Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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ADVANCES IN MOLECULAR PATHOLOGY

A PENNdemic Year in Review

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KEYWORDS
- Severe acute respiratory virus coronavirus 2 (SARS-CoV-2)
- COVID-19
- Clinical development
- Pandemic response

KEY POINTS
- A dedicated clinical development team is advantageous and allows flexibility during an emergency so that clinical work can continue uninterrupted.
- Supply chain restrictions required laboratories to validate multiple tests for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) detection to provide redundancy in testing and allow for flexibility based on clinical urgency.
- A comprehensive SARS-CoV-2 screening program can allow secondary institutions to reopen safely.
- Although surveillance-based sequencing can provide important information for public health initiatives, the clinical utility of sequencing individuals remains unclear.

INTRODUCTION

The novel coronavirus severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was first identified in Wuhan, China in December 2019 as the causative agent of the worldwide spread of coronavirus disease 2019 (COVID-19). By March 2021, greater than 120 million cases have been identified worldwide with approximately 25% of all cases occurring in the United States [1]. In addition, one-fifth of the total global deaths, which currently top more than 2.5 million, have occurred in the United States [1]. Several major cities, including the Philadelphia region, have been hard hit by COVID-19. Since the beginning of the pandemic, there have been 135,632 confirmed cases and 3333 deaths accounting for ~14% and ~13% of cases and deaths, respectively, in Pennsylvania [2,3].

In Philadelphia, like many cities across the nation, clinical laboratories faced unprecedented struggles and unpredictable changes while being at the forefront of the pandemic response. Although clinical testing challenges varied (limited vendor assays, Food and Drug Administration [FDA] regulations, supply chain disruptions on all aspects of the testing supplies, collection logistics, and personnel issues), over the course of the pandemic, the need for a swift response was imperative. This article highlights how a dedicated clinical development team (CDT), at a major academic institute in Philadelphia, was able to successfully navigate the shifting landscape of the COVID-19 pandemic while allowing clinical SARS-CoV-2 patient testing to continue and expand uninterrupted.

THE FIRST PHASE: NAVIGATING THE EMERGENCY

When the World Health Organization declared COVID-19 a global pandemic on March 11, 2020, clinical laboratories across the United States had already been hard at work, and Philadelphia had confirmed its first positive case 2 days previously [3,4]. Two months before that, the CDT, a group dedicated to molecular clinical test validation, had begun discussions with the

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faculty of the Molecular Pathology and Microbiology Laboratories regarding the response to increasing SARS-CoV-2 infections. An expanded COVID-19 research and development task force (CoV R&D) was developed in late February that consisted of faculty and fellows from the Molecular Pathology and Microbiology Laboratories, Pathology and Laboratory Medicine administrative staff, laboratory managers, and 2 members of the CDT from the Hospital of the University of Pennsylvania (HUP). A daily meeting was adopted to discuss operations and acute issues, regulatory guidelines, supply chain disruptions, guide the evolution of testing (new methods, specimen types, and media, and so forth), and review recently published literature. Members of CoV R&D participated in overlapping departmental and health system task force meetings in order to maintain awareness of the big picture as well as interact with operational and logistical teams across the enterprise of PennMedicine's 6 hospitals.

The sole responsibility of the CDT is to bring new clinical molecular testing in house and maintain all current molecular testing platforms. Consequently, the CDT has intimate knowledge of the assay development and verification process, including both the technical and the regulatory aspects, which allowed the rapid deployment of 7 SARS-CoV-2 testing methods in the first 8 weeks of our pandemic response, two of which were implemented in the Microbiology Laboratory, with the remaining deployed in the Molecular Pathology Laboratory. Assays were distributed based on the existing platforms in each laboratory and the technical expertise of the staff (ie, more manual or pipetting heavy assays in the Molecular Pathology Laboratory). The CoV R&D discussed and planned all verification studies to be consistent with the fluctuating regulatory guidelines. Because the Microbiology Laboratory does not have a dedicated development team, the tasks performed by the CDT became the responsibility of the fellow and the faculty laboratory director, who were already stretched thin.

New assay development can be disruptive to clinical testing, and when technologists are required to split their time, development work gets sidelined, as patient care is their primary responsibility. A CDT can quickly adapt to the changing needs of the health system and laboratory, and this allowed assay verifications and the supportive paperwork associated with implementing clinical testing, such as, but not limited to, verification documents, standard operating procedures, report interpretations, and maintenance log templates, to be completed on an abbreviated timeline. This was accomplished by pivoting assay development priorities; at the beginning of the pandemic, multiple non-COVID development projects were being juggled by the CDT, but by early February, all efforts completely shifted to development of SARS-CoV-2 testing in accordance with regulatory guidance. Having a dedicated development team allowed SARS-CoV-2 testing capacity to scale up to meet the increasing clinical needs without sacrificing patient testing. In addition, it provided the framework for coordinating decentralized rapid testing across our then 6 hospital health system.

