicians at UW Virology in Seattle were among those who first faced these challenges, when pre-pandemic regulations were still in effect and available tests failed (https://www.

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newyorker.com/news/news-desk/what-went-wrong-with-coronavirus-testing-in-the-us). Starting on Feb 29, the FDA has been revising its rules (https://www.fda.gov/news-events/press-announcements/coronavirus-covid-19-update-fda-issues-new-policy-help-expedite-availability-diagnostics), and this has allowed scientists to design and practice their own laboratory developed tests (LDTs) under an Emergency Use Authorization (EUA) process. Further, state-level departments of public health allowed institutions with an existing clinical diagnostic laboratory holding a CLIA license for high-complexity testing (such as a sequential RNA extraction and qPCR) to extend that license to a space that is also owned by the same institution (for example, a research laboratory). Finally, regulations now allow any individual with an advanced degree and proficiency in the relevant techniques to do the actual pipetting for a CLIA-grade test, following appropriate testing. This new regulatory environment remains in place as of mid-May 2020 and bodes well for any group in academia who wishes to set up their own testing capability. An essential logistical requirement for success is that academics must partner with an existing CLIA laboratory, either in-house or within the same institution. The molecular biologists will need continued guidance from a physician board-certified in laboratory medicine who heads the CLIA lab, and near-hourly help from clinical laboratory scientists (CLSs) who work there. It is noteworthy that both the Chair of Pathology and the Chief of Laboratory Medicine of Boston Medical Center are authors on the paper from Murphy and colleagues; they know, and practice, CLIA and HIPAA.

What are the key decisions one needs to make before embarking on this quest? The choice of the actual testing protocol is a good place to start. Using classical RT-qPCR to determine whether a specimen contains SARS-CoV-2 RNA remains the mainstay of clinical testing and was the method of choice for the academic groups listed earlier. In principle, no innovation is needed as an efficient and robust all-in-one-solution exists, developed and commercialized by Roche. It consists of the Cobas8000 device, using reagents also available from Roche, and for which an EUA approval has been granted by the FDA. The advantage of this approach is that the regulatory and logistical hurdles one needs to overcome are relatively minor, and for that reason, major healthcare providers such as Kaiser rely on the Roche solution. Of note, other commercial providers have also attempted to build an all-in-one system; one such device, sold by Abbott, was found to have a false negative rate of 25%–50% (https://www.wsj.com/articles/coronavirus-testing-hampered-by-disarray-shortages-backlogs-11587328441).

At the other end of the spectrum lies a fully DIY approach, which the Murphy lab chose. It is instructive and inspiring to read the EUA application for the LDT submitted by the Murphy group to the FDA and realize how much initiative and creativity the scientists showed. The LDT framework now accepted by the FDA means that you can use any combination of general reagents (RNA extraction, qPCR), specific reagents (e.g., primer-probesets for SARS-CoV-2), and hardware, as long as at the end of the process, your LDT meets certain performance metrics. For example, the FDA requires that you experimentally determine the analytical limit of detection (LoD) of your LDT, which is done using contrived samples (viral RNA spiked into mock specimens). The LDT from the Murphy group has an LoD of 1 viral genome per microliter, which is 10 times more sensitive than any of the LDTs evaluated in a recent study[5], and comparable to those reported by other academic groups[3,6]. Two other requirements that all groups had to meet are (1) demonstrating that this LoD can be attained 19 out of 20 times when analyzing 20 contrived specimens at that LoD and (2) evaluating analytical sensitivity and specificity of the LDT by using it to test a panel of clinical specimens known to be negative or positive for SARS-Cov-2 as gauged by a CLIA laboratory using a test with an issued EUA from the FDA.

It is also essential to understand one immutable feature of the current regulatory landscape: once you have formalized the details of your testing protocol in a standard operating procedure (SOP) and submitted it, along with the LDT validation results to the FDA, no substantial changes can be made without repeating the entire process. This holds true even if suppliers run out of reagents, testing equipment fails, or subsequent improvements to the protocol are found. The Murphy group wisely anticipated these issues and obtained validation for several versions of their LDT, granting them the flexibility to overcome unexpected challenges.

Even without this step, it is still possible to begin testing specimens under CLIA if you have finalized your SOP, generated an FDA-level validation report, and informed both the FDA and local state authorities of your intent to submit an EUA. The EUA must be submitted within 15 days, and during this time, testing is permitted until approval is granted. This is how Murphy et al. were able to begin CLIA testing within 7 days of project inception and develop multiple LDT protocols. This point should also be kept in mind when deciding which reagents to commit to and the testing capacity required. If it is not possible to anticipate how many tests will be run, consider providers with ample stocks of the required components.

In summary, at this point, a fully fleshed-out set of blueprints exists in the preprint and peer-reviewed literature on how to set up your own testing capability. The paper by Murphy and

colleagues[1] offers a clear and succinct introduction of what the key issues are and how to solve them in the context of an academic lab inside a medical center, with other examples providing additional specifics on how to do this if you do not have a medical center or school as your mother institution. It is abundantly clear from data across the nation that vulnerable communities are the ones most in need of such widespread testing and have at present the least access to it. Here, academic institutions have, and should continue to, stand up to their full height of serving the public good.

There is consensus among experienced and informed public health officials and physicians that widespread testing will continue to be necessary and the virus may return later in the year, or early in 2021. This presents a major national and global challenge—a challenge that academic scientists can play a key role in meeting. Some current efforts include pooling patient specimens to scale up testing[7] and building field-deployable tests derived from CRISPR-type bacterial adaptive immunity systems that are faster and more sensitive than qPCR-based methods[8,9]. The central point to keep in mind, as exemplified by Murphy and colleagues, is that basic science ingenuity and innovation can rapidly be advanced to the actual world of healthcare practice during the current pandemic of COVID-19, but also for other pandemics arising in the future.

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