Viral load of SARS-CoV-2 in adults during the first and second wave of COVID-19 pandemic in Houston, TX: the potential of the super-spreader

Vasanthi Avadhanula, Ph.D.,1 Erin G. Nicholson, M.D.,1,2 Laura Ferlic-Stark, M.S.,1 Felipe-Andres Piedra, Ph.D.,1 Brittani N. Blunck1, Sonia Fragoso, MPH1, Nanette L. Bond, P.A.,1 Patricia L. Santarcangelo, R.N.1, Xunyan Ye PhD1, Trevor J. McBride1, Letisha O. Aideyan1, Kirtida D. Patel1, Lauren Maurer1, Laura S. Angelo, Ph.D.1 and Pedro A Piedra, M.D.1,2

Departments of 1Molecular Virology and Microbiology, and 2Pediatrics, Baylor College of Medicine, Houston, TX, USA

Corresponding author: Pedro A. Piedra, M.D., Department of Molecular Virology and Microbiology, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, USA
E-mail: ppiedra@bcm.edu Telephone no.: 713-798-5240

Summary
A small group of individuals with extremely high viral load for SARS-CoV-2 and mild illness was detected in Houston, TX. Awareness of the social dynamics of these individuals is needed to understand their potential to be super-spreaders within the community.
ABSTRACT

**Background:** During the COVID-19 pandemic, a minority of index cases are associated with a majority of secondary cases suggesting that super-spreaders could drive the pandemic. We identified a phenotype in individuals with extremely high viral load who could act as super-spreaders.

**Methods:** Data were analyzed from individuals tested for SARS-CoV-2 from March 18 through August 15, 2020. Outcomes were compared using contingency table and quantile regression to test the equality of medians between the pandemic waves and by viral load groups.

**Results:** Of the 11,564 samples tested, 1,319 (11.4%) were positive for SARS-CoV-2. An increase in weekly median viral load occurred in the second wave of the SARS-CoV2 pandemic. This population was more likely to be women, outpatients, symptomatic and have an extremely high or high viral load. In patients with multiple RT-PCR positive tests, the duration of viral shedding was comparable between individuals with asymptomatic/mild and mild/moderate illness severity.

**Conclusions:** We detected a small group of individuals with extremely high SARS-CoV-2 viral load with mild illness. We believe that these individuals’ characteristics could be consistent with the super-spreader phenomenon and that greater awareness of the social dynamics of these individuals is needed to understand the spread of SARS-CoV-2.

**Key words:** SARS-CoV-2, super-spreader, extremely high viral load, viral load kinetics, cycle threshold
INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the etiological agent responsible for the coronavirus disease 2019 (COVID-19) [1]. Since the first documented case in Wuhan (Hubei province, China) in December 2019, SARS-CoV-2 has spread globally, leading to 58,570,555 cases and 1,386,596 deaths as of November 22nd 2020 [2]. The SARS-CoV-2 pandemic has caused an unprecedented public health crisis similar to the 1918 influenza pandemic; albeit with important epidemiological differences such as a higher reproductive number (R0) for SARS-CoV-2, and reduced hospitalization and mortality rates in children and young adults compared to older adults with COVID-19 [3–5].

A number of factors likely contributed to this rapid global spread of SARS-CoV-2, including its high transmissibility and a high proportion of asymptomatic illness [6]. The virus itself seems well adapted to human spread due in part to high affinity binding of the SARS-CoV-2 virus to the angiotensin converting enzyme -2 (ACE-2) receptors on a variety of host tissues and organs promoting efficient intra- and inter-host spread [7–9]. Environmental and other non-viral factors also play a role in transmission and disease. Transmission by droplets or aerosols can occur more efficiently in enclosed settings or in poorly ventilated areas [10–12]. Environmental conditions such as air pollution and comorbidities conducive to more severe disease lead to incommensurate levels of infection and severe disease within members of resource-poor and marginalized communities [13–16].

Super-spreading events result in large outbreaks and sustained spread of disease from a few individuals [17,18]. The duration of viral shedding and amount of virus a person sheds from the respiratory tract likely plays a vital role in transmission. Studies have shown that SARS-CoV-2 infected persons have peak viral loads 1-3 days before symptom onset, and can shed virus for three or more weeks [19,20]. Similarly, both symptomatic and asymptomatic individuals can transmit the virus efficiently and can have prolonged viral shedding [21–23]. Consequently, it is important to understand the role of viral load and
viral shedding in the transmission of SARS-CoV-2. In this report, we present data related to viral load, viral shedding and illness during two separate waves of the SARS-CoV-2 pandemic in Houston, Texas, USA. We describe a group of individuals with an extremely high viral load who have the potential to be super-spreaders within the community and become major drivers of the pandemic.