An unexpected outcome of the CoV R&D was that it provided a support system and a mechanism for assessing the overall well-being of all laboratory technologists, faculty, and staff. During a pandemic, many individuals experience heightened levels of stress, insomnia, alcohol and drug misuse, and symptoms of posttraumatic stress disorder [5]. In addition, health care workers often face stigma, because of working in a high-risk environment, which can have a negative effect on their psychological health and increase their risk of burnout, anxiety, and depression. Health care workers are hit especially hard because of extended working hours and social isolation that emergency situations create. This pandemic has had a profound effect on laboratory workers [5]. It is common for technologists to work with repetitive motion injuries, have feelings of guilt when they leave samples behind for the following shift, become instrument-troubleshooting experts, and experience emotional exhaustion from the never-ending specimen backlog [6]. Daily CoV R&D meetings provided an outlet to bring feedback from the technologists to laboratory administration and opportunities to work through difficulties.

THE SECOND PHASE: BUILDING A TESTING STRATEGY

Uniquely, during the COVID-19 pandemic, supply chain shortages of reagents and instrumentation were pivotal in method choice. The extent of disruption spanned all aspects of testing with deficiencies in collection kits, instrumentation, assay reagents and consumables, and personal protective equipment (PPE). In an April 2020 survey published by the Association of Molecular Pathology (AMP), 85% of respondents reported disruptions to supply chains, which caused either a delay in testing or a decrease in testing volume; by the August survey, this number was up to 93% [7,8]. Academic medical centers and community hospitals were disproportionately affected by shortages of testing kits compared with commercial reference laboratories [7,8]. Hospital laboratories are inherently different

from commercial reference laboratories. From early on, it was evident that assay-specific shortages would require hospital laboratories to implement multiple testing platforms for redundancy in testing and the flexibility to accommodate both rapid and routine testing to respond to various clinical scenarios. In the same April AMP survey, 57% of respondents from academic medical centers or community hospitals were running 3 or more assays compared with only 20% of commercial reference laboratories [8]. By August, these numbers jumped to 80% and 30%, for academic and commercial laboratories, respectively [7].

As manufacturers developed new assays, they were vetted by the CoV R&D to determine if implementation was necessary and feasible. Tests to implement were chosen based on the technical feasibility, projected daily capacity, cost, Emergency Use Authorization (EUA) approval status, the availability of instrumentation and reagents as well as alignment with the clinical needs of the 6 hospitals in our health system. CDT members maintained efficiency by dividing the laboratory and clerical work based on each member's strengths.

The timeline for clinical SARS-CoV-2 assay development is shown in Fig. 1A. On March 3, validation studies for the ePlex SARS-CoV-2 (GenMark Diagnostics, Carlsbad, CA) assay began while the assay was still under review for EUA, and by March 12, the first of many SARS-CoV-2 tests was performed. Although our theoretic testing capacity (based on instrument capacity) was several hundred per day, supply chain issues on test kits further limited the actual capacity to ~20 per day. Therefore, only the sickest patients and health care exposures from Occupational Medicine were tested following approval by hospital Infection Control. Hospital visitation was restricted, and multiple staff hotlines were set up 1 day following SARS-CoV-2 testing go-live to handle inquiries regarding clinical situations and staff questions about infection control, PPE, supplies, operational issues, and testing. By March 15, hospital visitation was completely suspended, and the laboratory was already hard at work bringing in new assay platforms for additional testing, which was complicated by supply chain shortages and regulatory oversight. The next day, statewide mitigations went

