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# Cycle Threshold Values from Severe Acute Respiratory Syndrome Coronavirus-2 Reverse Transcription-Polymerase Chain Reaction Assays

## Interpretation and Potential Use Cases



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### KEYWORDS

- COVID-19 • SARS-CoV-2 • Reverse transcription-polymerase chain reaction
- Cycle threshold value

### KEY POINTS

- The cycle threshold (Ct) value is a semi-quantitative value that is inversely related to the level of viral RNA in reverse transcription-polymerase chain reaction (RT-PCR) tests for severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2)
- The Ct value for SARS-CoV-2 is inherently variable due to the variability of RT-PCR, and further variability can be introduced by sample factors (collection, storage, sample type), and use of different RT-PCR tests.
- Potential clinical uses of Ct values for SARS-CoV-2 include the assessment of the progression of infection, prediction of disease severity, and determination of infectivity.
- Caregivers using Ct values for these purposes must understand the variability and limitations of Ct values, which can be facilitated by direct communication with the leadership of the clinical laboratory.

### INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic, caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has caused more than 381 million infections and more than 5.6 million deaths, worldwide, including more than 75 million infections and more than 890,000 deaths in the United States (US).<sup>1</sup> While the use

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of vaccines has slowed the mortality rates in many places, lack of access to vaccines and vaccine hesitancy has resulted in continued infections and deaths. Diagnostic testing for SARS-CoV-2 has been and will continue to be critical to addressing the pandemic.

The mainstay of diagnostic testing is nucleic acid amplified tests, primarily using reverse transcription followed by polymerase chain reaction (RT-PCR). RT-PCR tests for SARS-CoV-2 are designed and validated to be used as qualitative tests, reporting as positive if the virus is detected or negative if it is not. RT-PCR can be made quantitative if a standard curve is generated using known concentrations of virus or viral RNA, allowing the use of the sample cycle threshold (Ct) at which viral RNA is detected to estimate the quantity of virus present. Alternatively, the Ct value alone can be used directly, as a semi-quantitative way to compare the level of virus between 2 samples run using the same assay on the same platform.

While PCR and RT-PCR have been used to detect viral pathogens for several years, it is only during the COVID-19 pandemic that there has been serious consideration of using Ct values for clinical care and infection control measures. This has been an area of active debate. Professional organizations, including the Association for Molecular Pathology, Infectious Diseases Society of America, the Association of Public Health Laboratories, and the American Association of Clinical Chemistry have issued guidelines against using Ct values for clinical care.<sup>2-4</sup> Regardless of this, laboratory directors are frequently asked to supply Ct values for various purposes, discussed later in discussion, and need to be prepared for such requests. Furthermore, in at least one state in the US, Florida, clinical laboratories are required to report Ct values to the state Department of Public Health, but not in clinical reports to care providers.<sup>5</sup>

Before turning to a discussion of the potential uses of Ct values, it is important to understand the regulatory status of reporting Ct values for SARS-CoV-2. Because RT-PCRs for SARS-CoV-2 have been approved for use as qualitative assays, reporting a quantitative result, such as a Ct value, is a regulatory violation.<sup>3</sup> Reporting these values to providers outside of the laboratory report does not necessarily prevent them from appearing in the medical record, as they can be included in the clinical notes. Laboratory directors should understand the potential consequences of reporting Ct values and consider this in determining how and whether to provide these values for clinical care.

The remainder of this review will include 3 topics. First, the variability of Ct values for SARS-CoV-2 will be briefly discussed, as this is important to understanding the potential utility of these values. Second, the evidence for and against various uses of Ct values will be reviewed. Finally, specific recommendations will be made for those who have decided to provide Ct values to clinical caregivers or for infection control purposes.