MATERIALS AND METHODS

Subject enrollment and sample collection. Our diagnostic operation for SARS-CoV-2 in a Clinical Laboratory Improvement Amendments (CLIA) Certified Respiratory Virus Diagnostic Laboratory (ID#: 45D0919666) at Baylor College of Medicine (BCM) began on March 18, 2020. Our population consisted of individuals hospitalized or evaluated in the outpatient clinics at BCM and their affiliate institutions from March 18, 2020 through August 15, 2020. Mid-turbinate swab (MT) samples were collected at a drive through collection site while nasopharyngeal swabs (2.1% of total samples) were collected at a hospital, for real-time reverse transcription polymerase chain reaction (RT-PCR) testing. Four distinct adult populations were tested: 1) symptomatic employees utilizing occupational health services, 2) hospitalized patients, 3) patients evaluated at medical and surgical clinics, and 4) patients who required clearance for an out-patient surgical or aerosol generating procedure. RT-PCR testing was performed as a service to BCM and affiliated institutions, while the collection of metadata was performed under an Institutional Review Board approved protocol with waiver of consent.

SARS-CoV-2 RT-PCR. Viral RNA was extracted using the Qiagen Viral RNA Mini Kit (QIAGEN Sciences, Maryland, USA) with an automated extraction platform QIAcube (QIAGEN, Hilden, Germany) according to the manufacturer instructions. 280 µl of sample was extracted and eluted to 100 µl. All samples were tested by CDC 2019-novel coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic panel with primers and probes targeting the nucleocapsid genes, N1 and N2 [24]. Respiratory samples were also tested for a housekeeping gene, ribonuclease P (RNase P). RT-PCR reaction was set up using TaqPath™ 1-Step RT-qPCR Master Mix, CG (Applied Biosystems, CA) and run on 7500 Fast Dx Real-Time PCR
Instrument with SDS 1.4 software. Respiratory samples with cycle threshold (Ct) values <40 for both N1 and N2 primers were considered RT-PCR positive for SARS-CoV-2.

Relative copy number for N1 and N2 were extrapolated later from a standard curve run on separate plate using six 10-fold serial dilutions (1x10^6 to 1x10^4 copies/reaction) of a plasmid containing a complete N gene (IDT technologies).

**Statistical analysis**

**Comparison categories.** We defined the SARS-CoV-2 pandemic into two waves. The first wave occurred from March 18 to May 31, 2020, and the second wave from June 1 through August 15, 2020. A priori, we considered individuals as potential super-spreaders if they had either extremely high or high viral load, and/or prolonged viral shedding. Extremely high or high viral load was defined as Ct values <16 or 16 to <21, respectively. Prolonged viral shedding was defined as having two or more positive RT-PCR tests from an individual on two or more different days. The three categorical comparisons were 1) the first versus second wave, 2) viral load groups classified by their Ct value as extremely high (<16), high (16 to <21), medium (21 to <31) and low (31 to <40), and 3) individuals with single versus two or more RT-PCR positive tests. For all the data analysis involving viral load and viral shedding, N1 Ct value was used.

Statistical analysis and generation of the graphs were carried out using Stata 16.0 (Stata Corp, College Station, Texas). Continuous variables were summarized as median with interquartile range (IQR), or geometric mean with confidence interval (CI) and categorical variables as frequency with percentage of total. Demographic characteristics and RT-PCR outcomes were compared between waves, viral load groups, and single vs. multiple samples with the use of contingency table analysis of proportions and with quantile regression to test the equality of medians. Contingency table analysis was performed using either Fisher's exact or Pearson's chi-squared or Likelihood-ratio chi-squared tests, where appropriate.
Two summary statistics: median duration and cumulative percent at given duration were derived from the serial Ct values to describe viral load kinetics by group and analyzed using quantile regression. No multiple-comparisons adjustments were made to account for multiplicity in testing. Two-sided $P$ values were reported, with $P < 0.05$ considered significant.

RESULTS

First and second waves of SARS-CoV-2 pandemic. March 18 through August 15, 2020 (CDC week 12 to 33), we tested 11,564 samples of which 1,325 (11.5%) were SARS-CoV-2 positive. Six of the positive samples were excluded from further analysis because they represented the second MT swab specimen collected from six individuals on the same day.

During the first pandemic wave (March 18 to May 31) Houston was in lockdown. We tested 2,193 samples and 139 (6.3%) were positive. Peak weeks occurred in CDC weeks 16 and 17 when 11% and 15% of the respiratory samples were positive (Figure 1). Phase 1 reopening of Houston began May 1 and phase II on May 18, 2020. During the second wave (June 1 to August 31), we performed 9,371 RT-PCR tests, of which 1,180 (12.6%) were positive. Peak weeks occurred in CDC weeks 26 and 27 when 14% and 20% of the respiratory samples tested positive, respectively. The percent positivity dropped to 5% by week 33. During the two pandemic waves there were 828 unique individuals who were RT-PCR positive, with 328 having two or more positive specimens collected on different days.