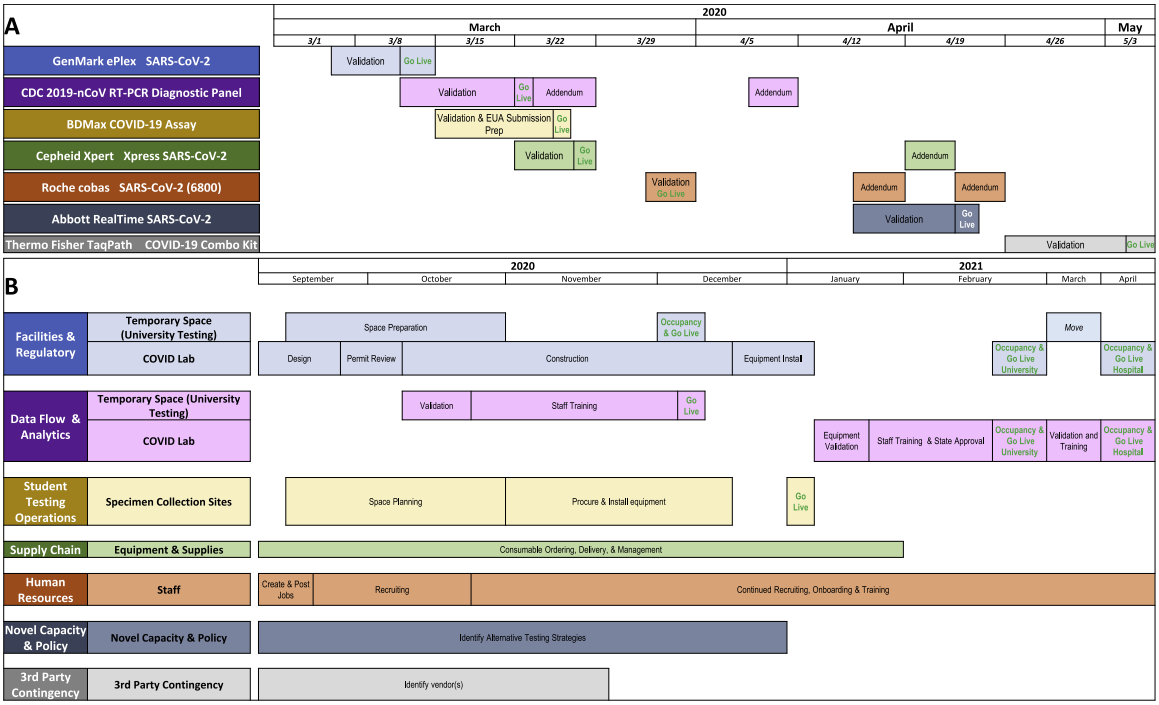

FIG. 1 Timelines for development and Project Quaker. (*A*) The timeline for initial clinical assay development from March to May 2020 in the Molecular Pathology and Microbiology Laboratories. (*B*) Timelines for Project Quaker from implementation of work groups to go-live of all clinical testing (September 2020 to April 2021).

into effect, and the confirmed cases in the Philadelphia region numbered 52 [2].

One week later, the CDT completed validation of the second method, the Centers for Disease Control and Prevention (CDC) 2019-nCoV Real-Time RT-PCR Diagnostic Panel (Atlanta, GA, USA). The next week, 2 tests, a laboratory-developed test (LDT) using the BD Max platform (BD Molecular Diagnostics, Franklin Lakes, NJ, USA) and the GeneXpert SARS-CoV-2 (Cepheid, Sunnyvale, CA, USA), went live within days of each other in the Microbiology Laboratory. The BDMax LDT was validated using archived residual specimens following the FDA's Molecular Diagnostic Template for Laboratories. The BDMax LDT assay was reviewed and approved for EUA by the FDA. The Cepheid platform was implemented at all hospitals in the health systems to address rapid community-based testing while centralizing routine testing at HUP. These 3 platform additions expanded theoretic instrument capacity, but all test requests were still being triaged through Infection Control because of continued supply chain shortages of assay kits, which limited capacity to ~200 tests per day. The subsequent week, the high-throughput cobas 6800/8800 SARS-CoV-2 assay (Roche Diagnostics, Indianapolis, IN, USA) was validated and nearly tripled capacity. With 5 validated methods and increasing volumes, it was no longer feasible for Infection Control to approve all test requests. Physician-based electronic medical record ordering was opened for inpatients and select groups of outpatients, which included organ transplant patients, hematologic malignancy patients starting therapy, radiation oncology patients, newborns, patients being discharged to a care facility, and patients being enrolled in COVID-related clinical trials.

As a multihospital institution with outpatient collection centers, PennMedicine received specimens arriving from within the city, tented collection sites, and other suburban hospitals in our health system. The logistics of collection and transportation created challenges and complicated laboratory workflows. Accommodating a normal day's collection hours at other locations and transporting specimens into Philadelphia meant many samples arrived at the laboratory late in the evening. The laboratory workflow shifted to monitoring the pending samples list and triaging samples to available and appropriate platforms in order to meet the fastest possible turnaround times. For example, batches $\geq$48 samples would be routed to the cobas 6800; batches under 48 would be routed to either the CDC assay or the ePlex or sent to the Microbiology Laboratory for testing on the BD Max with all urgent testing still occurring in the Microbiology Laboratory. By April 21, our sixth assay, the RealTi*me* SARS-CoV-2 (Abbott Molecular Inc, Des Plaines, IL, USA) was live, bringing our daily testing capacity to more than 1000 tests per day at HUP. Shortly thereafter, the seventh method, TaqPath COVID-19 Combo Kit (Thermo Fisher, Waltham, MA, USA), was validated. Both of the newest assays improved triage for batched workflows within the laboratory.

