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Making a Difference: Adaptation of the Clinical Laboratory in Response to the Rapidly Evolving COVID-19 Pandemic

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Abstract

The COVID-19 pandemic, caused by severe acute respiratory syndrome coronavirus 2, led to unprecedented demands assigned to clinical diagnostic laboratories worldwide, forcing them to make significant changes to their regular workflow as they adapted to new diagnostic tests and sample volumes. Herein, we summarize the modifications/adaptation the laboratory had to exercise to cope with rapidly evolving situations in the current pandemic. In the first phase of the pandemic, the laboratory validated 2 reverse transcription polymerase chain reaction–based assays to test ~1000 samples/day and rapidly modified procedures and validated various preanalytical and analytical steps to overcome the supply chain constraints that would have otherwise derailed testing efforts. Further, the pooling strategy was validated for wide-scale population screening using nasopharyngeal swab samples and saliva samples. The translational research arm of the laboratory pursued several initiatives to understand the variable clinical manifestations that this virus presented in the population. The phylogenetic evolution of the virus was investigated using next-generation sequencing technology. The laboratory has initiated the formation of a consortium that includes groups investigating genomes at the level of large structural variants, using genome optical mapping via this collaborative global effort. This article summarizes our journey as the laboratory has sought to adapt and continue to positively contribute to the unprecedented demands and challenges of this rapidly evolving pandemic.

Keywords

COVID-19, pandemic, clinical laboratory, testing, pooling strategy

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Introduction

Diagnostic clinical and translational laboratories across the globe have been the center point of unprecedented demands, particularly focused on diagnostic testing. The COVID-19 pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has forced laboratories to reassess their normal operations through innovative responses amid a range of challenges including test development and supply chain issues. Although the World Health Organization recognized COVID-19 as a global health emergency in late January, the

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magnitude of the disease was only felt a month later in the United States, as the US Food and Drug Administration (FDA) made a significant announcement that Clinical Laboratory Improvement Amendments (CLIA) laboratories can validate and perform COVID-19 testing under FDA-emergency use authorization (FDA-EUA). Testing for SARS-CoV-2 was identified as the most critical measure that needed to be implemented to curtail/manage the spread of the virus. The laboratories were required to make significant changes to adapt to the new diagnostic tests and sample volume. Unlike the large, commercial national laboratories, equipped with significant technological facilities and ample workforce, routine clinical diagnostic laboratories, such as ours had to make a significant leap to redirect the laboratory workflow and meet the expectations. The first step toward adaptation was to evaluate the commercially available COVID-19 diagnostic assays, assess their sensitivity, specificity, the limit of detection, and turnaround time.

Methods

Reverse Transcription Polymerase Chain Reaction–Based Severe Acute Respiratory Syndrome Coronavirus 2 Detection Assays

Luminex assay. The assay is a 1-step multiplex TaqMan-based reverse transcription polymerase chain reaction (RT-PCR) assay with RNA extraction, DNA amplification, and fluorescence detection occurring in a single tube. The assay detects N1 and N3 targets of the *nucleocapsid* (*N*) gene, with the *RNaseP* gene serving as the housekeeping control (ARIES® SARS-CoV-2 Assay [EUA]).

PerkinElmer Inc. assay. The assay is based on RNA extraction followed by TaqMan-based RT-PCR assay to conduct in vitro transcription of SARS-CoV-2 RNA, DNA amplification, and fluorescence detection (PerkinElmer Inc). The assay targets specific genomic regions of the SARS-CoV-2: nucleocapsid (N) gene and ORF1ab. The TaqMan probes for the 2 amplicons are labeled with FAM and ROX fluorescent dyes respectively to generate target-specific signals. The assay includes an RNA internal control (IC, bacteriophage MS2) that serves as assay control from nucleic acid extraction to fluorescence detection. The IC probe is labeled with VIC fluorescent dye to differentiate its fluorescent signal from SARS-CoV-2 targets (PerkinElmer New Coronavirus Nucleic Acid Detection Kit).

Overcoming Supply Chain Deficits

To eliminate testing constraints, we optimized key process variables namely: sample types (nasopharyngeal swabs [NPS], bronchoalveolar lavage [BAL], and saliva), swabs (ESwab or 3D-printed swabs) virus transport media (VTM, universal transport media [UTM], 0.9% NaCl), and RT-PCR reaction to facilitate SARS-CoV-2 testing. The process variables were optimized to include different sample types, using different swabs, VTM with reduced RT-PCR master-mix reaction volume.