Increased viral load during the second wave. During the second wave we noted a marked increase in weekly median viral load (Figure 2). To analyze both the phenotype of the RT-PCR positive individuals and the kinetics of the viral load over time, four categories were created representing individuals with varying viral loads. The extremely high (N1 Ct <16.0 or >9.74 log$_{10}$ copies/mL), high (N1 Ct 16 to <21 or 7.97 to 9.73 log$_{10}$ copies/mL), medium (N1 Ct: 21 to <31 or 5.70 to 7.96 log$_{10}$ copies/mL) and low (N1 Ct: 31 to <40 or 2.38 to 5.69 log$_{10}$ copies/mL) viral load. Interestingly, during the first wave the weekly viral load (median Ct was 21.3) was highest at CDC week 12, which was followed by the highest positivity.
rate of 15% at CDC week 15. Similarly, during the second wave the weekly viral load was highest (median Ct was 21.7) during CDC week 25 and was followed by the highest positivity rate of 20% at CDC week 27.

**Phenotype of individuals in the second SARS-CoV-2 pandemic wave.** Individuals during the second pandemic wave (n=751) were significantly more likely to be female, been seen at a clinic, have lower median Ct values, and be in the extremely high or high viral load groups (Table 1). Although not statistically significant, there was a trend for higher proportion of Hispanics and individuals with no-comorbidity to be positive during the second wave. The higher median viral load observed in the second wave was in the asymptomatic to mild and mild to moderate disease severity categories (Figure 3). The mean viral load in the second wave was ~ 7 Ct greater, i.e. there was 128-fold higher viral load than the first wave (n=77). The phenotype identified during the second wave suggested that healthy females, many asymptomatic, were contributing to the high viral load being shed in the community.

**Phenotype of individuals with extremely high and high viral loads.** To further define the phenotype of SARS-CoV-2 infected individuals, we analyzed the population by the four viral load categories based on the Ct distribution of the N1 primer (Table 2). Individuals in the extremely high and high viral load groups had on average 20 and 16 Ct difference, respectively, when compared to the low viral load group. This represents an average of approximately $1 \times 10^6$ and $6.6 \times 10^4$ fold-higher viral load in the extremely high and high viral load groups compared to the low viral load group. Significant differences were seen for illness severity, site of testing, number of RT-PCR tests performed. A non-significant trend was observed for race and ethnicity (Table 2). The extremely high viral load group phenotype was, in part, characterized by individuals presenting to the clinic or hospital with symptomatic illness, while individuals in the high viral load group were more likely to be asymptomatic at the time of diagnosis, evaluated at the occupational health clinic, and be RT-PCR positive two or more times.
Phenotype of individuals with two or more positive SARS-CoV-2 PCR tests. A priori, we considered individuals with two or more positive RT-PCR tests as those likely to shed the virus for longer duration compared to individuals with a single positive RT-PCR test (Table 3). The individual phenotype with two or more positive RT-PCR tests were more likely to be asymptomatic or have a mild illness at the time of the initial diagnosis, be seen at the occupational health clinic and have lower median Ct values, which translates to a 7 to 8-fold higher viral load compared to individuals with a single positive RT-PCR test. The individuals with 2 or more positive RT-PCR tests were overrepresented in the high viral load group.

Of note, the median time between the first two positive RT-PCR tests in the two or more positive tests group (N=328) was 12.3 days (IQR 7-15) compared to 20 days (IQR 14-28) for the 73 individuals in the one positive test group who had a subsequent negative test. The median time between two PCR tests was 19 days (IQR 7-34) for the 66 individuals in the one positive test group who had a negative test prior to their positive test.

Viral load kinetics. We next evaluated the viral load kinetics for individuals with three or more positive RT-PCR tests (n =118) by their viral load group category and illness severity. (Figure 4). In general, the second sample from a positive individual was obtained 5-10 days and the third sample obtained 10-20 days after the first positive RT-PCR test. Overall, the viral load decreased with time in all individuals independent of viral load grouping or illness severity. At days 10, 20, 30 and 40 post-first positive RT-PCR tests, 96%, 70%, 35% and 21% of individuals were still RT-PCR positive. Incredibly, one adult in the low viral load group shed SARS-CoV-2 RNA for at least 69 days.

The median duration of viral shedding for the extremely high (n=10), high (n=42), medium (n=28) and low (n=38) viral load groups was 28.5, 25, 21.5, and 26.5 days, respectively. For each viral load category, the duration of viral shedding was comparable between individuals with asymptomatic to mild and mild
to moderate illness severity. Remarkably, the low viral load group had limited fluctuation in their viral load even though they experienced prolonged viral shedding.