### ***Variability of Cycle Threshold Values***

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It is important to understand the sources and magnitude of variability of Ct values for SARS-CoV-2. This variability can be reduced or minimized, but it cannot be eliminated (**Table 1**). Clinical staff should understand that Ct values can be highly variable and this is one of the several reasons why these values must be interpreted with caution. Information about the variability of Ct values can be provided within the laboratory report or during consultation between the laboratory director and clinical staff, before the release of Ct values. These data should not be provided without a complete explanation of the expected variability and of what changes might be considered meaningful in the context of clinical care.

<b>Source of Variability</b>	<b>Magnitude of Variation</b>	<b>Mitigation Strategies</b>
Use of different RT-PCR Assays	Small to large	Use the same assay, performed in the same laboratory if values will be compared between different samples
Transport Media	Small	Not needed
Sample Storage	Small if samples are less than a week old at temperatures from room temperature to $-80^{\circ}\text{C}$ Moderate if samples are held longer than a week at room temperature or $4^{\circ}\text{C}$ (low-levels of RNA may become undetectable)	If sample retention is longer than a week, freeze samples, preferably at $-80^{\circ}\text{C}$
Sample type	Small to moderate, with nasopharyngeal swabs generally having lower Ct values than other samples from the upper respiratory tract	Use the same type of sample if values are compared between samples Nasopharyngeal swab samples may be preferred if Ct values are compared

Variations in Ct values are designated as small if they are 2 or smaller, moderate if they are greater than 2 but smaller than 7, and large if they are 7 or greater.

There are several sources of variability in Ct values, and these are discussed separately in the sections that follow. In considering these sources of variability, several things should be kept in mind. First, the methods used in these studies vary significantly. For brevity, the most important differences in methods will be discussed, but less important differences will not. Second, these papers often show summary data, such as means and standard deviations, graphically, but they may not provide the numerical values. In such cases, differences between Ct values have been estimated by the inspection of the figures. Third, these papers often include a separate analysis of the targets of amplification for those assays that include more than one target. For the most part, the different targets within a specific assay do not vary much in their Ct values, and so this level of detail will not be discussed except where necessary.

### ***Variability Associated with Severe Acute Respiratory Syndrome Coronavirus-2 Reverse Transcription-Polymerase Chain Reaction Tests***

Different RT-PCR tests for SARS-CoV-2 can yield very different Ct values. This is to be expected, given that the assays amplify different regions of the viral genome using different primers and probes and include different reagents, all of which will contribute to variations in the efficiency of the RT-PCR between assays. Reasonably comprehensive data on this issue were provided by the College of American Pathologists (CAP) Microbiology Committee in response to a paper in *Clinical Infectious Diseases*.<sup>6</sup> These data were collected from participants using CAP proficiency test materials. The median Ct values between different tests varied by as much as 14 cycles. Even within a single gene target on a single assay, between-laboratory results varied by up to 12 cycles. Papers in which various assays were validated or compared have similarly

found differences in Ct values that ranged from 5 to slightly more than 14 cycles.<sup>7,8</sup> Importantly, because of high test volumes and the need for reasonable turn-around-time, many laboratories have had to run several different assays. Potentially large differences in the Ct values between assays must be considered if values are compared between assays performed in different or the same laboratory.