The Mass Population Screening Approach

A 10-sample pooling approach was validated by testing 940 samples (934 negative and 6 positive NPS samples in VTM/UTM). In step 1: 94 pools of 10 samples each were created for the downstream test (extraction and RT-PCR). In step 2: Only the pools that showed positive were identified and each sample in those pools was traced and reanalyzed to identify the positive sample(s).

Research to Understand the Variability in Clinical Manifestation in Patients With COVID-19

The genomes of severely ill patients with COVID-19 were investigated using whole-genome optical mapping using Saphyr (Bionano Genomics). Briefly, whole-genome optical mapping involves isolating large molecules of the DNA (150 kb to $\geq$1 Mb), uniformly labeling them at a specific 6-base sequence motif, loading the labeled DNAs into a cartridge, where the molecules are electrophoretically linearized and imaged multiple times using the Bionano Genomics Saphyr platform. Using the captured images, a de novo genome map indicating the positions of the labels was constructed and compared to a reference genome to detect structural differences in the 2 maps. Chromosomal aberrations were detected by comparing optical maps to a reference and control dataset, and a coverage-based copy number variation calling was performed. Independently, the nasal microbiome was investigated by sequencing the microbial genome of severely ill versus asymptomatic patients with COVID-19.

Phylogenetic Evolution of Severe Acute Respiratory Syndrome Coronavirus 2

A high throughput next-generation sequencing (NGS) panel (Respiratory Viral Panel—Illumina) that includes 40 viral pathogens, analyze viral subtypes and mutational variants of SARS-CoV-2 in the state of Georgia, USA, and, to assess the other circulating viruses in the same population. Run metrics was evaluated on the Basespace app by analyzing cluster density and Q30 score. Sequences were then submitted for analysis to the Dragen pipeline for pathogen detection, available on the Basespace app. For phylogenetic analysis, a minimum of 60% coverage threshold was considered. In addition, sequences were also analyzed through the Dragen metagenomics pipeline for detection of viruses and bacteria, in addition to the 40 viruses.

Saliva Samples for COVID-19 Screening

We developed and optimized a protocol for saliva samples collected in both health care and community settings. We evaluated 429 matched NPS and saliva samples collected in either