Each assay was successfully implemented, allowing patient testing to expand concurrently. Gradually, the increased testing capacity facilitated the hospital to reopen at full capacity once the Governor lifted hospital admissions to non-COVID cases. With a testing capability of approximately 3000 tests per day (for both symptomatic and asymptomatic), testing was opened to all departments in the health system, and triaging workflows were adjusted accordingly. Because comparison studies showed that the analytical sensitivity of 6 of our 7 methods had similar limits of detection, samples could be stratified by clinical urgency without concern for loss of detection (Table 1) [9]. Following manufacturer workflow modifications, as described in the table legend, all 7 assays had similar limits of detection [9]. Table 1 compares chosen quality metrics of the first 7 methods [9]. Availability of laboratory staff and reagents also determined method choice. All samples were initially parsed into 4 groups based on the required turnaround time. Fig. 2 outlines the operations of triaging specimens within 2 laboratories. Urgent, moderate, and routine samples were triaged in the Molecular Pathology Laboratory, and STAT samples were triaged in the Microbiology Laboratory. For methods that required batching, samples were racked into appropriate run sizes according to their priority and started as soon as the batch size was reached. Approximate turnaround time for samples on these platforms was targeted for clinically relevant results. The random access nature of the ePlex proved valuable for minimizing turnaround time for repeat testing from our high-throughput systems, as batching is not required.

By the time the last method was implemented, the CoV R&D was running smoothly and efficiently; however, there were continuous assay modifications and updated regulatory guidelines. Supply shortages were also affecting collection devices. Therefore, as soon as testing was established, the CDT needed to have a swift response to alternative specimens (midturbinate and anterior nares swabs) and transport media (saline, phosphate-buffered saline, and so forth) as reagent supply and testing needs evolved. In addition, the CDT handled all validation related to changes in EUA

TABLE 1
Selected Quality Metrics for 7 Severe Acute Respiratory Syndrome Coronavirus 2 Methods (Top) and 5 Methods Added Later (Bottom)

Method	Batch Size	Approximate Assay Time (h)	Ease of Use	Technologists to Run Efficiently	Laboratory Assistants to Run Efficiently	Cost per Reaction	Approximate Laboratory-Established Sensitivity (Copies/mL)
Abbott RealTi*me* SARS-CoV-2	Up to 94	8	Moderate	1.5	N/A	$$	50
Cepheid Xpert Xpress SARS-CoV-2	1	0.83	Easy	1	N/A	$$$	100
GenMark ePlex SARS-CoV-2	1	1.75	Easy	1	N/A	$$$$	10,000[a]
Roche cobas SARS-CoV-2 (6800)	Up to 94	4	Moderate	1	1	$	500
Thermo Fisher TaqPathCOVID-19 Combo Kit	Up to 94	3.5	Difficult	2	N/A	$$	100
BDMax COVID-19 Assay (EUA LDT)	Up to 22	2.5	Easy	1	N/A	$$	1000
CDC 2019-nCoV RT-PCR Diagnostic Panel	Up to 29	7	Difficult	2	N/A	$	500
Additional added platforms							
Roche cobas SARS-CoV-2 & Influenza A/B (Liat)	1	0.20	Easy	1	N/A	$$$	Not assessed
DiaSorin Simplexa COVID-19 Direct	Up to 8	1.75	Easy	1	N/A	$$$	Not assessed
Fluidigm AdvantaDx SARS-CoV-2 RT-PCR Assay	Up to 186	5.5–8[b]	Difficult[b]	1–2	2	$	Not assessed
Thermo Fisher Amplitude Solution TaqPath COVID-19 High-Throughput Combo Kit	Up to 376	5	Moderate	2	2	$	Not assessed
Roche cobas SARS-CoV-2 (8800)	Up to 94	4	Moderate	2	1–2	$	Not assessed

Assay time accounts for preprocessing time, instrument time, and resulting. Sample collection, transport, and accessioning time are considered equivalent and are not included. The Roche 6800/8800, Amplitude, and Fluidigm platforms can be started in a staggered manner to increase throughput. The time to result staggered runs is decreased. Staff is split into laboratory assistants who perform the preprocessing steps, if included, and technologists, who perform the analytical and postanalytical steps. Analytical sensitivity was evaluated on the first 7 methods with a dilution series (50,000–1000 copies/mL) of quantified positive archived clinical specimens generated with pooled negative samples. Positive samples were quantitated on a CDC-based methodology with a standard curve generated using synthetic RNA of the SARS-CoV-2 N gene. Dilutions under 1000 cp/mL were evaluated on selected methods based on the stated limit of detection. The 5 additional platforms were implemented after comparison studies were completed.

Abbreviation: N/A, not applicable.

[a] Following workflow modifications, the ePlex SARS-CoV-2 had comparable sensitivity levels to the other assays.

[b] Fluidigm assay time and ease of use are dependent on the use of robotics, which both shorten the length of the assay and reduce the technical difficulties.

Modified from Gentile C, Richard-Greenblatt, M, Fink, J, et al. A Practical Comparison of Seven Molecular SARS-CoV-2 Methods. Paper presented at: Association for Molecular Pathology Annual Meeting 2020; November 16-20, 2020; with permission.

