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Detection of Higher Cycle Threshold Values in Culturable SARS-CoV-2 Omicron BA.1 Sublineage Compared with Pre-Omicron Variant Specimens — San Francisco Bay Area, California, July 2021—March 2022

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Before emergence in late 2021 of the highly transmissible B.1.1.529 (Omicron) variant of SARS-CoV-2, the virus that causes COVID-19 (1,2), several studies demonstrated that SARS-CoV-2 was unlikely to be cultured from specimens with high cycle threshold (Ct) values§ from real-time reverse transcription–polymerase chain reaction (RT-PCR) tests (suggesting low viral RNA levels) (3). Although CDC and others do not recommend attempting to correlate Ct values with the amount of infectious virus in the original specimen (4,5), low Ct values are sometimes used as surrogate markers for infectiousness in clinical, public health, or research settings without access to virus culture (5). However, the consistency in reliability of this practice across SARS-CoV-2 variants remains uncertain because Omicron-specific data on infectious virus shedding, including its relationship with RNA levels, are limited. In the current analysis, nasal specimens collected from an ongoing longitudinal cohort¶ (6,7) of nonhospitalized participants with positive SARS-CoV-2 test results living in the San Francisco Bay Area** were used to generate Ct values and assess for the presence of culturable SARS-CoV-2 virus; findings were compared between specimens from participants infected with pre-Omicron variants and those infected with the Omicron BA.1 sublineage. Among specimens with culturable virus detected, Ct values were higher (suggesting lower RNA levels) during Omicron BA.1 infections than during pre-Omicron infections, suggesting variant-specific differences in viral dynamics. Supporting CDC guidance, these data show that Ct values likely do not provide a consistent proxy for infectiousness across SARS-CoV-2 variants.

As part of an ongoing longitudinal cohort study, persons with documented SARS-CoV-2 infection (based on a positive clinical real-time RT-PCR test result) and their household members were recruited within 5 days of the first symptom onset in the household (or first RNA-positive test result if the infected person was asymptomatic). All participants self-collected nasal swab specimens once daily for 2 weeks from the first onset in the household; some participants also provided a serum specimen at enrollment to identify evidence of previous infection.†† In a single laboratory, real-time RT-PCR targeting SARS-CoV-2 nucleocapsid (N) and envelope protein (E) genes§§ (8) was used to detect RNA and to determine Ct values, whole genome sequencing was used to identify the infecting variant strain and sublineage, and the presence or absence of culturable virus was assessed by cytopathic effect observed in tissue culture.¶¶ Enrollment sera were tested for the presence or absence of anti-N immunoglobulin G (IgG) per manufacturer (Abbott) instructions at a clinical laboratory at the University of California, San Francisco.

Participants with confirmed infection (based on having at least one nasal specimen test positive by real-time RT-PCR for both
A total of 1,147 nasal swab specimens from 124 participants were analyzed; among 17 participants infected with Omicron variants (all BA.1 sublineages) and 107 infected with pre-Omicron variants, 149 and 998 specimens, respectively, were collected (Table). Timing of specimen collection after onset (in each participant) was similar in both groups (median = 8 days; IQR = 6–11 days). Among the 17 participants with Omicron BA.1 infections, nine (53%) were adults and 10 (59%) were fully vaccinated. Among 107 participants with pre-Omicron infections, 92 (86%) were adults and 35 (33%) were fully vaccinated. Nearly all participants were symptomatic (16 of 17 participants with Omicron BA.1 infection and 100 of 107 with pre-Omicron infection). No participants reported previous infection, and among 58 participants with available sera, none had detectable anti-N IgG at enrollment.

Accounting for age group and vaccination status, E-specific Ct values in all specimens were significantly higher in Omicron specimens than in pre-Omicron specimens (Ct difference = 4.45, p<0.001). Among all specimens, N-specific Ct values were also significantly higher in Omicron versus pre-Omicron infections (Ct difference = 3.27, p<0.001). When analysis was limited to RNA-positive specimens, a similar trend was observed (Ct difference = 3.90, p<0.001).

**Abbreviations:** Ct = cycle threshold; E = envelope gene.

* Fully vaccinated participants were defined as those who had received all recommended doses of a Food and Drug Administration–authorized or approved primary vaccine series (2 mRNA vaccine doses or a single dose of Johnson & Johnson [Janssen] vaccine) ≥14 days before either symptom onset or enrollment (whichever occurred earlier).

