

College of American Pathologists (CAP) Microbiology Committee Perspective: Caution Must Be Used in Interpreting the Cycle Threshold (Ct) Value

TO THE EDITOR—We read with great interest the article by Magleby and colleagues entitled “Impact of SARS-CoV-2 Viral Load on Risk of Intubation and Mortality Among Hospitalized Patients with Coronavirus Disease 2019” [1]. This article adds to the growing body of work on using the polymerase chain reaction (PCR) cycle threshold (Ct)-value associated with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA detection in clinical specimens as a prognostic indicator and to establish criteria for active infection and transmissibility. Although we recognize the importance of studying laboratory results and their relevance to care of patients with coronavirus disease 2019 (COVID-19), we wish to inform your readers of potential caveats that must be considered when applying published findings regarding Ct-values to their own patients’ results.

- 1) Specimen collection method, specimen source, transport media type and volume, duration from specimen collection to analysis, and days from infection to specimen collection can all impact the amount of viral RNA that could be detectable by an assay, and these variables are reflected in the Ct values.
- 2) No quantitative SARS-CoV-2 assays have received Emergency Use Authorization (EUA) by the Food and Drug Administration (FDA). Additionally, no international, commutable standardized reference material is currently available, which would be needed for validation of quantitative assays that generate comparable results across manufacturers and

- laboratories. Although specimens with lower Ct-values generally have more viral RNA than specimens with higher Ct-values, the quantitation and precision associated with those differences in Ct-values have not been determined.
- 3) Only traditional real-time PCR assays produce a Ct-value. Some diagnostic assays used to detect SARS-CoV-2 RNA use isothermal amplification methods, which do not produce a Ct-value. Other PCR platforms use nested PCR, which is not designed for quantitative interpretation.
- 4) Ct-values can vary significantly between and within methods. The College of American Pathologists (CAP) recently surveyed more than 700 laboratories using proficiency testing material produced from the same batch (Figure 1). The median Ct-values reported by the instruments for different FDA EUA methods varied by as much as 14 cycles. Within a single

test performed on the same instrument, the difference in the median Ct-values for different targets was as high as 3.0 cycles. Finally, within a single gene target for a single method, up to 12.0 cycle differences were seen across all laboratories. The assay and gene target used by Magleby et al, ORF1a detected by the Roche cobas system, differed by approximately 6.0 cycles across all laboratories responding to the survey. Many clinical laboratories are using multiple tests that assess different gene targets for SARS-CoV-2 and are performing testing on different platforms. This adds to the potential variability of Ct-values produced by a single laboratory.

The ongoing shortage of commercial testing reagents presents a major obstacle to conducting large research studies comparing testing platforms. We thus believe that data from the CAP proficiency testing survey

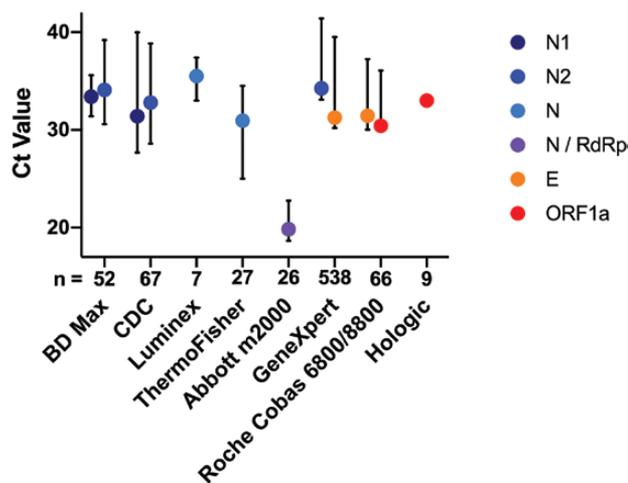


Figure 1. Ct values for gene targets and manufacturers for the same batch of testing material. Median Ct values (filled circles) and the range of Ct values from low to high (whiskers) are shown. The number of survey respondents using each method is indicated below the x-axis. Of note, the material used for the PT Survey did not contain all gene targets in use by commercial assays, and Ct values entered by laboratories under “Miscellaneous” were not incorporated into the data. Data from the users of the Cepheid GeneXpert and GeneXpert Xpress System were combined into a single category for the purposes of this visualization, as both systems employ the same test cartridge and there was likely misreporting between these 2 categories by survey participants. The Hologic category only includes values from the Panther Fusion SARS-CoV-2 assay, as the Hologic Aptima assay does not produce Ct values. Abbreviations: Ct, cycle threshold; PT, proficiency testing; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

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are extremely valuable in advancing our understanding of Ct-value commutability in SARS-CoV-2 molecular testing. If healthcare providers and researchers attempt to employ Ct-values as a component of their patient assessment, we caution them to consider the points described in this letter.

Notes

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