

## ENHANCED EPIDEMIOLOGICAL SUMMARY

# Cycle Threshold Values of S-Gene Target Failure COVID-19 Cases in Ontario: December 6 to December 30, 2021

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## Purpose

This report presents information on the cycle threshold (Ct) values of S-gene target failure (SGTF) COVID-19 cases detected by Public Health Ontario (PHO) Laboratory between December 6 and 30, 2021. During this time period, SGTF was utilized to screen all SARS-CoV-2 positive specimens meeting eligibility criteria, as this was a reliable proxy for identifying the Omicron variant and select sub-lineages (B.1.1.529, BA.1 and BA.1.1). In this report, we examine median Ct values for SGTF COVID-19 cases by demographic characteristics and COVID-19 vaccination status.

For more information on COVID-19 cases following vaccination, please refer to the [Confirmed Cases of COVID-19 Following Vaccination in Ontario](#)<sup>1</sup> report and the interactive [Ontario COVID-19 Data Tool](#)<sup>2</sup> to explore other recent COVID-19 data by public health unit, age group, sex, and trends over time.

## Background

Real-time reverse-transcription PCR (rRT-PCR) testing is used to detect SARS-CoV-2 infection and generates Ct values. Ct values represent the number of amplification cycles PCR tests have undergone until detection of viral RNA, where lower Ct values reflect higher relative viral RNA concentrations, but do not denote the actual quantity of virus in a sample. Higher SARS-CoV-2 viral loads may be associated with increased transmission probability, but it is unclear whether this relationship differs with the Omicron variant.<sup>3-6</sup> Ct values can be influenced by a number of factors including the PCR test used, time of sample collection relative to infection, sampling method, sample handling or processing, symptom status<sup>7,8</sup> and individual-level characteristics (e.g., age, inhibitors in the specimen matrix).<sup>7,9</sup> At PHO Laboratory, the majority of SARS-CoV-2 samples are tested using TaqPath™ COVID-19 PCR. For additional details on Ct values, please refer to the document [Overview of Cycle Threshold Values and their Role in SARS-CoV-2 Real-Time PCR Test Interpretation](#).<sup>10</sup>

PHO Laboratory utilizes an rRT-PCR assay targeting the N, ORF1ab, and S gene targets of SARS-CoV-2 (i.e., TaqPath™). SGTF is defined as non-detection of the S-gene target among samples with detection of both N and ORF1ab genes and with at least one of these gene targets (i.e. N or ORF1ab) having a Ct value  $\leq 30$  (see Data Sources and Caveats).<sup>11</sup>

In this report, Omicron variant samples refer exclusively to sub-lineages B.1.1.529, BA.1 and BA.1.1, due to very few BA.2 cases and no BA.3 cases being observed during our study period. SGTF is a useful screen to distinguish these Omicron variants (i.e., B.1.1.529, BA.1 and BA.1.1) from the Delta variant (B.1.617.2) which was circulating at the same time. Combined SGTF and whole genome sequencing (WGS) testing data from Ontario has confirmed that a SGTF positive result is a reliable proxy for Omicron B.1.1.529 lineage identification (98.9% sensitivity, 99.9% specificity, 99.5% positive predictive value, 99.7% negative predictive value).<sup>11</sup> From December 6 to December 30, 2021, all samples at PHO Laboratory that were positive for SARS-CoV-2 and had a Ct value  $\leq 35$  underwent SGTF screening.

In earlier waves of the pandemic, it appeared that SARS-CoV-2 positive individuals who were vaccinated had higher Ct values (lower relative viral RNA concentration) than those who were unvaccinated.<sup>12</sup> Upon the emergence of the Delta variant, the relationship between vaccination status and Ct values for those positive for SARS-CoV-2 became less clear.<sup>13, 14</sup> Most recently, there is a need to examine the relationship between Ct values and vaccination status amidst the surge in Omicron cases.<sup>15</sup>