† Participants were considered symptomatic if they reported one or more COVID-19 signs or symptoms consistent with those listed by CDC, including fever, chills, shortness of breath, fatigue, muscle or body aches, headache, loss of taste, loss of smell, sore throat, congestion, runny nose, nausea, vomiting, or diarrhea. https://www.cdc.gov/coronavirus/2019-ncov/symptoms-testing/symptoms.html

$ RNA-positive specimens are positive for both SARS-CoV-2 nucleocapsid and envelope gene real-time RT-PCR targets. Virus-positive specimens contain viable SARS-CoV-2 virus detected in tissue culture.

<table>
<thead>
<tr>
<th>Participant and specimen</th>
<th>Pre-Omicron</th>
<th>Omicron</th>
<th>Change in E-specific Ct value between pre-Omicron and Omicron variants</th>
</tr>
</thead>
<tbody>
<tr>
<td>All participants (N = 124)</td>
<td>107 (100)</td>
<td>17 (100)</td>
<td>—</td>
</tr>
<tr>
<td>Adults aged ≥18 yrs</td>
<td>92 (86)</td>
<td>9 (53)</td>
<td>—</td>
</tr>
<tr>
<td>Fully vaccinated*</td>
<td>35 (33)</td>
<td>10 (59)</td>
<td>—</td>
</tr>
<tr>
<td>Symptomatic†</td>
<td>100 (93)</td>
<td>16 (94)</td>
<td>—</td>
</tr>
<tr>
<td>Culturable virus detected</td>
<td>76 (71)</td>
<td>13 (76)</td>
<td>—</td>
</tr>
<tr>
<td>All specimens (N = 1,147)</td>
<td>998 (100)</td>
<td>149 (100)</td>
<td>4.45$</td>
</tr>
<tr>
<td>RNA-positive specimens§</td>
<td>539 (53)</td>
<td>72 (48)</td>
<td>3.90§</td>
</tr>
<tr>
<td>Virus-positive specimens¶</td>
<td>298 (30)</td>
<td>39 (26)</td>
<td>5.77¶</td>
</tr>
<tr>
<td>Median duration of virus detection after onset, days (IQR)</td>
<td>6 (5–8)</td>
<td>6 (5–8)</td>
<td>—</td>
</tr>
<tr>
<td>Median interval from onset to specimen collection, days (IQR)</td>
<td>8 (6–11)</td>
<td>8 (6–11)</td>
<td>—</td>
</tr>
</tbody>
</table>

**TABLE.** Characteristics of participants infected with SARS-CoV-2 pre-Omicron variants and Omicron BA.1 sublineage and nasal swab specimens evaluated for real-time reverse transcription–polymerase chain reaction cycle threshold values — San Francisco Bay Area, California, July 2021–March 2022

*** Participants were considered symptomatic if they reported one or more COVID-19 signs or symptoms consistent with those listed by CDC, including fever, chills, shortness of breath, fatigue, muscle or body aches, headache, loss of taste, loss of smell, sore throat, congestion, runny nose, nausea, vomiting, or diarrhea. https://www.cdc.gov/coronavirus/2019-ncov/symptoms-testing/symptoms.html

††† Fully vaccinated participants were defined as those who had received all recommended doses of a Food and Drug Administration–authorized or approved primary vaccine series (2 mRNA vaccine doses or a single dose of Johnson & Johnson [Janssen] vaccine) ≥14 days before either symptom onset or enrollment (whichever occurred earlier).

‡‡‡ Among all specimens, N-specific Ct values were also significantly higher in Omicron versus pre-Omicron infections (Ct difference = 3.84, p<0.001). When analysis was limited to RNA-positive specimens, a similar trend was observed (Ct difference = 3.27, p<0.001).**

§§§ Participants were considered symptomatic if they reported one or more COVID-19 signs or symptoms consistent with those listed by CDC, including fever, chills, shortness of breath, fatigue, muscle or body aches, headache, loss of taste, loss of smell, sore throat, congestion, runny nose, nausea, vomiting, or diarrhea. https://www.cdc.gov/coronavirus/2019-ncov/symptoms-testing/symptoms.html