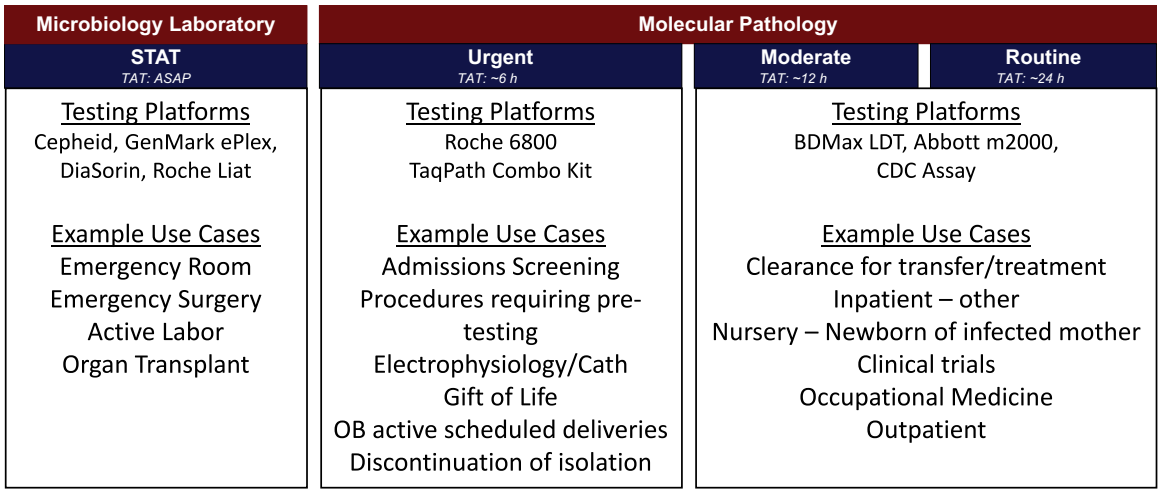

FIG. 2 Example workflow schematic when using multiple methods for testing. Outline of how testing was deployed across multiple laboratories to serve patients with various clinical needs. Figure does not include the new methods deployed at the COVID Testing Laboratory. ASAP, as soon as possible; OB, obstetrics; STAT, immediately (from the Latin "statim"); TAT, turnaround time.

manufacturer's instructions for use, such as a modified workflow for the ePlex that removed the use of the sample delivery device, a new internal control volume for the Abbott RealTi*me* assay, and new vortexing and centrifugation requirements, new extraction reagent manufacturer, and new analysis workflows for the Thermo Fisher TaqPath assay. These smaller validations were handled in a similar manner as the assay validations: working together as a team while catering to each member's strengths and allowing clinical SARS-CoV-2 testing to continue uninterrupted. Because of the prominent role the CDT plays in training, the team provided onsite training at HUP for one of the suburban entities on the Thermo Fisher TaqPath assay to support routine testing at that entity.

Following the initial implementations and even with an additional 2 platforms (DiaSorin Simplexa and Roche Liat), it was clear that the capacity for testing was limited by the both the methods and the technologist time required. The next phase of SARS-CoV-2 testing was expanding both the testing capacity and the staffing to better serve the health system's patients and the Philadelphia community, including the UPenn faculty, staff, and students. The solution was Project Quaker: 2 high-throughput COVID-19 testing laboratories for both swab and saliva testing. Timelines for Project Quaker are shown in Fig. 1B. Again, the CDT took an active role in vetting the instrumentation, including robotics, designing the laboratory space, and validating the new assays, while maintaining regulatory compliance. The CDT's role allowed existing swab-based testing to continue in the Molecular Pathology and Microbiology Laboratories.

THE THIRD PHASE: EXPANSION

By midyear 2020, with the testing and supply chain more stabilized, the focus of many academic institutes became how to safely open universities. Closures during the 2020 spring semester brought financial losses to institutions [10]. Psychological impacts of closure on faculty, staff, and students were evident by studies showing moderate to severe scores for anxiety, depression, and stress during the spring semester [11,12]. Academic centers needed to balance the financial pressure and continued strain on mental health with the safety of reopening. The Philadelphia area boasts 80 secondary education institutes, many of which reside in the dense urban setting. Therefore, considerations extend beyond the vulnerable members of the university to those within the surrounding community [10,13,14]. An analytical modeling study found that an effective screening design, robust testing supplies, results management strategy, and compliance to mitigation efforts were crucial to successful university openings and keeping the community safe [13]. A decision tree analysis by members of our institution determined the ideal testing strategy to both mitigate infection rates and minimize

inaccurate results [15]. Reopening designs incorporated the testing strategy and infection control measures that would need to be in place to bring students back to campus safely.