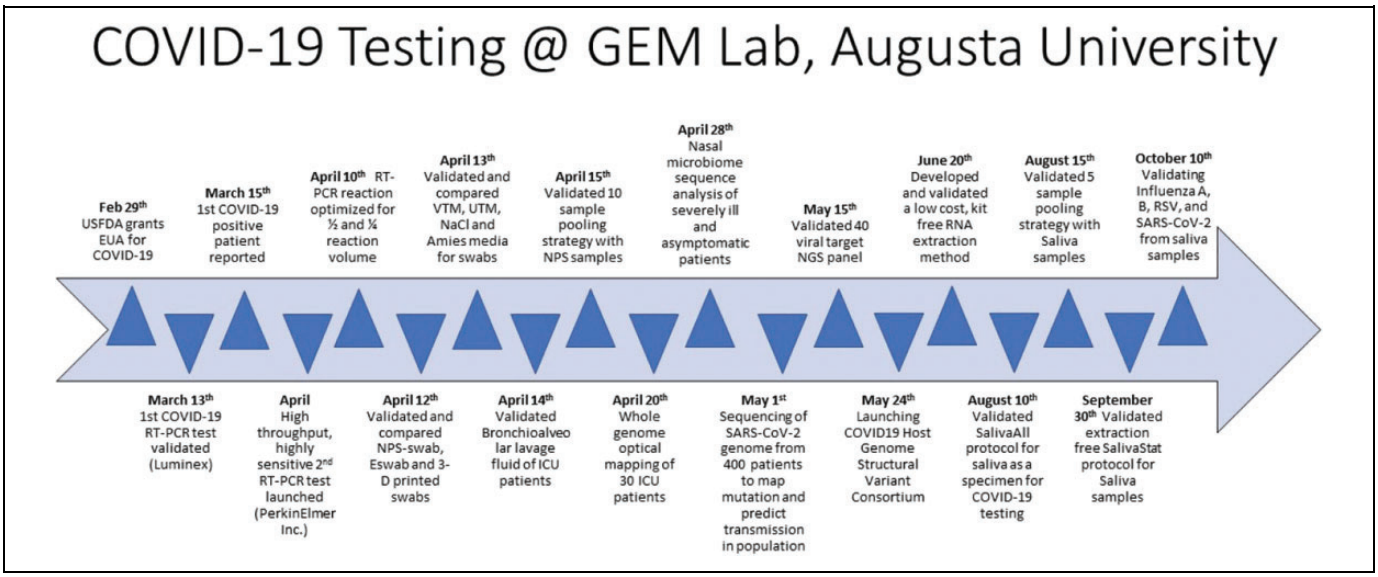

Figure 1. Chronologic sequence of key events in response to the COVID-19 pandemic in the Georgia esoteric molecular lab at Augusta University—Medical College of Georgia.

health care or community setting using the PerkinElmer Inc assay kit. Saliva samples were aliquoted from the collection tubes into Omni tubes (SKU: 19-628D), which were then placed in Omni bead mill homogenizer (Omni International). The samples were homogenized at 4.5 m/s for 30 seconds. Further, a 5-sample pooling strategy was also validated.

COVID-19 and Other Circulating Viruses

We validated an RT-PCR-based multiplex panel (PerkinElmer Inc) targeting SARS-CoV-2, Influenza A and B for saliva, and NPS samples. Further, 1500 clinical samples were evaluated for performance evaluation.

Data Analysis

Data were analyzed for descriptive statistics and presented as a number (%) for categorical variables and mean $\pm$ standard deviation for continuous variables. Regression analysis with slope and intercept along with a 95% CI was determined in the pooling sample study.

Results

Reverse Transcription Polymerase Chain Reaction–Based Severe Acute Respiratory Syndrome Coronavirus 2 Detection Assays

After the announcement made by US-FDA granting authorization to CLIA laboratories to validate and perform COVID-19 testing on February 29, 2020, we evaluated and started validating a one-tube RT-PCR-based detection assay (Luminex) on the ARIES Automated System (Figure 1). The assay was validated for NPS samples and BAL fluids with an LoD of 4500 copies/mL, PPA and NPA of 100%. The assay could run 6 samples in a batch, yielding a result in $\sim$2 hours. The laboratory started working around the clock reporting 72 cases per day for the initial week, and 216 cases for the following weeks.

However, 2 weeks into testing, the demand had substantially increased and we realized the need for a high-throughput assay that could cater to the need for this rapidly growing sample volume. Further, on evaluating several commercial SARS-CoV-2 RT-PCR-based assays, the assays that incorporated 2-step protocol (RNA extraction and RT-PCR) were found to have higher sensitivity and could significantly increase the number of samples resulted in a day. The CDC assay was widely available, where RNA could be extracted using viral extraction kits available from multiple vendors and the primers/probes for the RT-PCR assay were available from IDT. The assay targeted 2 regions of the virus *nucleocapsid* (*N*) gene, N1, and N3, with a housekeeping RNaseP target, having an LoD of 10 to 20 copies/mL. The assay could analyze 30 samples in a run, with each sample going into 3 wells. In addition to the CDC assay, Perkin Elmer Inc released a high-throughput multiplex assay targeting 2 virus genes N and ORF1ab, and internal control (IC). The assay had a sensitivity of 7.16 to 27.4 copies/mL and could analyze 94 samples per run, with each sample going into one well. We validated the PerkinElmer assay for NPS samples following FDA guidelines, with an LOD of 20 copies/mL, PPA and NPA of 100%, and launched the assay within a month of reporting the first positive case. With the 2 assays in the laboratory, we were able to report >1000 samples/day.

