

GENERAL INTEREST

Reproducibility of indeterminate results in SARS-CoV-2 PCR CT-value

Muneer Ahmad AlJamaan, Hussain Ali Al-Tufaif, Duaa Hassan Aldahan, Wassem Abdulmohsen Alali, Omar Mohammed Alsuliman, Mufeed Hassan Alyousief, Yousof Ayman Hassan, Ayman Mohamed Nabil

Dammam Regional Laboratory and Blood Bank, Ministry of Health, Dammam, Eastern Region, Kingdom of Saudi Arabia

Abstract

It is the global mission of the healthcare industry worldwide to prevent the re-emergence of another potentially lethal SARS-CoV-2 (COVID-19) pandemic. The PCR test for COVID-19 was the cornerstone of diagnosis and follow-up of suspected cases; however, there is a debate with regard to the interpretation of the results of the cycle threshold (CT) value of the PCR for the SARS-CoV-2 virus, especially, the indeterminate CT values between 30 and 35 or higher. The argument further extends to whether the test has to be repeated for confirmation or to interpret it as it is. The objective of our study was to determine the reproducibility of the indeterminate results of the SARS-CoV-2 PCR test values. Sixty (n = 60) indeterminate specimens with CT ranging from 32 to 40 were utilized in the study. The test was repeated twice. The results were read by two pathologists independently to decide whether they were positive or negative. The findings of this study were highly significant ($p < 0.05$) in favor of the conclusion that a CT value of 32 or more is to be considered positive. The patient with these CT values could transmit the disease and must be considered infected until a subsequent repeat negative PCR test proves the infection has subsided and receded completely.

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Correspondence: AlJamaan M; email: muneeraj@yahoo.com

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Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), also known as COVID-19, emerged in China at the end of 2019 and caused a large global outbreak that is considered a major public health crisis. SARS-CoV-2 is closely related to two types of bat-derived severe acute respiratory syndrome-like coronaviruses, bat-SL-CoVZC45 and bat-SL-CoVZXC21. The virus was confirmed to spread by human-to-human transmission either through droplets or direct contact, and the incubation period for its infection has been estimated to be 6.4 days with a basic reproduction number of 2.24–3.58 (Cheng et al. 2020). Currently, controlling infection to prevent the re-emergence of the pandemic is the cornerstone of primary intervention in healthcare. For this reason, it

remains important to reduce person-to-person transmission of SARS-CoV-2 on an individual level as well as on a public health level to prevent the re-emergence of the pandemic.

At the present moment, in addition to different types of rapid and comprehensive vaccination approved for all populations, politicians and health care experts have demanded various diagnostic strategies to achieve this goal. These diagnostic procedures have played a critical role in the diagnosis and follow-up of suspected cases. For this purpose, SARS-CoV-2 PCR testing is considered crucial not only for diagnosis and follow-up but also to ensure cases with low viral loads or a high CT-value are not overlooked. In contrast to Drancourt and

colleagues (Drancourt et al., 2021), who concluded that patients with high CT-values (CT-values ≥ 30) are no longer able to transmit infectious viral particles, Didier Raoult's group (Jaafar et al., 2021) showed that fifty percent of clinical specimens with CT-values ≥ 30 can be cultured and therefore may be potentially infectious. In general, the PCR CT values are used as surrogate markers for the claimed amount of virus in a given sample and, more importantly, to deduce the patient's infectivity. However, it is not yet known how many SARS-CoV-2 virions are required to cause an infection, how long the infective virus persists in patients through different stages of infection, or, as we have attempted to answer in this study, whether the CT values correlate with the number of infectious virus particles (Martin et al., 2021).

CT values between 17 and 32 represent culturable virus amounts and thus could be assumed to be infectious; whereas a CT value of 33.6 showed a positive culture test in some cases. Additional studies have also shown that culturing of SARS-CoV-2 is possible with samples containing significantly more CT than that, leading to the conclusion that CT values as a marker for infectiousness are still not reliable (V'kovski et al., 2021).

The report of Buchta and coworkers (Buchta et al., 2021) addressed this issue, specifically regarding the limitations of interpreting CT values for different SARS-CoV-2 target genes (that differ from one test kit to another) when different combinations of extraction and RT PCR platforms and reagents were used. When they analyzed the outcome with an external quality assessment challenge, it turned out that quantitative results deviated in 7.7% of cases by more than ± 4 cycles (that could go up to 18 cycles). This led to the conclusion that standardization is needed in the reporting of CT values with respect to the target gene (Buchta et al., 2021).

Disagreement exists over CT value representation and the inability to determine whether CT values of indeterminate results (between 30 and 37) were truly positive or negative. The goal of this study is to determine whether CT values between 30 and 37 are positive or negative, as well as the

reproducibility of an indeterminate SARS-CoV-2 PCR value.

Materials and Methods

A total of 60 specimens were selected with a CT result ranging from 32 to 40 that were considered indeterminate. These specimens were then read by two pathologists to determine whether the results were positive or negative. Statistical analyses were performed by standard methods. A "p" value of less than 0.05 is considered statistically significant.