Regulations on the testing programs complicated operational planning. The FDA and CDC differentiate screening and surveillance of SARS-CoV-2. Surveillance and screening both permit broad population testing, but where surveillance can only be reported as an aggregate data, screening results are reported on an individual level to allow isolation and contact tracing to further reduce spread [16,17]. Although the Molecular Pathology Laboratory was able to support UPenn student testing during the fall 2020 semester, this was not a durable solution nor could the capacity encompass all university testing at the effective screening rate [15]. UPenn partnered with Penn Medicine, with expertise in clinical testing, to develop a screening strategy for reopening and sustaining a safe learning environment designated PennCares. Fig. 3 shows the structure and diversity of the Project Quaker team to oversee both the PennCares testing program design and the expansion of hospital SARS-CoV-2 testing. Under the guidance of both university and hospital administration, specialty workstreams developed comprehensive operations for a spring 2021 opening. Campuses that remained open during fall 2020 cited that this integrative planning proved beneficial to mitigate spread [18].

The result of this multidisciplinary approach was the PennCares testing program using the EUA saliva-based platform manufactured by Fluidigm paired with Perkin Elmer Janus G3 liquid handlers to automate technically challenging steps. The PennCares program incorporates what Paltiel and colleagues [13] described as essential components to a screening program: ease of collection along with an accurate, cost-effective, and scalable testing method that could be turned around in a short amount of time. Members of the university community are screened 1 to 3 times per week depending on their risk categorization. The laboratory maintains a turnaround time of 24 hours or less for timely isolation and contact tracing of positive persons. During the spring semester thus far, the positivity rate among university screening tests has remained low, only spiking higher than 1.2% during 3 weeks in January, but positivity always remained below the percent positivity in Philadelphia [3,19]. Two of the high positivity weeks coincided with move-in weeks, indicating that students

FIG. 3 Structure of Project Quaker. Individual workstream teams were co-led with a particular goal to be accomplished. A project manager and executive lead oversaw the progress of each workstream weekly. The executive lead acted as a liaison between the stakeholders and the workstreams. Hosp, hospital; HR, human resources; IT, information technology; LIMS, Laboratory Information Management System.

were arriving on campus with existing infection. The third week with high positivity was 2 weeks after move-in, reflecting some initial noncompliance. Noncompliance, as outlined in agreements signed by students before returning to campus, was met with violation citations, which spiked in January, and steadily decreased thereafter [19]. Cumulative testing and positivity rates over the 2020 to 2021 academic year are presented in Fig. 4 [19]. Overall, implementation of SARS-CoV-2 monitoring has been successful at UPenn and throughout campuses across the United States.

Key struggles to the implementation of Project Quaker centered on the Human Resources workstream ranging from recruiting to training. With numerous clinical laboratories filling the testing demand for SARS-CoV-2, the pools of technical staff qualified to perform high complexity testing were limited. The candidate pool was expanded by splitting the preanalytical and analytical/postanalytical responsibilities into 2 well-defined roles, the laboratory assistant and the medical technologist. This splitting of responsibilities permitted hiring of laboratory assistants to perform preanalytical tasks, such as specimen accessioning and processing, as these are not considered high complexity by the FDA. An advantage of the laboratory assistant position was that hired persons could also staff specimen collection sites, building redundancy with cross-training. To obtain the large number of required staff, Penn partnered with the West Philadelphia Skills Initiative (WPSI) to hire 50 laboratory assistants for the expansion of university and hospital testing. WPSI is a

