Wide Variation in Cycle Threshold Values Cloud the Interpretation of COVID-19 Infectiousness

Short title: Ct Value Variability Clouds the Interpretation of COVID-19.

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Abbreviations
Cycle threshold: Ct
Real-time reverse transcriptase real-time polymerase chain reaction: rRT-PCR

To the editor:

There is continuing interest in using cycle threshold (Ct) values from real-time reverse transcriptase polymerase chain reaction (rRT-PCR) assays to determine infectiousness and timing of SARS-CoV-2 infection. Demand for reporting Ct values has been justified to guide the administration of therapeutics, prognostication, identifying reinfection, or removing isolation precautions (1). However, there are fundamental flaws when interpreting a qualitative test in a quantitative manner where Ct values vary on a multitude of factors, including sample collection, specimen type, reagents used, extraction/PCR platform(s), PCR targets, and laboratory practices (e.g., transportation and storage).

We analyzed the correlation between E gene Ct values and duration of symptom onset from confirmed COVID-19 cases in Alberta, Canada (population 4.4 million). Data recorded from March to May, 2020 from the Alberta Health Services Public Health and Alberta Precision Laboratory databases were linked. Symptom duration and status at the time of collection was determined during case investigation by Public Health using a standardized questionnaire. Cases with known symptomatology and tested with Ct values generated from Alberta Precision Laboratory's laboratory-developed E gene singleplex assay were included. Based on proficiency panels (published and unpublished), internal evaluations of our assay had similar sensitivity, specificity and precision to FDA approved assays (2,3).

Oropharyngeal, nasopharyngeal and nasal specimens for SARS-CoV-2 testing were collected in universal transport media (UTM-RT, COPAN Diagnostics, California, United States or UTM, Yocon, Beijing, China). Endotracheal tube aspirate specimens were collected in sterile containers. All specimens were stored at 4^{o}C prior to SARS-CoV-2 testing within ~72 h.

Of 7,974 cases, 5,756 met inclusion criteria. Oropharyngeal, nasopharyngeal, nasal, and endotracheal tube aspirate specimens made up 71.6%, 19.1%, 8.1% and 1.1% of specimens, respectively. At the time of collection, 787 (13.7%) were asymptomatic, 92 (1.6%) pre-symptomatic (symptom onset within 2 d after collection), 3,107 (54.0%) with symptom onset ≤7 d, and 1,770 (30.7%) with symptom onset >7 d. Excluding those tested on day 1 of symptom onset, a linear positive correlation between days of symptoms and median Ct values was observed ($R^2 = 0.970$, $p<0.001$, Figure 1).

Despite a linear positive correlation between days of symptom onset and median Ct value, Ct values ranged widely, regardless of symptom onset or specimen type. For example, >25% of individuals with symptoms ≤7 d had Ct values >29.1, indicating that a lower viral burden may be common early in disease. In contrast, over 25% of individuals with symptoms >10 d had Ct values <25.8, indicating that higher viral burden may be common later in disease. The median Ct value for asymptomatic individuals was 29.9, which was comparable to the median Ct value observed among pre-symptomatic individuals (28.3 at -2 d from symptom onset, 30.9 at -1 d). Twenty-five percent of asymptomatic individuals had Ct values ≤25.0.

As expected, we observed an increase in Ct values as time from symptom onset increased. However, our data demonstrates that Ct values cannot be used to determine the time from symptom onset since there was a wide variation in Ct values at all time points. Our findings also

highlight that using Ct value thresholds for infectiousness derived from other studies can be misleading. For example, a Ct value of 24 was determined to correlate with cultivability of virus (4); in our population using our laboratory-developed assay, this would represent a marked proportion of those with >10 d of symptom onset, which is inconsistent with the known period of infectivity for most cases of COVID-19. Furthermore, the higher median Ct values we observed among pre-symptomatic and early symptomatic individuals highlights that Ct value cut-offs alone cannot predict an individual's current or future contagiousness.

Our data was limited by its retrospective nature, the inclusion of multiple specimen types that may have contributed to Ct value variability (5), and the lack of validated methods for confirming infectiousness, such as viral culture.

COVID-19 has brought the microbiology laboratory and its processes into the spotlight, with reports about Ct values making front page news. Now, more than ever, it is important that the laboratory continue to uphold quality standards that have, and will continue to maintain, public trust in our laboratories. Reporting of Ct values to clinicians must be done cautiously and with the consideration of the assay and the pre-analytic and analytic factors that are associated with Ct value variability. Interpretation should be made within the clinical context by clinicians familiar with these caveats or in consultation with a laboratory physician/scientist. Since qualitative rRT-PCR assays were designed only for the detection of SARS-CoV-2, Ct values should not be reported on a patient's chart. Deviating from the intended use requires the appropriate validation studies demonstrating accurate and reproducible correlation of Ct values with SARS-CoV-2 viral loads, as determined by quantitative assays.