¶¶¶ Among all specimens, N-specific Ct values were also significantly higher in Omicron versus pre-Omicron infections (Ct difference = 3.84, p<0.001).
Ct values in Omicron than in pre-Omicron specimens, culturable virus was detected in specimens from a similar percentage of participants in both variant groups (Omicron = 76%; pre-Omicron = 71%), a similar percentage of total specimens (Omicron = 26%; pre-Omicron: 30%), and was detected for a similar duration following onset (median = 6 days, IQR = 5–8 days for both Omicron and pre-Omicron specimens). Among virus-positive specimens, E-specific Ct values were significantly higher in Omicron specimens than pre-Omicron specimens (Ct difference = 5.77, p<0.001). This difference was observed as early as day 3 after onset through day 8 after onset (Figure 1). When stratified by age group or vaccination status (Figure 2), virus-positive Omicron specimens were associated with higher E-specific Ct values than were virus-positive pre-Omicron specimens (p<0.01). Similar findings were observed in the N-specific analysis (p<0.001).

Among virus-positive specimens, N-specific Ct values were also significantly higher in Omicron versus pre-Omicron infections (Ct difference = 3.84, p<0.001). Further, no difference was detected in either E- or N-specific Ct values over time between Delta and pre-Delta specimens (E: Ct difference = −1.86, p = 0.25; N: Ct difference = −2.35, p = 0.14). Although not statistically significant, in the case of Delta infections, E- and N-specific Ct values were lower (suggesting higher viral RNA levels) compared with pre-Delta infections, hence the negative difference between Delta and pre-Delta specimens.

**Discussion**

In this study, and consistent with other published findings (9), Ct values detected in nasal specimens were higher (suggesting lower RNA levels) in those obtained from participants infected with SARS-CoV-2 Omicron BA.1 sublineage than in those from participants infected with pre-Omicron variants. However, despite these higher Ct values, culturable virus was detected from a similar proportion of participants in both variant groups, and for a similar duration following onset; consistent with a recent report (10), participants infected with Omicron BA.1 had detectable culturable virus for a median of 6 days after onset. Notably, among these virus-positive (i.e., potentially infectious) specimens, Ct values were higher than those for pre-Omicron specimens, especially during the first week of illness. In addition, these differences between Omicron and pre-Omicron infections were observed in adults and in children and adolescents and were irrespective of age group and primary COVID-19 vaccination status.

**FIGURE 2. Pre-Omicron and Omicron BA.1 envelope gene–specific* cycle threshold values among nasal specimens with culturable SARS-CoV-2 virus,† by age group§ and by primary COVID-19 vaccination status¶,** — San Francisco Bay Area, California, July 2021–March 2022

Abbreviation: E = envelope gene.
* Nucleocapsid-specific real-time reverse transcription–polymerase chain reaction results were similar.
† Displayed as 95% CIs. The mixed model used in this analysis included an interaction term between variant and time after symptom onset.
§ Adults aged ≥18 years included 21 Omicron and 273 pre-Omicron specimens. Children and adolescents aged <18 years included 18 Omicron and 25 pre-Omicron specimens.
¶ Fully vaccinated included 18 Omicron and 81 pre-Omicron specimens. Unvaccinated included 21 Omicron and 217 pre-Omicron specimens.
** Boxplots display the median, lower, and upper quartiles and 1.5 times above or below the lower and upper quartiles.
of vaccination status. Presence of culturable Omicron BA.1 in nasal specimens, despite high Ct values, might contribute to the high levels of Omicron transmission observed in other studies (2). Further, these findings highlight variant-specific differences in viral dynamics, specifically, differences in the relationship between RNA and shedding of infectious virus.

Strengths of this study include the robust prospective longitudinal nature of nasal swab specimen collection. Similar findings were observed from two distinct real-time RT-PCR targets, both of which have been shown to reliably amplify both Omicron and pre-Omicron variants.

The findings in this report are subject to at least three limitations. First, this is a single-site study with a small number of participants infected with the Omicron BA.1 sublineage; thus, these findings might not be representative of all infected persons. Replication of these findings with additional participants is necessary and is ongoing. Second, approximately one half of the participants did not provide an enrollment serum specimen; thus, it was not possible to comprehensively assess the incidence of previous infection. Finally, duplication was not carried out on multiple real-time RT-PCR platforms across laboratories.

Virus-positive (i.e., potentially infectious) specimens from participants infected with SARS-CoV-2 Omicron variants had significantly higher Ct values than did virus-positive specimens from participants infected with pre-Omicron variants. Supporting CDC guidance (4), these data highlight that Ct values likely do not provide a reliable or consistent proxy for infectiousness across SARS-CoV-2 variants.