### ***Variability Associated with Transport Medium and Sample Storage***

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The effect of dilution and storage of SARS-CoV-2 in different media, including M4, minimal essential media (MEM), phosphate-buffered saline, 0.9% saline, as well as in patient samples (sputum and bronchoalveolar lavage) has been evaluated. Briefly, 2 studies, each which included several prospective transport media or sample types, demonstrated that there was no increase greater than 2 in the Ct values for any media or sample type following storage at temperatures ranging from room temperature to  $-10$  to  $-30^{\circ}\text{C}$  during 7 days of storage.<sup>9,10</sup> Ct increases of slightly more than 2 were seen after storage of samples in saline after 14 days at refrigerator or freezer temperatures. In a third study, storage of SARS-CoV-2 in phosphate-buffered saline at room temperature,  $4^{\circ}\text{C}$ ,  $-20^{\circ}\text{C}$ , and  $-80^{\circ}\text{C}$  had little effect on the Ct at 7 days.<sup>11</sup> From 14 to 28 days, samples with a lower level of virus (500–1000 copies/mL) had increased Ct values of slightly more than 2, and some samples became negative at room temperature,  $4^{\circ}\text{C}$ , and  $-20^{\circ}\text{C}$  but not at  $-80^{\circ}\text{C}$ . Samples with a higher level of virus (5000–10,000 copies/mL) did not show significant changes in the Ct values at any of these temperatures. In summary, storage at room temperature or  $4^{\circ}\text{C}$  for up to a week has minimal effect on the Ct values for SARS-CoV-2, but if samples are stored longer there may be small increases of 2 to 3 Ct and lower levels of virus may become undetectable.

### ***Variability Associated with Sample Type***

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There are sizable differences in the Ct values of different types of respiratory samples. Several studies compare the Ct values between different specimens of the upper respiratory tract. Saliva will be included here, because it presumably represents a mixture of saliva and mucosal upper respiratory tract secretions, with the latter presumably containing SARS-CoV-2 RNA. Because there is so much variation in the methods of sample collection and processing, the findings are generally specific to the institution whereby the study was conducted, so only a few general points can be made. First, nasopharyngeal samples usually have Ct values lower than or equal to other sample types studied. Compared with nasopharyngeal samples, nasal swabs,<sup>12</sup> saliva,<sup>13,14</sup> and oral swabs<sup>14</sup> have values that are approximately 5 to 7 cycles higher in some studies. Other studies have found similar Ct values for nasopharyngeal and nasal swabs,<sup>13</sup> or for nasopharyngeal swabs, throat swabs, sputum, and dual throat/nasopharyngeal samples.<sup>15</sup> A small study found that Ct values of tracheal aspirates were on average 3 cycles higher than those of nasopharyngeal swabs.<sup>16</sup> When the Ct values were normalized to the human RNaseP gene that was included in the assay, there was no difference between the values from tracheal aspirate and nasopharyngeal swab specimens;<sup>16</sup> however, the meaning of standardizing Ct values of viral RNA to human RNA is not clear.

At the time of writing, there were no studies that compared Ct values for SARS-CoV-2 PCR between samples from the upper respiratory tract and the lower respiratory tract. There are case reports and case series that show that qualitative results of bronchoalveolar lavage samples differ from those of NP swabs or other upper respiratory samples.<sup>17</sup> These reports usually emphasize the importance of testing lower respiratory samples to optimize the detection of SARS-CoV-2. Given these discrepant

results, and the sample dilution required to collect a bronchoalveolar lavage sample, it is inevitable that there will be differences between Ct values of upper and lower respiratory samples. It is unlikely that the sample matrix will greatly affect the Ct values, at least if the method of RNA purification is adequate and consistent.<sup>11</sup> It is strongly recommended that the same sample type and assay be used to compare Ct values within a patient over time, unless there are sufficient local data to allow providers to make informed comparisons.

### ***Possible Use Cases for Cycle Threshold Values for Severe Acute Respiratory Syndrome Coronavirus-2***

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There are 3 potential uses of Ct values that are adequately discussed in the literature to merit review. These include, first, possible use of Ct values to evaluate the progression or course of COVID-19, second, the prognostic use of Ct values as predictors of clinical severity of infection and, third, use of Ct values to determine whether a patient is potentially infectious.

### ***Use of Cycle Threshold Values to Determine Progression of Infection***

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Caregivers may want to use the Ct value or multiple Ct values to determine whether the viral load is rising or falling, as a marker of whether the patient is recovering from infection. A similar, but perhaps less common use, is to use the Ct to try to determine approximately how long the patient has been infected. The utility of the Ct value for these applications depends on several things, including the average kinetics of the viral load (and associated Ct) in affected persons, the variability of these kinetics within an individual patient, and how the kinetics differ in patients who are immunocompromised. Each of these will be discussed.