DISCUSSION

The present study describes the first two waves of the SARS-CoV-2 pandemic in Houston, TX, USA. We observed an increase in the weekly median viral load that predated the onset of each wave by approximately two weeks. This was more evident during the second wave when the city of Houston was reopening from the initial lockdown. As the weekly median viral load increased, the percent positivity also increased with peak activity offset by two weeks. Similarly, as the weekly median viral load levels decreased, the percent positivity also decreased. This fluctuation in the weekly median viral load was, in part, the result of a subset of individuals detected with extremely high and high viral load levels. Individuals with extremely high and high viral load represented 7.1% and 20.8%, respectively, of the RT-PCR positives in our surveillance study. Such high viral load levels are infrequently observed with other respiratory viruses, even in children [25,26]. Our data support the concept that these individuals are potential super-spreaders for SARS-CoV-2 and major drivers of the pandemic waves. Recent studies document that a minority of index cases are associated with a majority of the secondary cases, consistent with the concept of the super-spreader being a major catalyst of the SARS-CoV-2 pandemic [27].

The extremely high viral load group’s phenotype was characterized by individuals presenting to the clinic or hospital with a mild symptomatic illness. These observations were similar to other studies where there was no relationship of high viral load to severity of disease [20] [21]. The median duration of viral shedding varied between 25-28.5 days for extremely high and high viral load groups when limited to individuals with 3 or more RT-PCR positive samples, and was greater than the median duration of 14.5 days observed in a systematic review [28]. In our individuals with prolonged viral shedding, the period with the highest viral load occurred early in their illness when they are most likely to be infectious. Our observations are consistent with reports that describe viral load shedding kinetics with the highest levels
occurring several days prior to and 7 to 10 days after illness onset[19,29]. In addition, the ability to isolate infectious virus occurred only in individuals with very high viral levels and within the first 10 days after illness onset[30,31].

In our study, a majority of individuals were evaluated at outpatient clinics and tested two or more times for SARS-CoV-2. This group appeared to maintain medium to high viral load for about 10 days from their first RT-PCR positive test suggesting they had the potential to remain infectious during this time period. In a recent report that described the transmission dynamics of SARS-CoV-2 in two Indian states, approximately 8% and 20% of the index cases were responsible for transmitting approximately 60% and 40%, respectively of the secondary cases[32]. Impressively, no positive cases were detected from contact tracing of approximately 70% of the index cases. Comparable results were reported from Shenzhen, China where 9% of index cases were responsible for 80% of secondary infections [33]. It is tempting to speculate that our population of extremely high (7.1%) and high viral load (20.1%) could be responsible for the majority of secondary cases.

The overall phenotype seen in the second pandemic wave shifted to women with no reported co-morbidity who were overrepresented in the extremely high or high viral load groups. It is apparent that extremely high and high viral loads do not translate to disease severity. Many were asymptomatic or had mild illness indicating that without appropriate viral detection, social distancing and quarantine, individuals who have extremely high or high viral load will be able to spread SARS-CoV-2 and sustain the current COVID-19 pandemic.

In addition to viral load, it is essential to evaluate other mechanisms potentially contributing to efficient viral transmission. The D614G mutation in spike protein was associated with high infectivity [34]. Our initial sequencing of 45 SARS-CoV-2 isolates during the first wave showed all had the D614G mutation[35]. Deep sequencing of isolates during the second wave will allow us to assess if there are
mutations associated with high viral load or adult phenotypes where the virus replicates efficiently and could thereby transmit easily.

This study has some limitations. First, the population reported was not representative of the community but rather of individuals who worked within the medical center or used the healthcare services of the medical center. Although these individuals reside within the greater Houston area, there may be confounders that place them at greater risk for SARS-CoV-2 infection. Secondly, the population represents a cross-sectional observational cohort rather than a prospective cohort, which limits the available clinical data. However, in approximately half of the positive individuals we were able to obtain two or more time points with metadata, which add to the strength of our clinical findings. Lastly, the viral kinetic data were limited to individuals who came in primarily to determine when they were negative for SARS-CoV-2. Although the timing of the second and subsequent RT-PCR tests were not performed within a set protocol, they were generally performed every 7 to 14 days until they cleared their infection.

In summary, we detected a marked increase in the median viral load of SARS-CoV-2 infected individuals during the second wave of the pandemic. The extremely high and high viral load groups in general were asymptomatic or had mild clinical illness. The duration of the high viral load and the mild nature of the illness suggest many individuals go undiagnosed. Greater awareness of the social dynamics of these individuals is needed to understand their potential to be super-spreaders of SARS-CoV-2.
Footnotes

**Conflict of interest:** The authors declare no conflict of interest

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**Corresponding author:**

Pedro A. Piedra, M.D.,

Department of Molecular Virology and Microbiology,

Baylor College of Medicine,

One Baylor Plaza, Houston, TX 77030

E-mail: ppiedra@bcm.edu

Telephone no.: 713-798-5240
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Figure legends

**Figure 1**: Number of samples tested and percent positivity over time by CDC week from our laboratory. The total number of positive and negative samples are shown as stacked bar graphs. The 7-day average percent positivity by CDC week is shown as line graph with second Y-axis. The dates of phased re-opening of greater Houston area from lockdown are shown.

