Early trends for SARS-CoV-2 infection in central and north Texas and impact on other circulating respiratory viruses

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Abstract
Rapid diagnosis and isolation are key to containing the quick spread of a pandemic agent like severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), which has spread globally since its initial outbreak in Wuhan province in China. SARS-CoV-2 is novel and the effect on typically prevalent seasonal viruses is just becoming apparent. We present our initial data on the prevalence of respiratory viruses in the month of March 2020. This is a retrospective cohort study post-launching of SARS-CoV-2 testing at Baylor Scott and White Hospital (BSWH), Temple, Texas. Testing for SARS-CoV-2 was performed by real-time reverse transcription polymerase chain reaction assay and results were shared with State public health officials for immediate interventions. More than 3500 tests were performed during the first 2 weeks of testing for SARS-CoV-2 and identified 168 (4.7%) positive patients. Sixty-two (3.2%) of the 1912 ambulatory patients and 106 (6.3%) of the 1659 emergency department/inpatients tested were positive. The highest rate of infection (6.9%) was seen in patients aged 25 to 34 years, while the lowest rate of infection was seen among patients aged <25 years old (2%). County-specific patient demographic information was shared with respective public health departments for epidemiological interventions. Incidentally, this study showed that there was a significant decrease in the occurrence of seasonal respiratory virus infections, perhaps due to increased epidemiological awareness about SARS-CoV-2 among the general public, as well as the social distancing measures implemented in response to SARS-CoV-2. Data extracted for BSWH from the Centers for Disease Control and Prevention’s National Respiratory and Enteric Virus Surveillance System site revealed that Influenza incidence was 8.7% in March 2020, compared with 25% in March 2019. This study was intended to provide an initial experience of dealing with a pandemic and the role of laboratories in crisis management. This study provided SARS-CoV-2 testing data from ambulatory and inpatient population. Epidemiological interventions depend on timely availability of accurate diagnostic tests and throughput capacity of such systems during large outbreaks like SARS-CoV-2.

KEYWORDS
COVID-19, diagnosis, epidemiology, rRT-PCR, SARS-COV-2
1 | INTRODUCTION

In December 2019, Wuhan city, the capital of Hubei province in China, became the center of an outbreak of pneumonia of unknown cause. By 7th January 2020, Chinese scientists had isolated a novel coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2; previously known as 2019-nCoV), from these patients with virus-infected pneumonia.\(^1\) Cases have now spread to 190 countries. As of 23rd March 2020 there were more than 372 000+ confirmed cases and 16 000+ deaths.\(^2\) Although the outbreak is likely to have started from a zoonotic transmission event associated with a large seafood market that also traded in live wild animals, it soon became clear that efficient person-to-person transmission was occurring.\(^3\) The clinical spectrum of SARS-CoV-2 infection appears to be wide, encompassing asymptomatic infection, mild upper respiratory tract illness, and severe viral pneumonia with respiratory failure and even death, with many patients being hospitalized with pneumonia.\(^4\) A global pandemic has erupted due to a high proportion of asymptomatic patients coupled with a high degree of viral shedding, long incubation period, and late clinical manifestations. Prolific testing coupled with case isolation and contact tracing, therefore, remains one of the most effective epidemiological interventions to stop early community spread. Unfortunately, the novelty of SARS-CoV-2 meant that no testing was immediately available, making it difficult for public health officials to get ahead of the pandemic curve.

As State public health laboratories became backlogged, Baylor Scott and White Health (BSWH), a large, not-for-profit integrated healthcare delivery system in north and central Texas, collaborated with the Luminex Corporation (Austin, TX) to implement a SARS-CoV-2 real-time reverse transcription polymerase chain reaction (rRT-PCR) assay using the genetic primers previously used in China to help relieve the bottleneck. The BSWH laboratory was one of the first laboratories in Texas to implement SARS-CoV-2 testing to assist state public health officials in their efforts to isolate patients and track their immediate contacts. As the pandemic continues to spread across the nation, goal of this study was to share the early clinical trends for coronavirus disease 2019 (COVID-19) in north and central regions of Texas, to encourage other laboratories to consider early implementation of testing during future pandemics. This study also reports the possible impact of SARS-CoV-2 on other seasonal respiratory viruses in this region, the spread of which may have been affected by epidemiological interventions introduced in response to SARS-CoV-2.