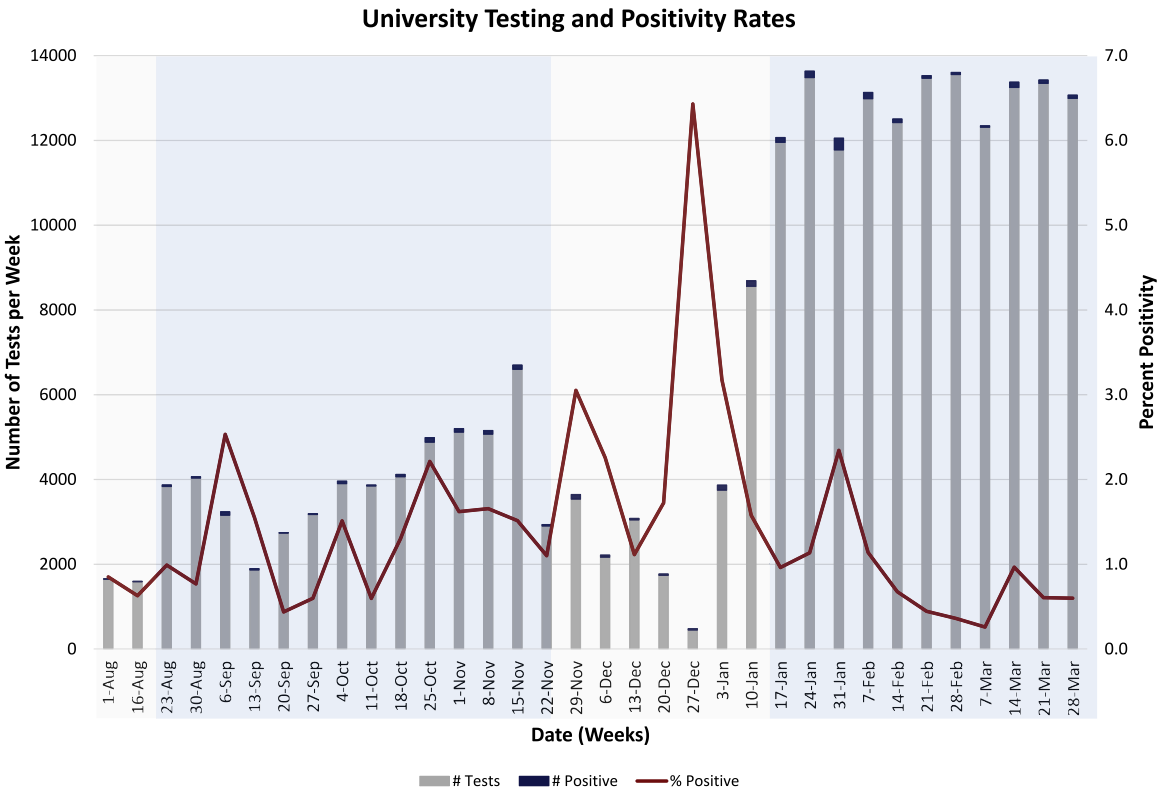

FIG. 4 Weekly university testing and positivity. The number of total (*gray bars*) and positive cases (*dark blue bars*) per week for university students, staff, and faculty. Gray-shaded areas represent dates in which instruction was held virtually or students were on holiday. Blue-shaded areas represent academic semesters. During the fall semester, classes were held virtually, but testing was available. During the spring 2021 semester, testing was mandated 1 to 3 times per week depending on the population risk category. The percent positivity is shown as a red line. (*Data from* COVID-19 Dashboard. University of Pennsylvania. https://coronavirus.upenn.edu/content/dashboard. Published 2021. Accessed 2021.)

program that helps link unemployed Philadelphians with employers through job-specific training models [20]. The partnership developed a 2-week training course, which all laboratory assistant candidates underwent before hire. This model of education and training became crucial to the success of the laboratory. Many of the new staff, both laboratory assistants and medical technologists alike, were in their first jobs and/or had not previously worked in a hospital or Clinical Laboratory Improvement Amendments (CLIA) laboratory environment. As such, job-specific education and training were developed and overseen by the CDT, which now included 2 recently gained full-time employees and several part-time volunteers. Instructional courses covering laboratory regulations (College of American Pathologists [CAP]/CLIA), basic virology, molecular biology, and SARS-CoV-2 diagnostics were required for staff. Additional assay-specific content, including instrumentation and maintenance, general and laboratory information systems workflows, assay performance characteristics, and analysis, was created. Knowledge retention was evaluated with content assessments. Without an experienced group of technologists to guide and train new staff, the CDT took on the role of trainers. Each new technologist began technical training with an in-house developed pipetting course and then assay-specific training, the length of which depended on the experience level of the person. Laboratory assistants and technologists were cleared for clinical work once the CDT deemed them competent in technical skills and assay-specific–based knowledge. We are not alone in recognizing the need for more robust and extensive training with such a new workforce. Recently, the CDC launched the OneLab initiative to develop training and strengthen learning communities to better prepare for emergency responses [21].

The coordinated efforts of Project Quaker greatly expanded SARS-CoV-2 testing capacities. Quality metrics for each of the expanded assays are shown in Table 1 [8]. Both UPenn and its population and the greater Philadelphia community have benefited. Mitigation of spread at the university level maintains a sense of security for the surrounding areas. Increased capacity at the hospital level allows expanded testing for the hospital, but also Philadelphians, hopefully curbing spread and threats of another wave.

THE FOURTH PHASE: LOOKING FORWARD

As the landscape of the SARS-CoV-2 pandemic is ever changing, the CoV R&D group continues to meet on a regular basis, albeit less frequently, to discuss workflow improvements, monitor regulatory policies, and forecast the fluctuating needs within the clinical laboratory. Although various topics, such as home collection, sample pooling, the utility of antigen and antibody testing, and the use of C_T values, are discussed, the recurrent debate is the utility of research and clinical sequencing.