**Figure 2**: RT-PCR Ct value (N1) of individuals by CDC week during the first and second wave of the SARS-CoV-2 pandemic. In the box plot, the median Ct value is represented by horizontal line, boxes represent 25% and 75% percentile and the whiskers represent 10\textsuperscript{th} and 90\textsuperscript{th} percentile. The four viral load groups are represented by decreasing shade of grey. Each individual sample is represented as a red dot.

**Figure 3**: Comparison of the viral load of individuals infected in the first and second SARS-CoV-2 wave by disease severity. In the box plot, the median Ct value is represented by horizontal line, boxes represent 25% and 75% percentile and the whiskers represent 10\textsuperscript{th} and 90\textsuperscript{th} percentile. The four viral load groups are represented by decreasing shade of grey. Each individual sample is represented as a red dot.

**Figure 4**: Viral load kinetics of individuals with three or more SARS-CoV-2 RT-PCR positive tests. The viral kinetics are shown by disease severity at presentation. X-axis shows days since the first positive RT-PCR test and Y-axis represents viral load by Ct value. The lines indicate Ct values of serially collected samples from the same person since their initial RT-PCR positive test (Day 0).
Table 1. Comparison of demographic and clinical characteristics between SARS-CoV-2 RT-PCR positive individuals infected during the first and second pandemic waves in Houston, TX, USA.

|                           | 1st Wave (N = 77) | 2nd Wave (N = 751) | P-value |
|---------------------------|-------------------|--------------------|---------|
| Age, years median (IQR)   | 46.0 (34.0-55.0)  | 41.0 (30.0-54.0)   | 0.136   |
| Age Group, years n (%)    |                   |                    |         |
| 0-17                      | 0 (0.0)           | 5 (0.7)            |         |
| 18-34                     | 20 (26.0)         | 269 (35.8)         |         |
| 35-49                     | 26 (33.8)         | 239 (31.8)         |         |
| 50-64                     | 24 (31.2)         | 159 (21.2)         |         |
| 65-79                     | 6 (7.8)           | 67 (8.9)           |         |
| 80 plus                   | 1 (1.3)           | 12 (1.6)           |         |
| Gender, n (%)             |                   |                    | 0.021   |
| Female                    | 33 (42.9)         | 425 (56.6)         |         |
| Male                      | 44 (57.1)         | 326 (43.4)         |         |
| Race, n (%)               |                   |                    | 0.730   |
| Asian                     | 3 (5.6)           | 38 (7.1)           |         |
| Black                     | 19 (35.2)         | 193 (36.1)         |         |
| White                     | 30 (55.6)         | 293 (54.9)         |         |
| Other/Multiracial         | 2 (3.7)           | 10 (1.9)           |         |
| [Unknown]                 | [23]              | [217]              |         |
| Ethnicity, n (%)          |                   |                    | 0.065   |
| Hispanic                  | 14 (21.9)         | 210 (33.2)         |         |
| Non-Hispanic              | 50 (78.1)         | 423 (66.8)         |         |
| [Unknown]                 | [13]              | [118]              |         |
| Illness Severity†, n (%)  |                   |                    | 0.001   |
| Asymptomatic/Mild         | 52 (67.5)         | 449 (60.7)         |         |
| Mild/Moderate             | 17 (22.1)         | 267 (36.1)         |         |
| Moderate/Severe           | 8 (10.4)          | 24 (3.2)           |         |
| [Unknown]                 | [0]               | [11]               |         |
| Co-morbidities, n (%)     |                   |                    | 0.064   |
| None                      | 34 (46.6)         | 435 (59.2)         |         |
| One                       | 25 (34.2)         | 225 (30.6)         |         |
| Two                       | 10 (13.7)         | 49 (6.7)           |         |
| Three or more             | 4 (5.5)           | 26 (3.5)           |         |
| [Unknown]                 | [4]               | [16]               |         |
| Site of Testing, n (%)    |                   |                    | <0.001  |
| Pre-Op                    | 3 (3.9)           | 62 (8.3)           |         |
| Clinic                    | 25 (32.5)         | 403 (53.7)         |         |
| Hospital                  | 2 (2.6)           | 40 (5.3)           |         |
| Occupational Health       | 31 (40.3)         | 240 (32.0)         |         |
| Other                     | 16 (20.8)         | 6 (0.8)            |         |
| Number of Positive Tests, n (%) |        |                    | 0.112   |
| One                       | 40 (51.9)         | 460 (61.3)         |         |
| Two or more | 37 (48.1) | 291 (38.7) |
|-------------|----------|------------|
| **RT-PCR** |          | <0.001     |
| N1          |          |            |
| Ct value, median (IQR) | 34.1 (27.2-36.5) | 27.3 (19.7-33.4) |
| Log₁₀ copies/mL, GM [95% CI] | 4.9 [4.5, 5.3] | 6.3 [6.2, 6.5] |
| N2          |          | <0.001     |
| Ct value, median (IQR) | 34.8 (27.8-37.1) | 27.8 (19.7-34.1) |
| Log₁₀ copies/mL, GM [95% CI] | 5.0 [4.6, 5.5] | 6.5 [6.4, 6.7] |
| RNase P     |          | 0.011      |
| Ct value, median (IQR) | 28.9 (27.7-30.2) | 27.9 (26.7-29.1) |
| **Viral Load Group†, n (%)** | <0.001 |          |
| <16         | 0 (0.0) | 59 (7.9) |
| 16 to <21   | 8 (10.4) | 164 (21.8) |
| 21 to <31   | 18 (23.4) | 248 (33.0) |
| 31 to <40   | 51 (66.2) | 280 (37.3) |