Results

The 60 SARS-CoV-2 PCR indeterminate specimens had a CT range of 32–40, with a mean of 32, a median of 35, an average of 35, and a SD of 1.9. The repeat analysis gave a mean of 17, the median was 33, the average was 32.8, and the SD was 7.8, with only 18 specimens concluded as negative and 42 specimens concluded as positive based on the repeat replication of all specimens with dual separate readings by two pathologists. The difference was highly significant ($p=0.004915$; Table 1).

Discussion

The present study attempted to define viral load thresholds above which infectivity of COVID-19 patients could be assumed. In daily practice, PCR CT-values are used as surrogate markers for the amount of virus in a given sample and more importantly, to deduce the patient's infectivity. However, it is not yet known how many SARS-CoV-2 virions are required to cause an infection, how long infective virus persists in patients through different stages of infection, or whether CT-values correlate with the number of infectious virus particles. In particular, regarding the limitations of interpreting CT-values with respect to different SARS-CoV-2 target genes, the importance to define whether CT indeterminate results have to be repeated or issued as it was emerged and to conclude the importance of reproducibility if those specimens. Our results showed a high significance difference between the indeterminate first results and results upon repeating and conceding with other studies that

Table 1 results of repeating the indeterminate specimens of SARS-CoV-2 PCR value

Description	First indeterminate specimens	Double checked specimens
Number of specimen	60	60
Number of positive	0	42
Number of negative	0	16
Number of indeterminate	60	0
Mean	32	17
Median	35	33
Average	35	32.8
STD	1.9	7.8
P value	P=0.004915	

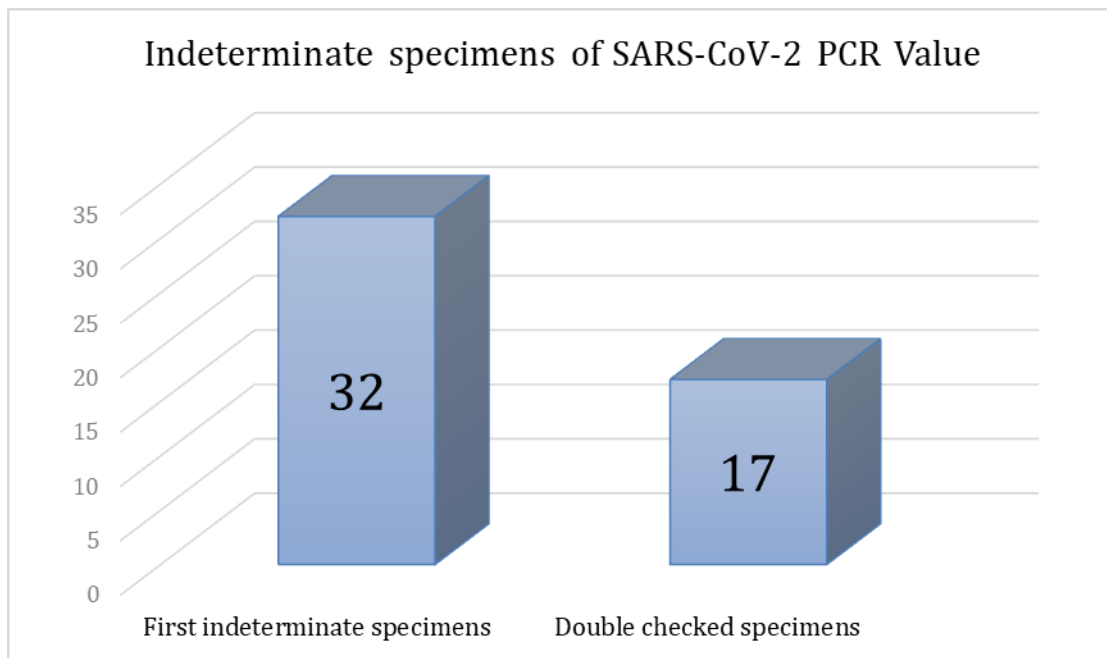


Figure 1: Results of repeating the indeterminate specimens of SARS-CoV-2 PCR value

concluded the significance of CT values even within the indeterminate values.

The findings of this study confirmed previous findings (Buchta et al., 2021; Arons et al, 2020) and contributed to the resolution of the debate. This is in agreement with the findings of Aron and coworkers (Arons et al, 2020). These workers reported they could identify viable virus in asymptomatic residents with a CT-value of 30 and below and were suffering from infection and could transmit the virus. Earlier, this debate intensified when Kujawski and coworkers (Kujawski et al., 2020) found that they were able to isolate culturable virus from samples with higher CT values (>30).

Based on these observations our findings support and enforced the suggestion that patients with significantly higher CT value of above 30 and more be considered positive, could transmit the virus and deal with such patients as infected.

Conclusion

In conclusion, The patient with these CT values could transmit the disease and must be considered infected until a subsequent repeat negative PCR test proves the infection has subsided and receded completely.

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