SARS-CoV-2 has a mutation rate of 2.5 nucleotides per month [22]. The clinical utility of sequencing for SARS-CoV-2 variants for individual patients is unclear at the time of writing this article. The Centers for Medicare and Medicaid prohibits lineage identification from surveillance efforts outside of the CLIA laboratory to be returned to patients or clinicians [23]. It remains important to correlate any lineage and variant information closely with clinical presentations and epidemiologic data to inform public health decisions [24,25]. Although no approved variant-specific treatments are currently available, it is difficult to envision a large demand for clinical sequencing without them [26]. Limitations to implementing clinical grade sequencing include costs with no current reimbursement plan, scalability, and informatics [24]. Clinical laboratories themselves benefit from sequencing by rapidly identifying variants that affect polymerase chain reaction detection that have the potential to escape detection [24]. Several identified variant changes lie within the primer/probe regions of commercial nucleic acid amplification tests (NAAT) with known target disruption [27–30]. Tests relying on a single gene target are especially vulnerable; however, most NAATs minimize the risk with multiple target designs. Emerging variants also pose a risk to monoclonal antibody treatments [31–33]. Rapid identification of drug-resistant variants allows health care providers to modify treatments appropriately. As more research is completed and identified variants have known advantages and disadvantages for clinical care, it is possible that sequencing will move into the clinical laboratory setting. However, without a clear utility, clinical-grade sequencing remains minimal. Therefore, sequencing of positive specimens suspected to contain variants of concern often occurs in a research setting as surveillance.

In late 2020, the B.1.1.7 UK lineage spread rapidly throughout England after being identified as a variant of concern [34]. The United Kingdom's centralized sequencing surveillance program documented the quicker spread of B.1.1.7 compared with other circulating strains [35]. Stricter mitigation efforts in England, such as social distancing and a new lockdown, reduced the transmission advantage of the B.1.1.7 lineage [34]. Around the same time, the P.1 and B.1.351 lineages with like variants emerged synchronously [34]. More

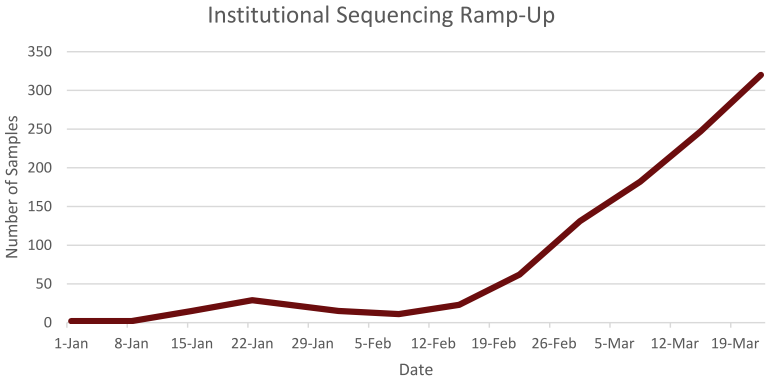

FIG. 5 Penn institutional sequencing. The ramp-up of sequencing efforts during 2021 with the goal of reaching 10% to 20% of all positive cases from the health system and university. A combination of biased and unbiased samples is included for public health initiatives, clinical laboratory quality assurance, and surveillance.

recently, the B.1.526 and B.1.427/429 were identified regionally in New York and California, respectively [36,37]. These discoveries highlighted the need for global lineage surveillance. Lineage and variant identification can inform public health authorities to circulating lineages in a region that have increased transmissibility or infectivity, can cause disease of greater severity, and can decrease treatment responses, and it can be used to identify origins, trace outbreaks, and monitor vaccine effectiveness [24]. Informed decisions, both locally and regionally, to improve protective measures, such as social distancing, gathering restrictions, and cleaning, may be guided by variant detection in monitored regions [22]. In addition, publicly available sequences may be used by manufacturers to pinpoint conserved regions of the genome for future vaccine targets [38]. Given the evolutionary trends of SARS-CoV-2, vaccine breakthrough and vaccine-driven virus evolution are anticipated [39,40]. For these reasons and others, the CDC, public health laboratories, and many institutions and developers have adopted a surveillance strategy, sequencing a percentage of the positive cases. These efforts are both decentralized and constrained in the United States [35,41]. A UPenn research laboratory, similar to efforts at other institutions, accommodates both unbiased surveillance and selective quality assurance and public health sequencing for hospital and university specimens [42]. As shown in Fig. 5, UPenn has been able to scale sequencing efforts over the past few months, but a limit to the capacity exists. Likewise, the GISAID public database shows a rapid increase in sequencing in the United States beginning in November [35]. The CDC has been seeking partnership with commercial diagnostic laboratories, clinical laboratories, and public health laboratories to expand sequencing capacity [43]. The importance of global surveillance intensifies to discover emerging lineages and moderate COVID-19 spread.

SUMMARY

The ability to quickly evolve clinical laboratory testing in a pandemic is essential to patient care. Here, a coordinated cross-disciplinary team and a dedicated CDT streamlined the validation process for SARS-CoV-2 detection implementation and expansion of capacity as well as aided in the design, building, and deployment of 2 new COVID-19 testing laboratories, in a rapidly changing environment. The expanded CDT also established and executed an enhanced training program for new employees. Both the CoV R&D and larger Project Quaker team supported all endeavors. Patient and university SARS-CoV-2 testing was uninterrupted even during expansion and relocation to the new laboratory, thus highlighting the important role of a dedicated CDT especially in the midst of an emergency.

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DISCLOSURE

The authors have nothing to disclose.

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