†Illness Severity: Asymptomatic/mild (contact/exposure assessment); Mild/Moderate (outpatient/discharged from the ED); Moderate/Severe (hospitalized/ICU)
‡Viral Load Group: grouped by N1 Ct value ranges

Abbreviations: IQR: interquartile range; GM [95% CI]: geometric mean with 95% confidence interval
Table 2. Comparison of demographics between SARS-CoV-2 RT-PCR positive individuals with varying viral loads during the first and second pandemic waves in Houston, TX, USA

|                      | Extremely high viral load group (<16 Ct) | High viral load group (16 to <21 Ct) | Medium viral load group (21 to <31 Ct) | Low viral load group (31 to ≤40 Ct) | P-value | p-value |
|----------------------|----------------------------------------|--------------------------------------|----------------------------------------|------------------------------------|---------|---------|
| **Age, years median (IQR)** | (N = 59)                               | (N = 172)                            | (N = 266)                              | (N = 331)                          | 0.058   |         |
|                      | 36.0                                   | 44.5                                 | 40.0                                   | 43.0                               |         |         |
|                      | (27.0-49.0)                            | (31.5-52.5)                          | (29.0-54.0)                            | (31.0-55.0)                        |         |         |
| **Age Group, years n (%)** |                                         |                                      |                                        |                                    | 0.277   |         |
| 0-17                 | 1 (1.7)                                | 0 (0.0)                              | 2 (0.8)                                | 2 (0.6)                            |         |         |
| 18-34                | 27 (45.8)                              | 55 (32.0)                            | 95 (35.7)                              | 112 (33.8)                         |         |         |
| 35-49                | 17 (28.8)                              | 64 (37.2)                            | 86 (32.3)                              | 98 (29.6)                          |         |         |
| 50-64                | 8 (13.6)                               | 36 (20.9)                            | 55 (20.7)                              | 84 (25.4)                          |         |         |
| 65-79                | 6 (10.2)                               | 12 (7.0)                             | 26 (9.8)                               | 29 (8.8)                           |         |         |
| 80 plus              | 0 (0.0)                                | 5 (2.9)                              | 2 (0.8)                                | 6 (1.8)                            |         |         |
| **Gender, n (%)**    |                                         |                                      |                                        |                                    | 0.225   |         |
| Female               | 34 (57.6)                              | 83 (48.3)                            | 152 (57.1)                             | 189 (57.1)                         |         |         |
| Male                 | 25 (42.4)                              | 89 (51.7)                            | 114 (42.9)                             | 142 (42.9)                         |         |         |
| **Race, n (%)**      |                                         |                                      |                                        |                                    | 0.070   |         |
| Asian                | 6 (15.0)                               | 9 (6.7)                              | 15 (8.0)                               | 11 (4.8)                           |         |         |
| Black                | 15 (37.5)                              | 27 (26.7)                            | 69 (36.9)                              | 91 (40.1)                          |         |         |
| White                | 19 (47.5)                              | 83 (61.9)                            | 98 (52.4)                              | 123 (54.2)                         |         |         |
| Other/Multiracial    | 0 (0.0)                                | 5 (3.7)                              | 5 (2.7)                                | 2 (0.9)                            |         |         |
| [Unknown]            | [19]                                   | [38]                                 | [79]                                   | [104]                              |         |         |
| **Ethnicity, n (%)** |                                         |                                      |                                        |                                    | 0.060   |         |
| Hispanic             | 17 (34.7)                              | 37 (25.2)                            | 86 (38.1)                              | 84 (30.5)                          |         |         |
| Non-Hispanic         | 32 (65.3)                              | 110 (74.8)                           | 140 (61.9)                             | 191 (69.5)                         |         |         |
| [Unknown]            | [10]                                   | [25]                                 | [40]                                   | [56]                               |         |         |
| **Illness Severity†, n (%)** |                                         |                                      |                                        |                                    | 0.011   |         |