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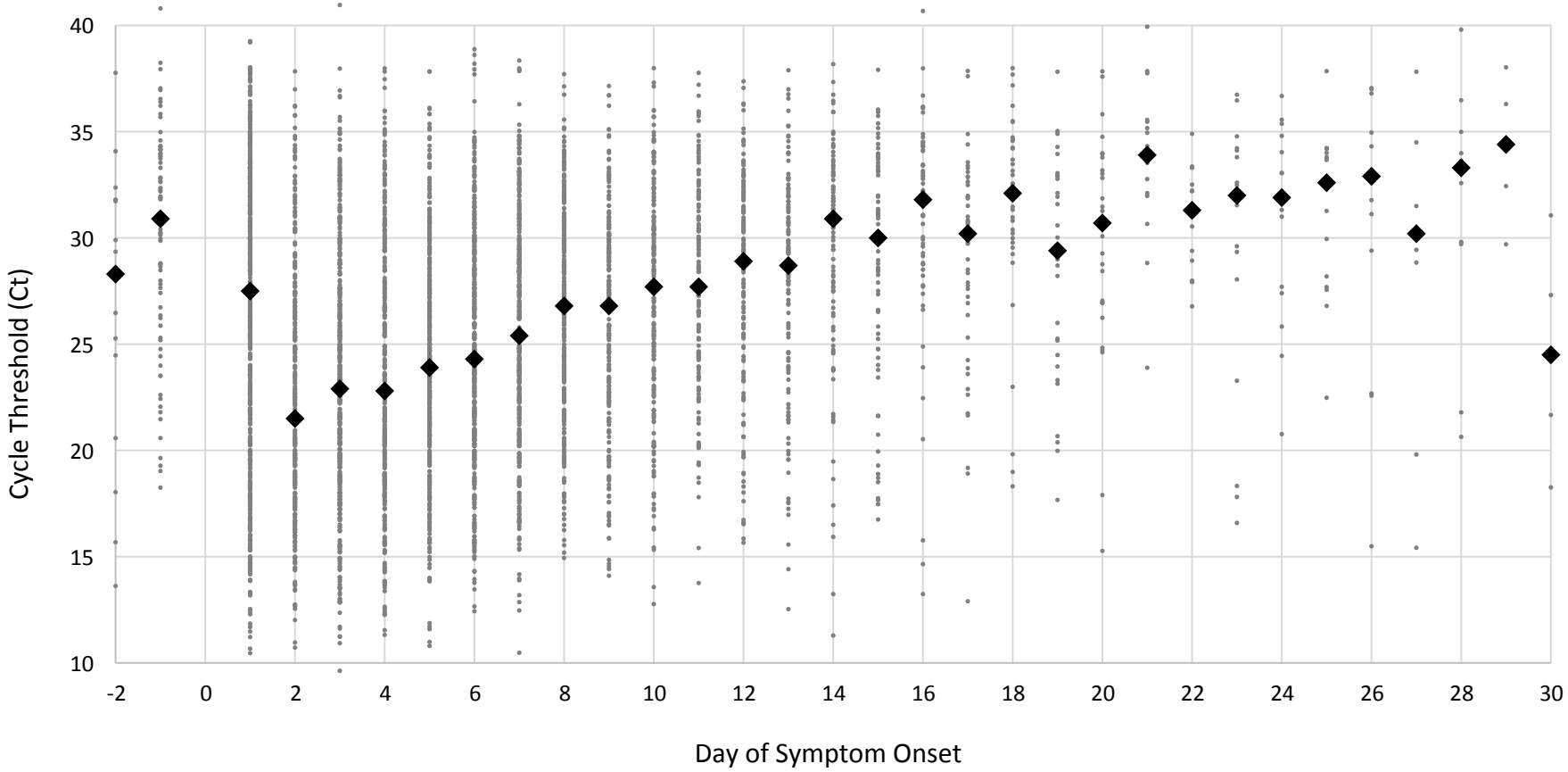
Figure Legend

Fig. 1. Scatter plot of E gene Ct value per symptom onset day. Black diamonds represent median E gene Ct values. From day 2-14, $R^2 = 0.970$, $p<0.001$.


Cycle Threshold (Ct)
40
35
30
25
20
15
10
-2
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
Day of Symptom Onset