## Summary

- Between December 6 and December 30, 2021, samples were collected from a total of 32,297 SARS-CoV-2 positive cases with SGTF. Of these cases, 13.2% (n=4,273) were unvaccinated and 80.1% (n=25,873) occurred among individuals who had completed their vaccination series ([Table 1](#)).
- Among SGTF cases, Ct values ranged from 17.8 to 18.7 across vaccination status ([Table 2](#)).
- There is slight variability in Ct values when stratified by age and vaccination status, however, all groups had a median Ct value < 25. Unvaccinated individuals aged 60 or older had the lowest Ct values compared to other groups ([Table 3](#)).
- Approximately 11.5% of cases (n=3,721) had a Ct value > 25. The majority of these were cases post-series completion (78.8%; n=2,933/3,721) followed by unvaccinated cases (13.6%; 507/3,721).
- For cases where symptom information was available (n=13,983/32,297), cases were further stratified by symptom status. Ct values appeared higher for asymptomatic cases relative to symptomatic cases in the same vaccination category ([Table A1](#)).
- An important mechanism through which vaccines prevent transmission is by reducing the likelihood of infection, which then interrupts further spread. Recent evidence suggests that a third COVID-19 vaccine dose increases protection against symptomatic Omicron infection and severe outcomes relative to a second dose.<sup>16,17,18</sup>

## Results

**Table 1. Summary of key demographic variables of S-Gene Target Failure detected cases stratified by vaccination status among samples collected between December 6 and December 30, 2021**

Case characteristics (N, %)	Unvaccinated	Post-series initiation	Post-series completion	Post-booster dose
Total	4,273	1,002	25,873	1,149
Sex: Female	2,047 (47.9%)	458 (45.7%)	13,345 (51.6%)	696 (60.6%)
Sex: Male	2,190 (51.3%)	539 (53.8%)	12,476 (48.2%)	452 (39.3%)
Age: 0 to 4	514 (12.0%)	NR	NA	NA
Age: 5 to 17	1,535 (35.9%)	665 (66.4%)	2,136 (8.3%)	NR
Age: 18 to 59	2,095 (49.0%)	311 (31.0%)	21,568 (83.4%)	596 (51.9%)
Age: 60+	128 (3.0%)	25 (2.5%)	2,166 (8.4%)	549 (47.8%)
Outbreak-related	274 (6.4%)	38 (3.8%)	1,227 (4.7%)	314 (27.3%)
Long-term care home resident	25 (0.6%)	7 (0.7%)	75 (0.3%)	149 (13.0%)
Health care worker	33 (0.8%)	0 (0.0%)	269 (1.0%)	41 (3.6%)
Ever hospitalized	12 (0.3%)	1 (0.1%)	36 (0.1%)	12 (1.0%)
Fatal outcome	3 (0.1%)	1 (0.1%)	8 (<0.1%)	12 (1.0%)

**NA:** Not Applicable. No cases were reported in this category. This group was not part of general population eligibility for a first dose (at the time of sample collection).

**NR:** Not Reported. Cases 0-4 years of age reported as post-series initiation cases as well as those 5-17 years of age reported as post-booster dose case are not shown due to small numbers < 5 and because these age groups were not part of general population eligibility for a first dose or booster dose, respectively (at the time of sample collection).

**Note:** Not all cases have an age or sex reported. Vaccine category definitions can be found in the technical notes.

Data Source: CCM, COVaxON, PHO Laboratory Information Management System

**Table 2. Summary of median cycle threshold value and inter-quartile range (IQR) results for S-Gene Target Failure detected cases stratified by vaccination status among samples collected between December 6 and December 30, 2021**

Blank cell	Unvaccinated	Post-series initiation	Post-series completion	Post-booster dose
N	4,273	1,002	25,873	1,149
Median Ct (IQR)	18.5 (15.6-21.9)	18.7 (16.1-22.3)	17.8 (15.3-21.6)	18.3 (15.4-22.4)

**Note:** Vaccine category definitions can be found in the technical notes.

**Data Source:** CCM, COVaxON, PHO Laboratory Information Management System

**Table 3. Summary of median cycle threshold value and inter-quartile range (IQR) results for S-Gene Target Failure detected cases stratified by vaccination status and age among samples collected between December 6 and December 30, 2021**

Age group (years)	Unvaccinated	Post-series initiation	Post-series completion	Post-booster dose
0 to 4	18.5 (15.7-22.3)	NR	NA	NA
5 to 17	19.2 (16.3-22.4)	19.2 (16.5-22.6)	18.4 (15.7-22.2)	NR
18 to 59	17.9 (15.2-21.6)	17.8 (15.6-20.9)	17.8 (15.3-21.5)	18.3 (15.5-22.3)
60+	16.9 (14.9-22.0)	20.4 (14.4-22.9)	17.5 (14.9-21.2)	18.5 (15.4-22.4)

**NA:** Not Applicable. No cases were reported in this category. This group was not part of general population eligibility for a first dose (at the time of sample collection).