2 | METHODS

2.1 | Study design, setting, and participants

This retrospective cohort study included all adult patients (age >18 years) tested for SARS-CoV-2 collected through BSWH testing sites from 11th March 2020 to 23rd March 2020. BSWH is a large, not-for-profit integrated health care delivery system, with 52 hospitals and more than 1000 care sites, serving patients in 47 counties in north and central Texas. All SARS-CoV-2 tests included in this study were performed at the molecular pathology laboratory located within BSWH, Temple.

All ambulatory care patients were prescreened according to World Health Organization and BSWH guidelines to be eligible for SARS-CoV-2 testing via the BSWH web portal, phone app and/or through an e-visit before making an appointment for specimen collection at one of the several designated BSWH locations. Patients were asked about current symptoms, such as fever, cough, and shortness of breath, as well as travel and other exposure history. When clinically indicated, SARS-CoV-2 testing was ordered by the attending physician or other care providers.

No prescreening was done for emergency department (ED)/inpatients. In these settings, a nasopharyngeal swab was collected for SARS-CoV-2 testing from all patients showing signs and symptoms of SARS-CoV-2 infection.

As BSWH laboratory continues testing, this study included data from the day testing began on 11th March 2020 to 23rd March 2020. These two hospital systems within BSWH represent central and north Texas population and are limited to these regions of Texas due to community outreach. Study includes data for SARS-CoV-2 testing from these two regions and seasonal respiratory virus testing data is limited to central Texas region.

2.2 | Data collection

Epidemiological, demographic, clinical, and laboratory data were extracted from the BSWH electronic medical records and laboratory information system. Seasonal respiratory virus data for BSWH, central Texas was collected from Centers for Disease Control and Prevention’s (CDC’s) National Respiratory and Enteric Virus Surveillance System (NREVSS) site.

2.3 | Laboratory procedures

2.3.1 | SARS-CoV-2 testing

Methods for laboratory confirmation of SARS-CoV-2 infection were based on the rRT-PCR technique approved by the US Federal Drug and Food Administration (FDA) under an Emergency Use Authorization (EUA).\(^5\) Briefly, all BSWH nasopharyngeal specimens were collected either at drive through collection sites or from inpatients using a flocked swab in Universal or Transport Media (Copan Technologies). Specimens were transported at 2 to 8 °C to the BSWH-Temple molecular pathology laboratory for processing and testing with less than 3 hours of transit time. The BSWH-Temple molecular pathology laboratory was responsible for SARS-CoV-2 detection in respiratory specimens by rRT-PCR methods (Luminex Corporation, Austin, TX).

The SARS-CoV-2 primers were designed by Luminex to detect RNA targets from the SARS-CoV-2 in respiratory specimens from
patients, as recommended for testing by public health authority guidelines. Luminex Aries employs primers for amplifying the ORF1 gene and the N gene from the SARS-CoV-2 virus, and the assay includes extraction and internal controls (Human RNAase P) built into the same cartridge, to verify sample lysis, nucleic acid extraction, and proper system and reagent performance. Luminex Aries offers true random-access testing, unlike the Luminex NxTAG platform, an assay for batched testing (offering high throughput capabilities) on which increased demand for testing necessitated validation. The Luminex NxTAG method also includes an additional Envelope (E) gene target for SARS-CoV-2 detection.

2.3.2 Other respiratory virus testing

BSWH-Temple utilizes a respiratory virus syndromic panel, also from Luminex and run on the NxTAG platform, to diagnose upper respiratory infections. The assay detects influenza A and B, respiratory syncytial virus (RSV), parainfluenza 1 to 4, human metapneumovirus, rhinovirus/enterovirus, adenovirus, bocavirus, coronaviruses HKU1, NL63, 229E, OC43, chlamydophila pneumoniae, legionella pneumophila, and mycoplasma pneumoniae, with automated DNA/RNA extraction. This test is based on Luminex’s respiratory pathogen panel technology to amplify multiple targets within a single tube and is read on the Luminex MagPix workstation. The BSWH-Temple laboratory also uses standalone PCR tests for Influenza and RSV on the Roche LiAT system (Roche Molecular, Indianapolis, IN) or Luminex Aries, and the tests performed on these instruments were included in this study. A $\chi^2$ test was used to compare the rate of infection for each virus between 2019 and 2020. Statistical significance was set at $P < .05$. In addition to investigating differences in seasonal respiratory virus infections between 2019 and 2020, we examined co-infections rates for SARS-CoV-2 positive patients.