Overcoming Supply Chain Deficits

Only a week into reporting $\sim$1000 to 1200 samples/day, we were faced with supply chain constraints pertaining to laboratory supplies that included NPS, VTM, and RT-PCR kits. To

eliminate testing constraints, we optimized both preanalytical and analytical laboratory variables. The preanalytical constraints emerged as the VTM used for collecting NSP swab samples (most common sample type) became exhausted, forcing laboratories to holdup sample collection, or revert to other collection methods (in different media or sample types). To validate the alternate transport media and sample types, we performed "bridging studies" as per FDA recommendations, using 3 serial dilutions of the SARS-CoV-2 viral material in UTM, VTM, 0.9% NaCl, Amies media and BAL samples, which demonstrated comparable results with these modifications. In addition, 3D printed swabs were validated as a sample collection tool by comparing NPS and 3D print swab data from 20 patients. The validation of BAL samples helped us to screen intensive care unit patients on ventilators, as NPS samples could not be collected from this subgroup of patients. In the analytical stage, we optimized the RT-PCR reaction for 1/2 (30 μL) and 1/4 (15 μL) reaction volume, compared with the manufacturer's protocol (60 μL). The results with both 1/2 and 1/4 RT-PCR reactions were comparable with the manufacturer's protocol (60 μL).[1]

The Mass Population Screening Approach

To maximize the yield from the diagnostic assay and to meet the testing requirements of the population in our region, we investigated the feasibility and accuracy of the sample pooling approach and validated a 10-sample pooling protocol for wide-scale population screening for SARS-CoV-2, with PPA of 91.6% and NPA of 100%. The sample pooling strategy tested 940 samples in 148 reactions compared to 940 reactions in routine screening. In terms of cost analysis, 1 million individuals could be tested for $9.1M with the proposed mass population screening approach compared to $58M with routine screening. More important, however, was the potential to massively increase the number of people tested using the same quantity of reagents/test kits, thus limiting supply chain constraints. The sample pooling strategy demonstrated significant potential to catchup with testing needs with minimal turnaround times and facilitate enormous savings on laboratory supplies, extraction, and PCR kits that were in short supply. Further, the strategy might be important as we are now in a phase of reopening business and monitoring cases are necessary to frame policies to manage the pandemic.[2]

Research to Understand the Variability in Clinical Manifestation in Patients With COVID-19

Since the emergence COVID-19 pandemic, SARS-CoV-2 has infected more than 40 997 453 individuals across the globe, with at least 1 127 637 COVID-19-related deaths (https://coronavirus.jhu.edu/map.html, last accessed October 21, 2020). Interestingly, the clinical manifestations in patients with COVID-19 have varied from no symptoms, mild, moderate to severe illness leading to death.[3-7] The extreme diversity frames the premise for the investigation as to why some individuals experience severe symptoms as compared to others. Several groups began to address this underlying phenomenon using varied approaches that included, bioinformatics modeling, transcriptomic, proteomics, genetic susceptibly studies using cell lines, and/or human samples. In this global effort, we searched for knowledge gaps in the approaches/data that emerged from around the world, and to contribute to this global effort, we began distinct research projects in our laboratory.

Several groups had investigated the host genome of severely ill COVID-19 patients and associated single nucleotide variants to the disease severity by comparing with the massive population datasets available because of the decade-old use of NGS technology. Although this approach certainly led to several associations, it was limited to investigating the genome at the level of base pair length variants. A significant percentage of variations in the human genome that includes large structural variants (SVs) remained inaccessible with the sequencing technology. To investigate the human genome at the level of large SVs, we began optical mapping of human genomes using the Bionano Inc Saphyr platform. Given the need to investigate a significant number of human genomes and combined with intriguing preliminary findings, we were able to establish a global consortium, bringing together world experts that were studying the role of structural variations in contributing to the host's immune and overall response to the infection. Since its conception, the consortium has grown and has become a multi-institutional and multiplatform agnostic group dedicated to identifying genetic factors driving discrepancies in patient outcomes.[8]

Apart from the host genome, the human microbiome has been investigated in several diseases, and the implications of the nasal microbiome in a respiratory tract infection might be significant and warrant a thorough investigation. Age has been identified as an independent risk factor for COVID-19 disease severity; therefore, we have investigated the nasal microbiome in COVID-19 patients who become severely ill compared to those who remain asymptomatic [Kolhe et at. 2021].