| Asymptomatic/Mild    | 26 (44.1)                              | 100 (58.5)                           | 156 (59.1)                             | 219 (67.8)                         |         |         |
| Mild/Moderate        | 30 (50.8)                              | 67 (39.2)                            | 95 (36.0)                              | 92 (28.5)                          |         |         |
| Moderate/Severe      | 3 (5.1)                                | 4 (2.3)                              | 13 (4.9)                               | 12 (3.7)                           |         |         |
| [Unknown]            | [0]                                    | [1]                                  | [2]                                    | [8]                                |         |         |
| **Co-morbidities, n (%)** |                                         |                                      |                                        |                                    | 0.139   |         |
| None                 | 31 (52.5)                              | 106 (61.6)                           | 167 (63.5)                             | 165 (52.5)                         |         |         |
| One                  | 20 (33.9)                              | 51 (29.7)                            | 73 (27.8)                              | 106 (33.8)                         |         |         |
| Two                  | 7 (11.9)                               | 8 (4.7)                              | 14 (5.3)                               | 30 (9.6)                           |         |         |
| Three or more        | 1 (1.7)                                | 7 (4.1)                              | 9 (3.4)                                | 13 (4.1)                           |         |         |
| [Unknown]            | [0]                                    | [0]                                  | [3]                                    | [17]                               |         |         |
| **Site of Testing, n (%)** |                                         |                                      |                                        |                                    | <0.001  |         |
| Pre-Op               | 1 (1.7)                                | 7 (4.1)                              | 16 (6.0)                               | 41 (12.4)                          |         |         |
| Clinic               | 31 (52.5)                              | 93 (54.1)                            | 141 (53.0)                             | 163 (49.2)                         |         |         |
| Hospital             | 9 (15.3)                               | 5 (2.9)                              | 15 (5.6)                               | 13 (3.9)                           |         |         |
| Occupational Health  | 17 (28.8)                              | 64 (37.2)                            | 90 (33.8)                              | 100 (30.2)                         |         |         |
| Other                | 1 (1.7)                                | 3 (1.7)                              | 4 (1.5)                                | 14 (4.2)                           |         |         |
| **Number of Positive Tests, n (%)** |                                         |                                      |                                        |                                    | <0.001  |         |
| One                  | 39 (66.1)                              | 78 (45.3)                            | 165 (62.0)                             | 218 (65.9)                         |         |         |
| Two or more          | 20 (33.9)                              | 94 (54.7)                            | 101 (38.0)                             | 113 (34.1)                         |         |         |
| **RT-PCR** N1        |                                         |                                      |                                        |                                    | <0.001  |         |
| Ct value, median (IQR) | 14.9                                   | 18.2                                 | 26.2                                   | 34.5                               |         |         |
|                      | (13.6-15.5)                            | (17.0-19.5)                          | (23.4-28.5)                            | (33.0-36.2)                        |         |         |
| Log₁₀ copies/mL, GM [95% CI] | 9.9                                    | 8.8                                  | 6.5                                    | 4.0                                |         |         |
|                | N2                                    | [9.8, 10.0] | [8.7, 8.8] | [6.4, 6.6] | [3.9, 4.0] |
|----------------|---------------------------------------|-------------|------------|------------|------------|
| Ct value, median (IQR) | 14.8 (13.9-15.5)                      | 18.3 (17.0-19.6) | 26.5 (23.8-29.1) | 35.6 (33.7-37.5) | <0.001     |
| Log_{10} copies/mL, GM [95% CI] | 10.3 (10.2, 10.4)                     | 9.1 (9.0, 9.2) | 6.7 (6.6, 6.8) | 4.0 (4.0, 4.1) |
| RNa$\text{se}$ P | 26.9 (24.9-27.9)                       | 28.2 (27.1-29.3) | 28.0 (26.8-29.2) | 28.1 (26.7-29.4) | 0.020      |

†Illness Severity: Asymptomatic/mild (contact/exposure assessment); Mild/Moderate (outpatient/discharged from the ED); Moderate/Severe (hospitalized/ICU)

Abbreviations: IQR: interquartile range; GM [95% CI]: geometric mean with 95% confidence interval
Table 3. Comparison between persons with single or subsequent positive tests for SARS-CoV-2