A study early in the pandemic provided pooled and individual patient data on the Ct values of patients with acute COVID-19.<sup>18</sup> While the number of patients is small at 14, the study is valuable because the trends over time after the onset of symptoms are shown for individual patients. Nasal (mid-turbinate and nasopharyngeal) swabs or throat swabs were analyzed separately, using RT-PCR which detected regions of the N and ORF1b genes. Ct values for ORF1b were tracked over time for each patient. From this study, it is clear that aggregated Ct values are lower in the first week after symptom onset, and then higher thereafter for both specimen types, with nearly all patients having negative results by 21 days after symptom onset. However, there is marked variability in Ct values between and within patients. From the inspection of the graphs, for each of the 2 specimen types, at least 5 patients had samples that were negative followed by samples that were positive for SARS-CoV-2. Furthermore, although the aggregate Ct values rose over time, indicating falling levels of viral RNA, several patients had decreases in the Ct values of greater than 8 to 10, followed by rising Ct values or negative results. Taken together, these results indicate that results within an individual patient can be highly variable, and so using a small number of Ct values to track a patients' course may be misleading.

Numerous case reports and case series show that people who are immunocompromised can have prolonged infection with SARS-CoV-2. The largest of these includes 20 patients receiving immunosuppressive therapies for various cancers.<sup>19</sup> Unfortunately, the publication does not include Ct values; however, RT-PCR results are summarized and detection of replication-competent virus is presented in some detail. Viral RNA could be detected as late as 78 days after onset of symptoms (interquartile range, 24–64 days). Three of 20 patients shed replication-competent virus for more than 20 days. A smaller series, including only 3 patients with various causes of immunosuppression and prolonged infection with SARS-CoV-2, includes Ct values.<sup>20</sup>

Again, as has been discussed above, there is marked variation in the Ct values over time, with apparent reductions in viral RNA being followed by increases, so that one could be misled by trying to track the course of infection by using these values. Perhaps the longest documented shedding of replication-competent SARS-CoV-2 was 238 days in an immunocompromised patient was seen in a patient with mantle cell lymphoma receiving treatment with rituximab, bendamustine, and cytarabine.<sup>21</sup>

### ***Use of Cycle Threshold Values to Predict Severity of Coronavirus Disease 2019***

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The prognostic utility of the Ct value at or shortly after patient admission was assessed in a<sup>22</sup> retrospective study was performed at 2 hospitals in New York City.<sup>22</sup> Hospitalized patients tested positive for SARS-CoV-2 by RT-PCR using nasopharyngeal swab specimens collected within a day of admission were included. RT-PCR was performed using an assay that detects regions of the E gene and the ORF1ab gene, and Ct values from the ORF1ab gene were used to divide the patients into 3 roughly equally sized groups, with Ct values of less than 25 (high viral load), 25 to 30 (medium viral load) and greater than 30 (low viral load). Patients whose samples were positive for the E gene but negative for the ORF1ab gene were included in the low viral load group. A total of 678 patients were studied. There was a strong relationship between the viral load and in-hospital mortality, with mortality rates of 35%, 17.6%, and 6.2% for the high, medium, and low viral load groups, respectively. The proportion of patients who were intubated was similarly related to viral load, with 29.1%, 20.8%, and 14.9% for the 3 groups. Finally, multivariate analysis which included multiple risk factors such as age, race, and several comorbidities revealed a significantly increased risk of mortality in the high viral load group compared with the low viral load group, with an odds ratio of 6.05 (95% confidence interval 2.92–12.52).