Phylogenetic Evolution of Severe Acute Respiratory Syndrome Coronavirus 2 Virus

The current COVID-19 testing is utilizing the majority of nationwide resources, which has led to serious knowledge gaps that are required to manage and contain the ongoing pandemic. The signatures of geographic movement and epidemic dynamics are carried in the viral genomes.[9] Consequently, genomic sequencing and comparative genetic analyses can provide actionable information to prevent or mitigate emerging viral threats. Keeping this in view, we have begun to sequence the SARS-CoV-2 genome to gain insights into the phylogenetic evolution of the virus. In addition, with NGS 96 to 384 samples using NextSeq500/550 system can be reported in 72 to 96 hours in our laboratory. In addition to this, we sequenced individuals who seemed to be reinfected to confirm reinfection by comparing the SARS-CoV-2 genome in the 2 infection events. To our

knowledge, only a few cases of reinfection have been reported and sequencing has remained the central approach to confirm these cases.[10]

Saliva Samples for COVID-19 Screening

Saliva samples are of significant interest owing to their ease of collection and alleviating some of the challenges associated with NPS sampling. However, the clinical performance of saliva compared to NPS has shown conflicting results in both health care and community settings. Although different collection devices, media, sample handling, extraction procedure, and RT-PCR methods have accounted for the discrepancies in the literature, validating saliva samples in our laboratory, we identified a critical facet of the saliva samples that if accounted for, renders the saliva samples more sensitive than NPS samples. We introduced a simple bead beating homogenization step in our saliva sample protocol that not only led to the higher sensitivity of saliva samples but also helped us to validate a 5-sample pooling strategy for wide-scale screening.[11] In addition to the validation of saliva samples for COVID-19 screening, we developed and validated a SalivaSTAT protocol where direct PCR could be performed on saliva samples without the need for extraction. The protocol is a rapid, sensitive, and cost-effective method that can be adopted globally, and has the potential to meet testing needs, and may play a significant role in the management of the current pandemic.[12]

COVID-19 and Other Circulating Viruses

The Global Burden of Disease (2017) data demonstrated that influenza contributed 11.5% of the total lower respiratory tract infections, leading to over 9 million hospitalizations and 145 000 deaths across all age groups. Due to the diversion of testing resources and supplies to SARS-CoV-2 testing, the testing of viral pathogens that normally cause seasonal respiratory tract infection has almost been neglected causing serious public health gaps both at a clinical and epidemiological level. To circumvent this crisis, we have validated an NGS assay that targets 40 different viruses including SARS-CoV-2 using NPS samples. The assay could run 96 samples during library preparation and could batch up to 384 samples in a single flow cell on the NextSeq instrument. The panel has the potential to identify co-circulating viruses in the population and help define the transmission dynamics of SARS-CoV-2 at community and state levels, which can provide public health agencies actionable information to prevent or mitigate emerging viral threats. Although NGS is very useful, it has a slightly higher turnaround time. In the pursuit to develop a rapid assay for screening the common flu alongside SARS-CoV-2, we have recently validated an RT-PCR-based multiplex assay targeting SARS-CoV-2, Influenza A/B, and RSV, with RNaseP as a housekeeping gene and expect to publish this data shortly.

Discussion

The SARS-CoV-2 pandemic has placed significant unexpected demands on clinical diagnostic and translational research laboratories. Compounded by supply chain constraints and the need to validate multiple alternatives and create new testing modalities, laboratories have not only been placed front and center of this pandemic but have been tasked with providing perhaps the most significant piece to understanding the dynamics of this pandemic through testing. Laboratories have adapted to these challenges by adopting a multifaceted strategy including assay development and validation, that is, cost-effective, accurate, time efficient and can cater for mass testing in the face of significant supply chain failures. The Laboratory responded to the COVID-19 pandemic's challenges to pathology education with a novel virtual teaching approach. On the initial part of the Pandemic response, all the laboratory rotations and research elective were postponed, cancelled, or kept on hold. Laboratories adapted their rotations to ensure students received meaningful curricular experiences throughout the pandemic. The Virtual teaching approach was an effective solution with a safe, virtual alternative to traditional wet bench experiences. Noteworthy, the laboratory was short-staffed to perform such testing and to cater the community, but the dedication of the staff and managing the laboratory with a positive environment were key ingredients to meet the expectations. The staff was managed with different shifts and soon additional staff were hired/transferred from other department to be trained and give competency to contribute to the laboratories efforts. The laboratory enrolled in College of American Pathologist proficiency testing for SARS-CoV-2 and is monitoring and maintaining the accuracy of the testing. With humility and a sense of accomplishment, we can say that like many other laboratorians around the world, we have stepped up and adapted to meet this unprecedented challenge.

Declaration of Conflicting Interests

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