|                                | One Positive Test (N = 500) | Two or more Positive Tests (N = 328) | P-value |
|--------------------------------|-----------------------------|--------------------------------------|---------|
| **Age, years median (IQR)**    |                             |                                      |         |
| 0-17                           | 4 (0.8)                     | 1 (0.3)                              | 0.297   |
| 18-34                          | 170 (34.0)                  | 119 (36.3)                           |         |
| 35-49                          | 156 (31.2)                  | 109 (33.2)                           |         |
| 50-64                          | 114 (22.8)                  | 69 (21.0)                            |         |
| 65-79                          | 49 (9.8)                    | 24 (7.3)                             |         |
| 80 plus                        | 7 (1.4)                     | 6 (1.8)                              |         |
| **Gender, n (%)**              |                             |                                      | 0.624   |
| Female                         | 280 (56.0)                  | 178 (54.3)                           |         |
| Male                           | 220 (44.0)                  | 150 (45.7)                           |         |
| **Race, n (%)**                |                             |                                      | 0.230   |
| Asian                          | 21 (6.1)                    | 20 (8.3)                             |         |
| Black                          | 136 (39.2)                  | 76 (31.5)                            |         |
| White                          | 184 (53.0)                  | 139 (57.7)                           |         |
| Other/Multiracial              | 6 (1.7)                     | 6 (2.5)                              |         |
| [Unknown]                      | [153]                       | [87]                                 |         |
| **Ethnicity, n (%)**           |                             |                                      | 0.844   |
| Hispanic                       | 132 (32.4)                  | 92 (31.7)                            |         |
| Non-Hispanic                   | 275 (67.6)                  | 198 (68.3)                           |         |
| [Unknown]                      | [93]                        | [38]                                 |         |
| **Illness Severity**, n (%)    |                             |                                      | <0.001  |
| Asymptomatic/Mild              | 273 (55.7)                  | 228 (69.7)                           |         |
| Mild/Moderate                  | 187 (38.2)                  | 97 (29.7)                            |         |
| Moderate/Severe                | 30 (6.1)                    | 2 (0.6)                              |         |
| [Unknown]                      | [10]                        | [1]                                  |         |
| **Co-morbidities, n (%)**      |                             |                                      | 0.230   |
| None                           | 270 (55.9)                  | 199 (61.2)                           |         |
| One                            | 157 (32.5)                  | 93 (28.6)                            |         |
| Two                            | 34 (7.0)                    | 25 (7.7)                             |         |
| Three or more                  | 22 (4.6)                    | 8 (2.5)                              |         |
| [Unknown]                      | [17]                        | [3]                                  |         |
| **Site of Testing, n (%)**     |                             |                                      | <0.001  |
| Pre-Op                         | 47 (9.4)                    | 18 (5.5)                             |         |
| Clinic                         | 249 (49.8)                  | 179 (54.6)                           |         |
| Hospital                       | 41 (8.2)                    | 1 (0.3)                              |         |
| Occupational Health            | 146 (29.2)                  | 125 (38.1)                           |         |
| Other                          | 17 (3.4)                    | 5 (1.5)                              |         |
| **Viral Load Group, n (%)**    |                             |                                      | <0.001  |
| <16                            | 39 (7.8)                    | 20 (6.1)                             |         |
| 16 to <21                      | 78 (15.6)                   | 94 (28.7)                            |         |
| 21 to <31                      | 165 (33.0)                  | 101 (30.8)                           |         |
| 31 to <40                      | 218 (43.6)                  | 113 (34.5)                           |         |

† Multinomial logistic regression analysis
‡ Additional analysis not possible due to small sample size
### RT-PCR

|            | N1                  | N2                  | RNase P               |
|------------|---------------------|---------------------|-----------------------|
| Ct value, median (IQR) | 28.7 (21.4-34.1)       | 29.3 (21.7-35.0)       | 27.8 (26.6-29.1)       |
| Log$_{10}$ copies/mL, GM [95% CI] | 6.0 [5.8, 6.2]       | 6.2 [6.0, 6.4]       | 6.7 [6.4, 6.9]       |
| Ct value, median (IQR) | 25.9 (18.7-33.1)       | 25.9 (18.9-33.9)       | 28.2 (27.1-29.4)       |
| Log$_{10}$ copies/mL, GM [95% CI] | 6.5 [6.3, 6.7]       | 6.7 [6.4, 6.9]       |                       |

† Illness Severity: Asymptomatic/mild (contact/exposure assessment); Mild/Moderate (outpatient/discharged from the ED); Moderate/Severe (hospitalized/ICU)

‡ Viral Load Group: grouped by N1 Ct value ranges

Abbreviations: IQR: interquartile range; GM [95% CI]: geometric mean with 95% confidence interval
Figure 1

The graph shows the number of samples and the percentage positivity over CDC weeks from 12 to 33. The x-axis represents the CDC week, while the y-axis shows the number of samples and the percentage positivity. There are two phases of reopening marked: Phase I on May 1st, 2020, and Phase II on May 18th, 2020. The graph indicates a significant increase in positivity during these phases.
Figure 2

![Box plot of N1 Ct values over CDC weeks, with two waves indicated.]
Figure 3

Box plots showing the distribution of N1 Ct values across different SARS-CoV-2 pandemic waves. The plots are categorized by severity status: Asymptomatic/Mild, Mild/Moderate, and Moderate/Severe. The data points are differentiated for the 1st and 2nd waves.
Figure 4