Another approach has been to evaluate the prognostic value of a rising or falling Ct value over time. This was investigated in a retrospective study at a single institution.<sup>23</sup> Patients who presented to the Emergency Department with radiological and clinical evidence of pneumonia who had 2 or more positive SARS-CoV-2 RT-PCRs with the same assay more than 24 hours apart were included. RT-PCR was performed using an assay that amplifies the N2 and E genes, and the Ct values for the N2 results were analyzed. Clinical status was determined using the sequential organ failure assessment (SOFA) score, which includes scores for 6 organ systems and predicts clinical outcomes in critically ill patients. Only 42 patients met the inclusion criteria, which is not surprising as there was no systematic retesting required. The number of tests performed and time between tests varied, as these depended on the needs of clinical care. With these caveats, there was a relationship between the change in Ct value and the change in the SOFA score. Overall, an increase of 1 Ct value, indicating a reduction in the viral load, was associated with a decrease in the SOFA score of 0.05, indicating clinical improvement. It should be noted that many of the repeat tests were performed in patients as part of discharge planning, and this may have biased the patients studied to include those with clinical improvement. The results of this small study indicate that further research into the relationship between changes in Ct scores and changes in clinical outcomes is warranted.

A number of small studies have been conducted to evaluate the utility of the Ct value or viral load for predicting the severity of illness. The Ct value of saliva was retrospectively evaluated, using a nonstandardized clinical score, and lower Ct values were found in patients with more severe manifestations of COVID-19.<sup>24</sup> One study did not find a strong relationship between the Ct value and clinical outcome in 875 patients with COVID-19.<sup>25</sup> Patients with SARS-CoV-2 detected by RT-PCR for regions of the N1 and N2 genes were classified as having mild (no hospital admission), moderate

(hospitalized in nonintensive care units) or severe (admitted to the intensive care unit) disease. The Ct values of those with moderate disease were slightly higher, on average, than those with mild or severe disease, but there was a significant overlap in the Ct distribution of the 3 groups.

Taken together, these studies indicate that patients with more severe COVID-19 tend to have lower Ct values early in the course of illness or at the time they present for medical care. However, the utility of the Ct value is limited as values overlap between groups classified by disease severity such that the values are unlikely to be useful for the care of individual patients. Furthermore, there are no data supporting the use of Ct values in making therapeutic decisions. Instead, decisions about therapy are generally guided by the clinical severity of disease.

### ***Use of Cycle Threshold Values to Determine Infectivity***

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A number of studies have assessed whether lower Ct values can be used to predict who is more likely to transmit SARS-CoV-2. There have been 2 approaches to this question. Initial studies looked at the relationship between Ct values and viral culture as a proxy for infectivity. These studies required containment at BL-4 or, more recently, BL-3, and facilities for viral culture, so they could only be performed at a limited number of sites. It is important to bear in mind that the accuracy of viral culture as a surrogate for infectivity of human contacts with an index case is not known, and it is possible that viral culture overestimates or underestimates infectivity of an index case for contacts. The second, more recent approach is to link Ct values determined in routine laboratory testing to transmission events detected in epidemiologic programs meant to reduce transmission. This approach is clearly more powerful than the use of viral culture, but it also has limitations. Specifically, it cannot be definitively determined whether those contacts who become infected after exposure to an index case acquired their infection through that contact or through another contact. This could be evaluated by typing the virus, for example, with viral genome sequencing; however, such a study has not been conducted.

One of the earliest studies to evaluate the relationship between Ct values and viral culture results included 183 nasopharyngeal and sputum samples that were positive by RT-PCR for the E gene of SARS-CoV-2.<sup>26</sup> Samples were stored at 4°C for up to 10 hours before being processed for viral culture. Culture was performed with Vero cells, with blind subculture twice for those viral cultures not demonstrating the cytopathic effect. All samples with Ct values below 17 had growth of SARS-CoV-2 in culture, while none with Ct values greater than 34 did. No samples collected greater than 8 days after the onset of symptoms had growth of the virus in culture. A subsequent study by the same investigators expanded these data to include 3790 samples, selected and processed as above and demonstrated that samples with higher Ct values were less likely to contain SARS-CoV-2 detectable by culture.<sup>27</sup> However, 3% of samples with a Ct value of 35 contained detectable virus in culture. No samples with Ct values greater than 35 had positive viral cultures for SARS-CoV-2.