**NR:** Not Reported. Cases 0-4 years of age reported as post-series initiation cases as well as those 5-17 years of age reported as post-booster dose case are not shown due to small numbers < 5 and because these age groups were not part of general population eligibility for a first dose or booster dose, respectively (at the time of sample collection).

**Note:** Vaccine category definitions can be found in the technical notes.

**Data Source:** CCM, COVaxON, PHO Laboratory Information Management System

# Technical Notes

## Data Sources

- COVID-19 case data for samples collected between December 6 and 30, 2021 are based on information successfully extracted from the Public Health Case and Contact Management Solution (CCM) for all PHUs by PHO as of **January 24, 2022 at 1 p.m.**
- COVID-19 vaccination data were based on information successfully extracted from the Ontario Ministry of Health's COVaxON application as of **January 24, 2022 at approximately 7 a.m.** and was subsequently linked to the CCM data.
  - Clients in COVaxON and CCM were linked using health card number as well as other personal identifiers, including name, date of birth, gender, and postal code. Linkage was done using processed COVaxON and CCM data. Methods for processing COVaxON vaccination data are described in the Technical Notes of the [COVID-19 Vaccine Uptake Report](#)<sup>19</sup> and in the Technical Notes of the [COVID-19 Daily Epidemiological Summary](#)<sup>20</sup> for CCM case data.
- Ct value data were extracted from the PHO Laboratory Information Management System (LIMS) on **January 26, 2022 at 5 a.m** and was subsequently linked to the linked CCM and COVaxON data.
  - LIMS records were linked to the linked CCM and COVaxON data if they had the same health card number or if they had the same first name, last name, and date of birth. Cases may not have linked to CCM due to discrepancies in patient identifiers or if they were not residents of Ontario (diagnosing health unit was reported as MOH).

## Data Caveats

- Samples meeting the following criteria were deemed to have SGTF detected:
  - The sample was tested by PCR using an assay targeting both the SARS-CoV-2 S gene and two additional gene targets (ORF1ab and N).
  - The S gene target was not successfully amplified.
  - The ORF1ab and N gene targets were detected (i.e., Ct < 37), with at least one target having a Ct value ≤ 30.
- Samples without S gene detection for which the ORF1ab and N gene targets were detected with Ct > 30 were considered inconclusive for SGTF. If the S gene was not detected and the ORF1ab and/or N gene was not detected, SGTF testing was considered unable to be completed. In specimens tested in this time period, a higher proportion of specimens unable to be completed or inconclusive were post-booster dose cases (11.1% and 6.1%, respectively) compared to the proportion of detected specimens that were post-booster dose cases (3.6%).
- As per the preceding definition for SGTF detection, restricting analyses to cases with SGTF detected resulted in exclusion of cases with Ct values > 30. Median Ct values for cases with inconclusive SGTF results or where SGTF screening was unable to be completed are provided in [Table A2](#). This bias toward lower Ct values may have affected the comparison of median values across certain age or vaccination strata.

- SGTF as a proxy for Omicron variant cases would not identify BA.2, however we do not expect the exclusion of these cases to substantially impact our findings due to low prevalence of this sub-lineage during the study period.
- Sample collection date is the date the sample was collected. If sample collection date was missing, the sample received date was used. For cases with multiple samples, the sample with the earliest date was included.
- Cases where the difference between the earliest positive lab collection date (from CCM) and the earliest sample date (sample collection date where available, else sample received date from LIMS) were greater than +/-2 days were excluded from the analyses (6.5%) to align sample and case information.
- Case characteristic information (sex, age, severity, risk factors) in this report are sourced from case investigation fields in CCM. Further details on CCM case data are described in the Technical Notes of the [COVID-19 Daily Epidemiological Summary](#).<sup>20</sup>
- ‘Outbreak-related’ cases include cases linked to a confirmed outbreak as declared by the local medical officer of health or their designate in accordance to the Health Protection and Promotion Act and criteria outlined in [Ministry guidance documents](#).<sup>21</sup>
- These analyses do not account for previous infection among cases.
- Ct values reported for each vaccination group may be impacted by differences in the characteristics of individuals in those groups. For example, the majority of post-initiation cases during this timeframe were cases aged 5 to 17 (noting that due to program rollout and eligibility for vaccination, the majority of these individuals were 5 to 11 years of age) and therefore any differences in Ct value by age should be considered when interpreting the data. In addition, vaccine eligibility criteria during this timeframe may impact the characteristics of individuals in each of the vaccination groups. For example, post-booster dose cases included in this report may not be representative of the population currently eligible for a booster dose as the time period included in this report was early in the provincial booster dose program. Further, time since latest dose was not accounted for in this analysis and may be an important consideration when interpreting Ct values.
- Cases post-two booster doses were not included in this report as a second booster dose was not yet offered to eligible populations during this time period.