3 RESULTS

3.1 BSWH testing burden during initial periods of community spread

The BSWH-Temple laboratory was one of the first few laboratories in Texas to start testing for SARS-CoV-2 using the Luminex Aries system. The assay was developed and validated on 10th March 2020, and an application for an EUA submitted to the FDA. Relying on the FDA guidance issued on 29 February 2020, allowing laboratories certified under the Clinical Laboratory Improvement Amendments to perform high-complexity testing to use internally validated tests while awaiting an EUA, the BSWH-Temple laboratory accepted Luminex’s claims regarding the Aries and NxTAG systems’ performance with limited internal verification, and started patient testing on 11th March 2020. It became the central testing location supporting all BSWH hospitals across central and north Texas. Data presented in Figure 1 includes daily test volumes, combined from both Luminex Aries and NxTAG platforms. A total of 3571 nasopharyngeal specimens were tested between 11th March and 23rd March 2020. The north Texas region contributed 1219 specimens, while the central Texas region contributed 2352 specimens.

FIGURE 1 Baylor Scott and White Memorial hospital initiated testing on 11th March 2020 following an Emergency Use Authorization submission to Food and Drug Administration (FDA). The data shown represents more than 3500 tests were performed between 11th and 23rd March 2020 for the two different regions of Texas State (Central and North)
The typical turnaround time from specimen collection to verification of test results was less than 15 hours.

Luminex received EUAs for its NxTAG and ARIES platform Sars-CoV-2 assays on 27th March and 3rd April 2020, respectively.

### 3.2 SARS-CoV-2 positive cases and age distribution

A total of 3571 SARS-CoV-2 rRT-PCR tests were performed at the BSWH-Temple laboratory, 1912 specimens were received from ambulatory or drive-through collection sites, and 1659 from ED/inpatient settings. Sixty-two (3.2%) ambulatory patients were tested positive, as did 106 (6.3%) ED/inpatient population, noted in Figures 2 and 3, respectively.

BSWH initial data showed that the age group with the highest percentage of positive tests was patients 25 to 34 years (7.4%) followed by 6.9% in 55 years to 64 years age group. Lowest incidence (2%) was seen among those aged <25 years old (Figure 4).

Clinical symptoms and underlying morbidities for limited number of ED/inpatients are presented in Table 1. More than 75% of the patients who presented in the ED had fever and cough.

### 3.3 Number of confirmed cases per specific county in north and central Texas

An appropriate epidemiological intervention requires identification of patient demographics for public health officials to track and trace positive cases. Therefore, it is prudent that SARS-CoV-2 testing laboratories work closely with local epidemiologists for effective communication of test results. BSWH had previously built an electronic bridge with Texas Department of State Health Services for instant communication of all notifiable conditions. SARS-CoV-2 results were added to this electronic health reporting system for efficient communication.

Major metropolitan areas in both central and north Texas saw higher numbers of positive cases than other regions served by BSWH. Dallas county (north Texas) and Travis county (central Texas) had the highest numbers of positive cases while this manuscript was under preparation (Figure 5).

### 3.4 COVID-19 impact on other circulating respiratory viruses

As local, state, and national epidemiologic countermeasures were enacted, this study observed an interesting correlation between SARS-CoV-2 positive cases and the incidence of other seasonal circulating respiratory viruses during the same timeframe. Data extracted for BSWH from the CDC’s NREVSS site revealed that Influenza incidence was 8.7% in March 2020 compared with 25% in March 2019 (\(P < .0001\)). A declining trend was observed over the last few weeks of March coincides with a sharp uptick in the SARS-CoV-2 incidence in the region. We also observed that bocavirus and para-influenza virus infections were significantly lower in March 2020 compared with March 2019 (\(P < .05\)); similar declines were seen in adenovirus, common cold coronavirus, human metapneumovirus, and...
rhinovirus, and RSV infections for March 2020 compared with March 2019 (Figure 6).

This study also looked at co-infections rates from SARS-CoV-2 positive patients. We searched for 262 patient records that had concurrent testing requests for SARS-CoV-2 and other respiratory virus infections. Contrary to several other reports from other parts of United States, this study did not find any cases of co-infection.