A separate study by Bullard and colleagues found similar results, but with some important differences. This study used 90 nasopharyngeal or endotracheal samples that were positive for SARS-CoV-2 using an RT-PCR assay targeting the E-gene.<sup>28</sup> The samples were in viral transport medium and they were stored at 4°C for 2 to 3 days and then frozen at -80°C for 2 to 4 weeks. Viral culture with serial dilution of the sample was performed using Vero cells. The authors showed that virus could be detected by culture as long as 8 days after the onset of symptoms and that samples positive by culture had mean Ct values of 17, compared with those that were negative by culture, which had mean Ct values of 27. All samples with virus that was detectable



by culture had Ct values below 24. An excellent editorial commentary that accompanied this article pointed out that the Ct value above which no samples are positive by viral culture varies between studies, and that the effect of storing the samples on the sensitivity of viral culture is unknown.<sup>29</sup>

A different group evaluated the relationship between the Ct value and viral culture using 234 samples of several types, 97% of which were from the upper respiratory tract.<sup>30</sup> RT-PCR was performed using 5 primer-probe sets (E, RdRp, N, M, and ORF1ab) for patients in the intensive care unit and various subsets of these for other patients. Viral culture was performed using Vero cells, with terminal RT-PCR for cultures that did not show cytopathic effect. Unlike the studies discussed above, this study found a small number of samples positive in viral culture from patients with symptom onset at 17 and 18 days. For the N gene, the mean Ct value for samples with SARS-CoV-2 detectable by cytopathic effect was 25.0, while samples without cytopathic effect or terminal RT-PCR positivity had a mean Ct value of 36.9. The highest N gene Ct value for which virus could be detected in viral culture was 32. Although numerical values are not provided for the other primer-probe sets, there were much smaller differences than those for the N gene, with the exception of the ORF1ab primer-probe set. Finally, a study that used quantitative RT-PCR to measure the number of copies of viral RNA/mL found results similar to those in the studies already discussed, confirming that the Ct value is a reasonable surrogate for the burden of replication-competent virus.<sup>31</sup>

Taken together, these studies show that the Ct values used to predict infectivity in viral culture vary greatly, with the relevant values ranging from 24 to 35. Thus, using a specific Ct value to predict whether viral culture will be positive would have to be informed by local data. However, the culture of SARS-CoV-2 requires a biosafety level 3 laboratory according to the CDC recommendations,<sup>32</sup> and similar practices are recommended by the World Health Organization.<sup>33</sup> Many laboratories lack BSL-3 facilities, and those that have such facilities may not perform viral culture within them. Therefore, in practice, collecting such data is impractical at most institutions.

Two recent studies evaluated the relationship between Ct values for SARS-CoV-2 and transmission of infection using RT-PCR results and information from contact tracing to track transmission of infection. The smaller of the studies was conducted at Tulane University, in New Orleans, LA.<sup>34</sup> It included college students less than 23 years of age. Students were tested twice weekly for a 2-month period, using nasopharyngeal swab specimens that were tested within 24 hours of sample collection. The RT-PCR assay had primer-probe sets to amplify regions of the N, S, and ORF1ab genes. The Ct values for the 3 were averaged for data analysis. A total of 61,982 tests were performed on 7440 students, 602 of whom had at least one positive result. Of these, 195 were identified as index cases with one or more close contacts; 94 spread the infection to one or more close contacts and 101 did not. The surprising result was that the mean Ct value for those who spread infection was essentially the same as for those who did not (23.99 and 24.02, respectively), with very similar distributions of the Ct values. The median Ct values differed slightly, at 22.47 for those who spread infection and 24.43 for those who did not. The authors conclude that it is not practically feasible to predict who will spread infection using the Ct values as determined by the methods used.

