Caveats of Reporting Cycles Threshold from SARS-CoV-2 Qualitative PCR Assays: A Molecular Diagnostic Laboratory Perspective

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Dear Editor,

We read the two recent commentaries published in *Clinical Infectious Diseases* by Binnicker 2020, and Tom & Mina 2020, respectively with great interest [1, 2]. Binnicker pointed out the potential pitfalls when interpreting real-time polymerase chain reaction (PCR) cycle threshold (Ct) values in his response to the study by Bullard et al. 2020 [3]. Tom & Mina in their response to the study by Xiao et al. 2020 [4] suggested the implementation of reporting actual Ct values along with reference ranges or viral load equivalents from Ct conversion, or tiered Ct categories as high, medium or low. These discussions on Ct values raised concerns on the caveats of reporting Ct values generated by qualitative PCR assays in the clinical setting.

From the clinical laboratory perspective, a high-throughput and fully-automated assay is favored against small-scale and manual ones to meet the diagnostic needs during current SARS-CoV-2 pandemic. At the time of writing this manuscript, there was no quantitative assay for the detection of SAR-CoV-2 RNA received Food and Drug Administration (FDA) emergency use authorization (EUA) (https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/vitro-diagnostics-euas#individual-molecular, accessed on Sep 08, 2020). Unlike quantitative viral load assays which report absolute viral copy numbers in clinical samples, a Ct value from a qualitative assay, although is reflective of viral load is not directly comparable between different assays even when the same viral genes are targeted. Adding to the complication is that in a dual-target assay, the Ct values of both targets are concurrently available as the assay's result outputs, which are target gene-dependent, and may not be consistent. When the diagnostic samples are referred to a laboratory which utilizes multiple assays for COVID-19 testing, clinicians must take note that the reported Ct values from different assays may not be in correlation, especially when trending of Ct values is monitored in the follow-up of a patient. Apart from these caveats, Ct values are also sensitive to preanalytical vulnerabilities such as swabbing technique, swab

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sites (nasopharyngeal versus oropharyngeal) and input volume for nucleic acid extraction. Variability in different specimen types, for example, swabs from upper respiratory tracts versus lower respiratory specimens like sputum, bronchioalveolar lavage and endotracheal aspirate, may contribute to challenges in interpreting Ct values [5], especially in the cases with pre-treatment of specimen or when dilution of nucleic acid is required to mitigate effects from inherent PCR inhibitors. A quantitative assay reporting SARS-CoV-2 viral load may seem to overcome the shortcomings associated with direct interpretation of Ct values, however, it remains sensitive to afore-mentioned preanalytical and analytical issues. In addition, there are reports on persistence of SARS-CoV-2 RNA in stool relative to respiratory samples [6], and a specific SARS-CoV-2 target, for example the N gene [7] compared to ORF1a sequences for longer duration. Although Ct values, in theory could serve as a surrogate indicator of 'quantity' in a qualitative PCR assay, the results are not portable across different assays, different gene targets and different specimen types.

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Declaration of Competing Interest

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