4 | DISCUSSION

The SARS-CoV-2 literature is evolving at breakneck speed, but there is a paucity of literature detailing experiences with the in-house testing solutions implemented to combat the national delays in turn-around time and the shortages of testing kits. Real-time rRT-PCR is already widely deployed in diagnostic virology laboratories; our experience demonstrates that institutions with molecular testing
TABLE 1 Demographic, comorbidity, symptoms, other social, and past medical history of BSWH inpatient population

| Baseline characteristics (total n = 14) |
|----------------------------------------|
| Demographics                          |
| Sex, % (n)                             |
| Male                                   | 29 (4) |
| Female                                 | 71 (10) |
| Age, y                                 |
| Mean                                   | 51.6 |
| Range                                  | 20-81 |
| BMI                                    |
| Mean                                   | 32 |
| Median                                 | 28 |
| Range                                  | 22-63 |
| Comorbid conditions                    |
| Type 2 diabetes mellitus               | 28 (4) |
| Asthma, % (n)                          | 36 (5) |
| COPD, % (n)                            | 21 (3) |
| CHF, % (n)                             | 14 (2) |
| HTN, % (n)                             | 50 (7) |
| Medications, % (n)                     |
| ACE inhibitor                          | 28 (4) |
| Angiotensin receptor blocker           | 7 (1) |
| Social history                         |
| Tobacco, % (n)                         |
| History of use                         | 57 (8) |
| Current use                            | 21 (3) |
| Vaping, % (n)                          | 7 (1) |
| Marijuana, % (n)                       | 7 (1) |
| Subjective symptoms                    |
| Symptom time prior, d                  |
| Mean                                   | 4 |
| Median                                 | 3 |
| Range                                  | 1-21 |
| Fever/chills % (n)                     | 79 (11) |
| Headache % (n)                         | 36 (5) |
| Rhinorrhea % (n)                       | 28 (4) |
| Cough % (n)                            | 79 (11) |
| Otalgia/pressure % (n)                 | 21 (3) |
| Odynophagia % (n)                      | 7 (1) |
| Mild dyspnea % (n)                     | 64 (9) |
| Severe dyspnea % (n)                   | 21 (3) |
| Diarrhea % (n)                         | 7 (1) |
| Clinical characteristics, % (n)        |
| Abnormal chest x-ray                   | 50 (7) |
| Peripheral pulse oximetry <94          | 28 (4) |
| Lymphopenia                            | 21 (3) |
| Temperature 38°C/100.4°F               | 50 (7) |

Abbreviations: ACE, angiotensin-converting enzyme; BMI, body mass index; BSWH, Baylor Scott and White Health; CHF, congestive heart failure; COPD, chronic obstructive pulmonary disease; HTN, hypertension.

capabilities should consider proactively reaching out to manufacturers to improve testing capabilities and turn-around time should a similar situation recur. In the race against this pandemic, real-time data empower epidemiologists and public health officials to identify, track, and contain spread as much as possible. Integrating laboratory-based reporting with epidemiologic surveillance registers will only improve public health outcomes.

The intent of this study was not to assess the performance characteristics of the rRT-PCR assay for the detection of SARS-CoV-2 infection. Accurate determination of test performance characteristics will require appropriate distribution of cohorts among the general population. This is specifically true in the context of virus shedding, transmission dynamics, asymptomatic carriers, and specimen requirements that are still being debated and investigated. SARS-CoV-2 has exhibited a great degree of plasticity in all of the above properties; hence it may take additional time and understanding to determine the performance characteristics of the assay.

The evidence available from the literature at the initiation of the public health emergency was sparse for US healthcare systems. The only country with published data and epidemiological or management studies was China. However, the structure of both the political and the health care system in China is very different from the United States, where healthcare is regional and private for most part, and much of the public health authority is decentralized and vested in state and local governments. While these structures create challenges for the coordinated, authoritarian type of response China implemented to control and mitigate its. SARS-CoV-2 outbreak, the substantial autonomy they provide creates opportunities to try to improve and optimize diagnosis, management and partnership with public health officials. In this context, BSWH ramped up efforts in laboratory diagnosis and collegial collaboration with public health officials to support effective epidemiological interventions.

Because SARS-CoV-2 infection symptoms range from nonspecific mild respiratory symptoms to acute respiratory distress, and because these symptoms are very similar to those of many seasonal viruses, BSWH implemented outpatient screening protocols hosted on the BSWH web portal, phone app, and e-visit sites to ensure appropriate prescreening of individuals for targeted laboratory testing. Real-time RT-PCR testing for various other infections is widely deployed in most diagnostic laboratories. In the case of a public health emergency, proficient diagnostic laboratories can rely on this robust technology and infrastructure to establish new diagnostic tests within their routine services before pre-formulated assays become available. In addition to information on reagents, oligonucleotides, and positive controls, laboratories working under quality control programs need to rely on documentation of technical qualification of the assay formulation as well as data from external clinical evaluation tests.

Everything listed above can be true for a laboratory-developed test; however, if commercial manufacturers design assays under FDA oversight, all of the above requirements can be mitigated. The available genome sequence of SARS-CoV-2 has
enabled several diagnostic kit manufacturers to design primer sets for rRT-PCR diagnostic test builds, in addition to other respiratory pathogen testing.