The second study came to different conclusions, finding a strong correlation between lower Ct values in index cases and risk of transmission.<sup>35</sup> Data were collected from 3 high-throughput testing centers performing community testing over a 6-month period in England with linked contact tracing. The test, performed using combined nose and throat swabs, included the detection of regions of the S, N, and ORF1ab

genes. Lack of amplification of the S gene was used as a surrogate for the detection of the alpha or B.1.1.7 variant of SARS-CoV-2. This large study included 1,064,004 index cases with 2,474,065 contacts, 231,498 of whom had positive RT-PCR for SARS-CoV-2. Different types of contact (household, household visitor, events/activities, work/education, and outdoors) were considered separately, and are listed in descending order of the risk of transmission. For each category of contact, there was a roughly linear relationship between the Ct value and the proportion of contacts with SARS-CoV-2 detected within 1 to 10 days following contact after the initial diagnosis of the index case. Among household contacts, for example, rates of positive SARS-CoV-2 PCR positivity were 11.7% when the index case had a Ct value of 15%, and 4.5% when the index case had a Ct value of 30. Failure of S gene amplification, indicating the likely presence of the alpha variant of SARS-CoV-2, increased the risk of transmission by 1.44- to 1.55-fold depending on the Ct value of the sample. The strengths of this study are large size and the careful analysis by contact type and SARS-CoV-2 variant, which make the results more robust than the previous study and highly generalizable. Similar results were found in a study of the risk of transmission from individuals with different viral loads (not Ct values) in respiratory samples: the secondary attack rate was 12% for index cases with  $10^6$  or fewer copies of RNA per mL, but 24% for index cases with  $10^{10}$  or greater.<sup>36</sup>

### ***Recommendations on Reporting Cycle Threshold Values***

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The decisions of whether and when to report Ct values should be made by the laboratory directors in consultation with local experts in compliance and departmental leadership. If the leadership group decides that Ct values should be reported, several other decisions should be made before proceeding. The first task is to decide whether all results will be reported or if they will be selectively reported, that is, on request. Reporting of all results may cause confusion among caregivers who do not know how to interpret the information; therefore, selective reporting would seem to be the better choice. Second, if reporting is to be conducted selectively, it must be determined who may request the information. One option would be to work with the infectious diseases practitioners at the institution and provide them with the information needed to understand and use these results. If caregivers from other specialties request Ct values, they could be directed to involve infectious diseases specialists so that the information is used appropriately. The third task involves providing the supporting information necessary to help providers correctly interpret a Ct value. Basic information to be provided includes the fact that lower Ct values indicate higher concentrations of viral RNA and that a difference of 3 Ct values indicates approximately a 10-fold different level of viral RNA. Local data about RT-PCR efficiency might be used to provide a more precise relationship between these values. An understanding of the factors underlying the variability of Ct values is essential, so that simple mistakes, such as comparing Ct values from different specimen types, are avoided. Finally, basic statistical values of the Ct values obtained in the laboratory should be used to understand the Ct value. These could include, for example, the mean, median and interquartile ranges for symptomatic patients' results.

### **CLINICS CARE POINTS**

- Because there are several sources of variability in the Ct values for SARS-CoV-2, clinical staff should work closely with laboratory staff to interpret Ct values for individual patients.

- The sources of variability in Ct values for SARS-CoV-2 vary in the size of variability that they introduce. The greatest potential for variability in the Ct values is comparison of Ct values from different assays. Variability of Ct values can be mitigated by comparing values only when they are from the same assay, and by taking steps to ensure that samples tested are of the same kind and are stored in a manner that will preserve the viral RNA.
- Potential applications of Ct values for SARS-CoV-2 in patients include evaluation of the course of the infection, prognosis of the infection and assessment of infectivity.

## DISCLOSURE

The author has nothing to disclose.

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