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Reproducibility of cycle threshold values from severe acute respiratory coronavirus virus 2 (SARS-CoV-2) reverse-transcription polymerase chain reaction (RT-PCR) assays

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To the Editor—To diagnose severe acute respiratory coronavirus virus 2 (SARS-CoV-2) infection, nucleic acid amplification is frequently used. Many such assays yield not only a detected or not detected result but also a cycle threshold (Ct) value. The Ct represents the cycle number needed to cross the positive (detected) signal threshold. This value is sometimes considered a surrogate for viral load because, in general, a lower Ct value suggests a higher viral concentration (and vice versa) in the specimen.[1]

Several proposals have been made for using Ct cutoffs to help determine the need for patient isolation.[2,3] However, before a test value can be used for clinical purposes, it must be determined to be reproducible; that is, similar results would be obtained regardless of the collector or across clinically insignificant time points. We sought to determine the reproducibility of Ct values to assess for discrepancy rates between sample collection variables and molecular assay performed.

The study was approved by the institutional review board (#infoEd record no. 2002107). We included patients aged >18 years who were inpatients at Creighton University Medical Center–Bergan Mercy (CUMC-BM) in Omaha, Nebraska, with a diagnosis of COVID-19 and a first positive PCR or antigen test for SARS-CoV-2 ≤5 days from the date of sampling. In total, 10 patients agreed to participate, and each underwent 4 nasal swabs. The first swab was performed by researcher A in the right naris (patient A0), and the second swab was performed by researcher B in the left naris (patient B0). After 10 minutes, 2 additional swabs were obtained: researcher A from the left naris (patient A10) and researcher B from the right naris (patient B10). The swabs were then stored at −80°C until all study swabs were collected.

Once collection was complete, swabs were processed at the CUMC-BM molecular laboratory. Samples were run on both the Abbott m2000 System (Abbott RealTime SARS-CoV-2 assay, dual target RdRp and N genes, Abbott Laboratories, Chicago, IL) and the LIASION MDX System (DiaSorin Molecular Simplexa COVID-19 Direct assay, dual target ORF1ab and S genes, Cypress, CA). The Ct values were recorded for each assay, with nondetectable values set to 40 cycles.

To account for the right censoring of Ct values at 40 cycles, we used a mixed-effects Tobit model that included a random intercept to account for the correlation due to repeated measurement of the same patient as well as fixed effects for the researcher collecting the specimen (A vs B), naris sampled (left vs right), time (0 vs 10), and assay (Abbott m2000 vs Simplexa S vs Simplexa ORF1ab). We used a top–down modeling approach that evaluated fixed interaction effects between researcher, naris sampled, time, and assay, and systematically removed nonstatistically significant interaction effects to arrive at the final model. All analyses were conducted using SAS version 9.4 software (SAS Institute, Cary, NC) with 2-tailed $P < .05$ indicating statistical significance.

The patient-specific Ct values are reported in Table 1. No statistically significant mean differences in Ct values were indicated between researchers A and B (22.9 vs 22.0; $P = .055$), left and right naris (22.2 vs 22.7, $P = .346$), or time 0 and time 10 (22.3 vs 22.7; $P = .429$). Although there was no overall mean difference between the 2 gene targets for the DiaSorin Molecular Simplexa S and ORF1ab assays (25.3 vs 25.8; $P = .457$), significant differences were observed between both DiaSorin Molecular Simplexa targets (S and ORF1ab; 25.3 vs 16.3; $P < .001$) and the Abbott RealTime SARS-CoV-2 assay (25.8 vs 16.3; $P < .001$).

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Table 1. Cycle Threshold (Ct) Values by Patient, Researcher, Nares, and Time

Sample Designation[a]	Simplexa S	Simplexa ORF1ab	Abbott m2000
1A0	23.3	24.4	14.72
1A10	31.5	33.6	20.48
1B0	27.4	28.2	18.02
1B10	22.3	23.0	14.72
2A0	30.2	32.0	23.23
2A10	30.3	31.2	21.35
2B0	26.0	27.1	17.04
2B10	30.3	31.8	22.24
3A0	21.4	22.4	12.09
3A10	24.9	25.4	15.37
3B0	22.4	22.6	12.38
3B10	23.0	23.5	14.05
4A0	26.0	26.4	15.89
4A10	25.4	26.2	15.93
4B0	29.8	30.3	20.86
4B10	25.5	25.9	16.92
5A0	32.3	32.3	23.61
5A10	27.4	28.7	17.47
5B0	30.2	31.4	22.45
5B10	31.2	32.5	22.85
6A0	24.0	24.6	15.24
6A10	27.5	28.6	19.1
6B0	24.3	25.5	14.83
6B10	21.6	22.5	13.45
7A0	30.4	30.8	23.9
7A10	ND	ND	30.49
7B0	ND	31.9	23.87
7B10	ND	ND	26.74
8A0	21.1	21.4	12.64
8A10	16.1	16.8	6.35
8B0	14.0	14.3	5.72
8B10	15.5	15.7	7.53
9A0	28.2	27.8	18.25
9A10	25.7	26.2	17.58
9B0	24.1	24.0	15.4
9B10	22.8	22.9	14.32
10A0	14.4	15.0	7.43
10A10	11.3	11.7	4.64
10B0	14.4	15.1	6.63
10B10	13.9	14.6	5.56

Note. ND, nondetected (values for a system were set to 40).
[a]Patient number (1–10), researcher (A or B); naris and relative time of collection (time 0 or 10 minutes later; AO & B10-right naris; A10 and B0 left naris).

Our results are consistent with a study from College of American Pathologists, which urges discretion regarding the reliability of Ct values.[4] Rhoads et al[4] also reported significantly different values for Ct with standardized samples between platforms and even when the same platform was used at different laboratories. As an extreme example of result discrepancy, for patient 7 in our study, 2 of 4 swabs tested negative for both gene targets on the DiaSorin Molecular Simplexa assay, and 3 of the 4 swabs would still warrant ongoing patient isolation if the Abbott RealTime SARS-CoV-2 assay had been performed and an absolute Ct cutoff of 30 had been used to determine isolation need.

Singanayagam et al[5] reported that 5 (8.3%) of 60 samples with a Ct >35 had culturable virus. Although a patient with culturable virus is not necessarily able to infect others, this is concerning if a single cutoff value is used to determine need for patient isolation. In our results, 5 (50%) of 10 patients had some swab values >30 and some <30, suggesting that determining isolation need based on such a cutoff would be swab variable and not based on any patient-specific factor. A more nuanced approach, such as that described by Mowrer et al[6] in which Ct might play a role in conjunction with expert consultation and evaluation of clinical status, might be better if the Ct is to be used clinically. However, to use Ct in such a fashion, the Ct would need to be correlated with culturable virus for each platform in use at an institution because the absolute value of Ct is not reproducible between assays or even with the same assay between institutions. This is likely not possible for many clinical laboratories. We would therefore recommend caution if Ct is being considered for use for clinical or patient isolation purposes without such correlation having been performed.

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