- The following definitions were used to describe COVID-19 infection following vaccination. For additional information and technical notes related to COVID-19 infection following vaccination please refer to the [Confirmed Cases of COVID-19 Following Vaccination in Ontario](#)<sup>1</sup> report.
  - **Unvaccinated case:** Cases that have not received a dose of a COVID-19 vaccine, as well as cases that are not yet protected from vaccination: cases with a symptom onset date that was 0 to <14 days following the first dose of a Health Canada authorized COVID-19 vaccine. This time period from vaccination is not sufficient to develop immunity, therefore these cases are not considered protected from vaccination.
  - **Cases post-series initiation (i.e. cases following initiation but not completion of their primary series):** Cases with a symptom onset date that was 14 or more days following the first dose of a two-dose series of a Health Canada authorized COVID-19 vaccine or 0 to <14 days after receiving the second dose of a two-dose Health Canada authorized COVID-19 vaccine series.
  - **Cases post-series completion (i.e. cases following the completion of their primary vaccine series):** Cases with a symptom onset date that was 14 or more days following a receipt of the first dose of a one-dose series or the second of a two-dose series of a Health Canada authorized COVID-19 vaccine, or 0 to <14 days after receiving a Health Canada authorized booster dose following their primary series.
  - **Cases post-booster dose (i.e. cases following completion of their primary series and a booster dose):** Cases with a symptom onset date 14 or more days following receipt of a Health Canada authorized COVID-19 booster dose following their Health Canada authorized primary series, or 0 to <14 days after receiving a second Health Canada authorized booster dose following their primary series.
- For certain populations (e.g. immunocompromised individuals) three doses are recommended to complete the primary series. Due to challenges in identifying these individuals in the COVaxON data, it was not possible to account for a three-dose primary series in the analysis, and these individuals are classified as per the definitions above.

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## Appendix A

**Table A1. Summary of median cycle threshold (Ct) value and inter-quartile range (IQR) results for S-Gene Target Failure detected cases stratified by symptom status and vaccination status among samples collected between December 6 and December 30, 2021**

Symptom and vaccination status	N	Median Ct (IQR)
Symptomatic		
Unvaccinated	1,600	18.2 (15.4-21.9)
Post-series initiation	328	18.5 (16.1-21.3)
Post-series completion	9,770	17.5 (15.0-21.1)
Post-booster dose	398	18.1 (15.3-22.0)
Asymptomatic		
Unvaccinated	315	19.3 (16.5-22.4)
Post-series initiation	74	21.3 (18.1-24.7)
Post-series completion	1,407	18.6 (15.9-22.3)
Post-booster dose	91	18.8 (15.4-23.2)
Missing symptom information*		
Unvaccinated	2,358	18.5 (15.6-22.0)
Post-series initiation	600	18.7 (16.0-22.3)
Post-series completion	14,696	18.0 (15.5-21.7)
Post-booster dose	660	18.3 (15.5-22.2)

**Note:** Symptomatic cases were defined as those with a reported symptom onset date. Asymptomatic cases were defined as those where asymptomatic was reported as 'yes' and no symptom information (e.g. cough) was reported. \*Symptom information may be incomplete for cases reported particularly after December 18, 2021 due to changes in case-follow up guidance.

**Data Source:** CCM, COVaxON, PHO Laboratory Information Management System

**Table A2. Summary of median cycle threshold (Ct) value and inter-quartile range (IQR) results for cases stratified by S-Gene Target Failure result among samples collected between December 6 and December 30, 2021**

SGTF Result	N	Median Ct (IQR)
Detected	32,297	17.9 (15.4-21.7)
Inconclusive	2,280	32.8 (31.5-34.3)
Unable to complete	739	35.4 (34.4-36.2)

**Data Source:** CCM, COVaxON, PHO Laboratory Information Management System

## Citation

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