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COMMENTARY

The First Quarter of SARS-CoV-2 Testing: the University of Washington Medicine Experience

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ABSTRACT In early March 2020, the University of Washington Medical Center clinical virology laboratory became one of the first clinical laboratories to offer testing for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). When we first began test development in mid-January, neither of us believed there would be more than 2 million confirmed SARS-CoV-2 infections nationwide or that we would have performed more than 150,000 real-time PCR (RT-PCR) tests, with many more to come. This article will be a chronological summary of how we rapidly validated tests for SARS-CoV-2, increased our testing capacity, and addressed the many problems that came up along the way.

KEYWORDS SARS-CoV-2, Seattle, coronavirus, history, pandemic

We first heard of the virus, which had not yet been named, when many others did, at the end of 2019, when the first report came out of China to the World Health Organization (WHO). Looking back at our e-mail in-boxes from that time shows only the usual diversity of viruses that come up in clinical and research work: enteroviruses, *Bunyavirales*, Epstein-Barr virus, human parainfluenza viruses, herpes simplex virus, HIV, measles virus, and so on. There was no mention of a coronavirus (CoV). At a meeting of faculty interested in pandemic preparedness on 6 January 2020, we briefly covered the potential of what we thought could be a new virus. At the time, it was not clear if the outbreak represented a new virus or a cluster of a known virus. The postmeeting e-mail exchange includes, "Hopefully the sequence goes up and we can all design primers/amplicons overnight. We might have to worry about setting up a qRT-PCR for this asap, given all the flights into SeaTac, if it does show human-to-human transmission."

The sequence was posted on 10 January 2020, and the world saw another new *Betacoronavirus*. It was remarkable how similar the genome of the new virus was to that of severe acute respiratory syndrome (SARS)-CoV. The E gene in particular stuck out for its almost complete identity to the SARS-CoV E gene. The identity to SARS-CoV was so notable, Dr. Greninger somewhat flippantly texted movie sequel titles to viral genomics colleagues, such as, "Seriously Dude, where's my SARS?" Soon after, the International Committee on Taxonomy of Viruses coronavirus group settled on SARS-CoV-2 as the official name for the virus (1). While taxonomically correct, this name has proven cumbersome given the independent naming by the WHO of the disease as coronavirus disease 2019 (COVID-19).

Within 3 days of the release of the genome, there was already a quantitative real-time PCR (qRT-PCR) protocol released by multiple European groups led by Dr. Christian Drosten (2). Their work was both elegant and important. They took advantage of the high similarity between SARS-CoV and SARS-CoV-2 to use the SARS-CoV genome as a template for development and validation of a qRT-PCR protocol. To this day, their E gene primer and probe set remains one of the most sensitive, and it is included in

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some of the most widely used commercial assays. Hundreds of thousands of people are tested every day with this primer set, which was developed within hours of the genome sequence becoming available. As with the CDC assay, Dr. Drosten's group also designed three primer and probe sets across the viral genome, though they rapidly removed one primer and probe set after it had unfavorable performance characteristics. Unfortunately, as is well known now, it took approximately 3 weeks to remove the N3 primer set from the CDC assay in February 2020.

Three days after the WHO protocol was posted online, we ordered primers, and they arrived the next day. The same day that the primers arrived, the first known person with COVID-19 in the United States was developing symptoms, just a few miles north of Seattle, WA. The patient presented to a different hospital, and their samples went to a different clinical laboratory. When the CDC rapidly detected the infection, it seemed a victory for modern science and information sharing. The first genome had been publicly shared only about 10 days earlier, and the CDC already had a workable qRT-PCR assay that could diagnose COVID-19. Meanwhile, our county hospital laboratory received blood samples from the patient to perform chemistry testing but no samples that contained SARS-CoV-2. We tried to obtain any positive samples from the hospital laboratory where the patient received care, but all of them were sent to the CDC headquarters. In retrospect, we might have tried harder to obtain these samples to speed test development, given that they were only a few miles away.

Throughout late January, Dr. Arun Nalla, a research scientist at the University of Washington Medical Center clinical virology laboratory (UW Virology), studied several different primer sets and versions of an assay. We had only synthetic genes to use to optimize the assay but could at least perform specificity testing. We thought that that there would be resurgent interest in coronaviruses in basic science and clinical trials but that it was unlikely that the virus would establish itself in Seattle. Dr. Greninger was working in his research lab, assembling data for an early February grant for funding to understand the function of his favorite coronavirus gene (orf3a) in SARS-CoV-2, while Dr. Jerome continued splitting time between clinical virology and his work at the Fred Hutchinson Cancer Research Center on gene therapies for persistent viruses. Our expectations were based on the lessons of the 2003 SARS-CoV outbreak, and we thought that with better technology, early openness from China, and international cooperation, we would swiftly send SARS-CoV-2 back to the bats. From mid-January until the end of February, we received respiratory specimens from about 15 different persons under investigation for COVID-19 for respiratory virus panel testing, but each of them tested negative for SARS-CoV-2, not only at the CDC but also in our lab as we continued the evaluation of our assay. We could not report results from our assay development and validation studies, but our clinicians soon became disappointed by the 5- to 7-day turnaround time for clinical results from the CDC, during which patients waited in isolation for test results.