BSWH worked diligently with Luminex Corporation to submit an FDA emergency use authorization application for their assay during early phases of SARS-CoV-2 community spread in Texas. This early adoption of rRT-PCR assay led to improved turnaround times of SARS-CoV-2 test results, reduced testing burden on public health laboratories, and won praise from the local public health officials for efficient communication of test results for appropriate interventions.

To the best of our knowledge, this is the first report on SARS-CoV-2 testing from Texas, and the first report of a private FIGURE 5  Epidemiological data reported to specific public health departments. Patient demographics were extracted from the laboratory information system and segregated number of positive tests for specific counties within central (S40) and north (S45) Texas regions.

FIGURE 6  Decline in seasonal influenza cases. Following the enactment of epidemiological interventions by the state of Texas, large gatherings were banned and public practicing social distancing may have led to the decrease in the number of Influenza positive cases. Data shown are from March 2019 to March 2020 for percent positive test results for each virus target. A $\chi^2$ test was used to assess the association between the rate of infection for each virus between 2019 and 2020. Statistical significance was set at $P < .05$.
laboratory’s experience with early implementation of SARS-CoV-2 testing in the context of relieving bottlenecks related to testing shortages and delays at the national level. While we hope never to find ourselves again in the situation of a rapidly spreading pandemic and inadequate testing resources and supplies within the traditional public health infrastructure, the reality is that COVID-19 may not be an isolated occurrence. Our results show the capabilities of private laboratories, partnering with both industry and public health stakeholders, to provide alternative mechanisms to support the early adoption of testing so necessary for effective management and control measures in a pandemic situation.

Our results also provide limited insights into the clinical manifestations of patients with COVID-19 who either presented to an ED or were admitted to a BSWH hospital for further evaluation. Major symptoms included were fever and cough, with more than 75% of the patients reporting these symptoms. Khuwara et al\textsuperscript{11} reported similar findings in Wuhan outbreak, with greater than 90% and 75% of the patients exhibiting fever and cough, respectively.

Interestingly, data mining did not yield any co-infections with SARS-CoV-2 in our study population, in contrast to the Stanford Medicine data.\textsuperscript{12} It is likely, however, that the lack of co-infections in our data is attributable to the limited concurrent test ordering for other respiratory viruses in an ambulatory setting. A general impression of the prohibitive cost of respiratory syndromic panels among our providers may have led to the limited ordering in outpatient settings.

SARS-CoV-2 has spread rapidly around the world, posing enormous health, economic, and social challenges to societies. As there are no proven drug or vaccine treatments,\textsuperscript{13} non-pharmaceutical measures are essential to slow the spread of the epidemic.\textsuperscript{14} Social distancing (eg, cancellation of large gatherings, closures of schools and non-essential businesses) is an essential part of public health measures for infection control.\textsuperscript{14}

The non-pharmaceutical measures may have additional impacts on public health. For example, our data indicated significantly lower incidence of other respiratory viruses, such as influenza viruses typically circulating during this time of the year. This observation may be merely coincidental, however, we suspect that the general epidemiological measures, such as social distancing, and cancellation of large gatherings, as well as large portions of the general population being extra-careful about practices, such as handwashing or avoiding handshakes to prevent SARS-CoV-2 infection may have led to the decrease in influenza cases compared last year. However, our results regarding influenza are not intended to, nor should they be interpreted as, conclusive on this point, especially while data for 2019 to 2020 flu vaccine effectiveness is still evolving.

5 | CONCLUSION

We provide results from a molecular diagnostic laboratory’s initial experience with dealing with a pandemic, and an example of how laboratories can contribute to management of public health crises. We demonstrate that proactive collaboration with assay manufacturers can enable laboratories to be prepared for—or respond rapidly to—emerging diseases like COVID-19. Epidemiological interventions depend on availability of accurate diagnostic tests and high throughput capacity during large outbreaks like SARS-CoV-2. It is also important to have a well-organized plan to report the test results to public health officials to initiate counter measures to control the infections, and to build a diagnostic algorithm to include testing for other seasonal respiratory viruses, especially most common viruses like influenza and RSV, which may require medical attention.

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CONFLICT OF INTERESTS

All authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

MBM, AR, and ACA conceived and designed the study. KW and AM performed the experiments. MBM, MMB, RMB, and JKM helped in data analysis and IRB submissions. MBM wrote the paper. All authors read and approved the final manuscript.

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