The pace of test development picked up in February, and we faced two closely related challenges: first, meeting the regulatory requirements of the FDA and, second, getting the materials that we needed to validate and perform our tests. We first reached out to the FDA on 4 February 2020, after learning that the CDC had received emergency use authorization (EUA) for their assay and that all laboratory-developed testing would require federal authorization. Our clinical virology laboratory had not submitted EUA paperwork to the FDA in previous public health emergencies, opting instead to use FDA-authorized tests from commercial manufacturers. This outbreak seemed like an excellent opportunity to see how the FDA might regulate laboratory-developed tests, something that has been long debated in the clinical laboratory community. The FDA was quite responsive to Dr. Greninger's many e-mails, but it was clear that the process was not going to be completed quickly. Talking to several large commercial manufacturers indicated that, as of mid-February, they did not expect to receive an EUA until late April or early May.

By far the biggest roadblock for starting testing was obtaining positive-control material for validation. Cultured virus from the CDC became available from BEI Re-

sources on 12 February 2020, but receiving it required both a biosafety level 3 (BSL-3) laboratory and multiple institutional approvals. It was not clear when control materials that could be handled in clinical laboratories, which are BSL-2, would become available. At the time, there were fewer than 14 confirmed cases of COVID-19 in the United States, yet the EUA validation recommendations from the FDA suggested obtaining at least 25 positive clinical specimens.

After receiving our first pre-EUA comments from the FDA during the last week of February, we pushed the ball forward on specificity and the informatics portions of the document and continued to search for positive-control material. We screened past specimens sent for respiratory virus testing and began prospectively screening every respiratory specimen sent to our laboratory for SARS-CoV-2. We heard through the grapevine that viral whole-genomic RNA was available from UTMB Health—Galveston, and we worked aggressively to obtain it, including a record 59-minute signoff from the materials transfer agreement (MTA) and biosafety offices at the University of Washington. At the same time, Dr. Greninger called offices of our U.S. congressperson and senators to detail what we perceived as an overall lack of preparedness and exchanged e-mails with IDSA and ASM policy offices. After talking with Dr. Melissa Miller, the clinical microbiology director at the University of North Carolina School of Medicine, we wrote a letter to Congress asking for the reinstatement of laboratory-developed tests in high-complexity laboratories following the Clinical Laboratory Improvement Amendments (CLIA; https://www.cms.gov/Regulations-and-Guidance/Legislation/CLIA). That letter was signed by more than 100 laboratory directors in under 8 h. Dr. Greninger believes that he averaged around 70 phone calls a day that week.

On Friday, 28 February 2020, we detected our first SARS-CoV-2-positive specimen. It was from a person under investigation for COVID-19 for whom respiratory viral panel testing was also ordered. Within 20 minutes of the result, we learned that the patient had also tested positive at the Washington State Public Health Laboratory, which had gone live with testing only in the previous 24 h. The next morning, we awoke to news that the FDA was permitting CLIA high-complexity labs to start testing. The weekend was a blur of nonstop final validation studies and preparation of an online test guide, test documentation, and electronic test build, led by our laboratory manager, Greg Pepper. The only break for either of us was Dr. Greninger's hurried visit to his mother's assisted-living facility on Sunday evening and then his helping her move into his apartment. Neither of us have taken a day away from our clinical work, 13 weeks later.

Testing began slowly, as we focused on our inpatient populations. During the first days of testing, we were strongly supported by the Washington State Public Health Laboratory, which had identified a number of additional positive samples. We were also supported by our infectious disease clinicians, who told us how important it was to have local testing available as the hospital filled with patients with COVID-19. We began a daily department-wide operations conference call at 7:30 a.m. As testing volume grew, we transitioned the lab to 24-h, 7-day/week operations and called back any non-virology staff in the department who had previously worked in virology. Our informatics division set up shop in the virology laboratory, along with client support services and specimen-processing personnel. Dr. Patrick Mathias, Dr. Nik Krumm, and Dr. Noah Hoffman quickly built multiple internal and external dashboards that would allow us to track samples, orders from different clients, turnaround times, and positivity rates. Dr. Jerome took UW Virology's large conference room as an office and "situation room," while Dr. Greninger took a supply closet. Having everyone in one location was critical to ensuring constant and accurate communication in the face of the growing test demand.

As testing volume increased, supply chain considerations quickly became apparent. Within 4 days of testing, it became clear that we did not have enough of the WHO assay probe on hand for the next week and that it would take too long for more to be synthesized. We quickly validated the CDC assay and began using it, mainly because of the reliable and seemingly inexhaustible supply chain and overnight shipping of premade CDC primers and probes from IDT. However, soon after, we found ourselves

short of supplies for RNA extraction. A shipment arrived just in time to replace instrument fluid, but unfortunately, we failed to recognize that part of that shipment was missing tips and other consumables, beginning a new game of supply chain whack-a-mole. Dr. Jerome, who had taken to Twitter to post the number of positive results and the test volume, began using it as a megaphone to signal the need for specific consumables and reagents. Other laboratories from the University of Washington and even one from the San Francisco Bay area led by a UW alum provided voluntary donations of tips, which allowed our testing to continue without interruption during those critical early days.

Less than 10 days after testing started, our daily volumes were already surpassing annual volumes of most of our virology test menu. The chair of the Department of Laboratory Medicine, Dr. Geoffrey Baird, plotted the volume data for the first 2 weeks of testing and showed that growth was essentially exponential. We assigned two full-time employees to manage orders and shipments and to clear the hallway, which became nearly impassable most mornings by 10.30 a.m. when shipments arrived. We also began ordering sample-to-answer platforms for which we anticipated that EUAs would soon become available (3). The early arrival of the virus in Seattle allowed us to get ahead of supply chain issues before they became apparent across the rest of the country. The university administration allowed us to e-mail the entire University of Washington system repeatedly to ask for consumables, qPCR instruments, and labor. A bioinformatics Ph.D. friend from Dr. Greninger's graduate school flew up from San Francisco just to help process and decant samples for 2 weeks. We reduced the frequency of testing for the rest of our test menu and even chose to temporarily send out a few of our lower-volume tests. Somehow the reagents continued to arrive just in time, but we knew we could not double our supply chain every 5 to 6 days as the spread of the virus demanded, and for about 2 days, we exceeded our analytical capacity as median turnaround times rose from 12 h to 24–36 h.

Two weeks after we began testing, we had a Saturday morning meeting in which we developed a triage system to ensure that inpatient testing took priority over outpatient testing. Our first sample-to-answer platform arrived that weekend, and that helped expand capacity beyond the 2,500 to 3,000 specimens we had been processing each day using our laboratory-developed test. Still, we did not have enough preanalytical capacity either at the hospital or at the clinical virology lab to handle the volume. It would be too hard to expand at the hospital given the space available, and the need to increase testing was acute. After a 5-min negotiation, we rented several thousand square feet of additional space in our building on the floor below our existing lab; ironically, it was a BSL-3 that we rapidly decommissioned into additional molecular testing space. A little over a week later, we completed the initial steps to create a new COVID-19-dedicated laboratory with additional specimen accessioning stations, 10 new BSL-2 hoods for processing samples, and two new sample-to-answer instruments. By the end of March, our laboratory analytical capacity reached approximately 7,000 tests per day.

No one wanted or needed any more to do, but it was time to provide accurate serology testing. Our Immunology Division colleagues in the department, led by Dr. Mark Wener, had validated a couple of early accurate platforms for serology testing. However, these platforms could not scale with the likely demand for SARS-CoV-2 testing. We obtained an early high-throughput kit that used the SARS-CoV-2 nucleocapsid protein and were impressed with the early results. The first 100 pre-COVID-19 serum specimens in our lab gave no false positives, as did the next hundred, and the next hundred. It found all of the known positives sent from Immunology, with positivity occurring 24 to 48 h earlier than the spike protein, just as had been found with SARS-CoV and Middle East respiratory syndrome CoV (MERS-CoV) (4). To truly push the test, Dr. Andrew Bryan brought in more than 600 serum samples from RT-PCR-positive patients at Northwest Hospital, and it detected all of them after 17 days post-onset of symptoms (5). It was off to our second press conference in a month. The news reporters

jumped forward when Dr. Jerome pulled out an empty reagent box from his jacket and lined up to film their spots with it.

Many others, not yet mentioned, contributed to our laboratory testing for COVID-19. Our administrative staff brought on many new hires and handled onboarding, while also responding to outside requests for visits. These visits became a highlight for many members of the press or university who had not been to our laboratory before. The outpouring of support from the community was incredible, and lunch and dinner were donated to the clinical laboratory staff for more than 2 months straight. Girl Scouts sent care packages to the staff. In addition to kind Twitter messages, old-school handwritten notes arrived at the laboratory every day. Everyone wanted to help, and we tried to return the favor. Given the difficult process of obtaining the first specimens, our MTA office processed more than 70 requests for SARS-CoV-2-positive specimens during this time, and we fulfilled nearly all of them, sending out package after package of specimens to laboratories to help increase testing capacity across the United States.

Looking back, it is incredible to see everything that happened so quickly. In the month of March, we went from testing only at the CDC to testing at the public health laboratories to testing with laboratory-developed tests in high-complexity laboratories to using FDA-authorized high-throughput analyzers to using point-of-care tests. For several months running, our job has been the subject of mainstream media and nonstop cable news. For clinical microbiologists and virologists, the job has certainly followed us home. While it is not the best of times when clinical microbiologists and virologists are in the news, it has been awesome to be able to contribute to the control of this virus.

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