Can the SARS-CoV-2 PCR Cycle Threshold Value and Time from Symptom Onset to Testing Predict Infectivity?

Matthew J. Binnicker

Division of Clinical Microbiology, Department of Laboratory Medicine and Pathology,

Mayo Clinic, Rochester MN 55905

To curb the spread of SARS-CoV-2, a three-pronged strategy is recommended, which consists of testing, isolating those who are infected, followed by contact tracing. To date, the vast majority of testing for SARS-CoV-2 has been performed using real-time PCR. This diagnostic tool is inherently sensitive, but positive results may be due to the presence of active, replicating virus or residual viral nucleic acid (i.e., non-infectious virus). Despite this important limitation, real-time PCR is commonly being used as a '*test of cure*' following a diagnosis of COVID-19. For symptomatic patients, the U.S. Centers for Disease Control and Prevention (CDC) recommends either a symptom- or test-based strategy to guide decisions regarding the discontinuation of transmission-based precautions (i.e., isolation). When following a test-based strategy, the criteria for discontinuing isolation requires negative results for SARS-CoV-2 RNA from at least two consecutive respiratory samples collected ≥24 hours apart (1). Although this is a conservative approach, it has been justified during the early stages of the COVID-19 pandemic due to the lack of data on the period of infectivity of SARS-CoV-2, as well as the significant repercussions associated with discontinuing transmission-based precautions prior to confirming that an individual is no longer infectious. However, this strategy has increased the use of limited resources (e.g., tests, swabs, transport media), has led to some patients testing positive for weeks following their initial diagnosis (2, 3), and therefore, has significantly extended the period of isolation and/or quarantine for many individuals.

In this issue of *Clinical Infectious Diseases*, Bullard *et al* (4) assessed the correlation of the real-time PCR cycle threshold (Ct) value, as well as the time from symptom onset to testing, with growth of SARS-CoV-2 in cell culture. The study included 90 samples (nasopharyngeal swabs and endotracheal specimens in viral transport media) that were positive for SARS-CoV-2 RNA by a real-time PCR assay targeting the Sarbecovirus envelope (E) gene (5). Among the 90 samples that were inoculated into a Vero cell culture

line, cytopathic effect consistent with SARS-CoV-2 was observed in 26 (28.9%) cases. Notably, positive cultures were not observed in samples collected from patients with >8 days of symptoms, and a PCR Ct value >24 showed a strong correlation with reduced recovery of SARS-CoV-2 in cell culture. If confirmed, these results add to our growing understanding of the period of SARS-CoV-2 infectivity and have important implications on isolation, quarantine and return-to-work decisions. However, there are several significant limitations that should be carefully considered when interpreting the results.

First, Bullard *et al* tested for SARS-CoV-2 RNA using a single real-time PCR assay targeting the E gene of the virus. The data suggest that SARS-CoV-2 infectivity was reduced when the Ct value was >24, and that for every single unit increase in the Ct, the odds ratio for recovering the virus in cell culture decreased by 32%. Although real-time PCR Ct values can be used to estimate the relative concentration of target nucleic acid in clinical samples, Ct values are not interchangeable between assays. The PCR Ct value can be impacted by the assay's gene target(s) and by factors affecting the efficiency of the PCR reaction, including the nucleic acid extraction system and PCR amplification chemistry. Prior studies have demonstrated that Ct values may vary by up to 5 cycles when the same samples are tested by different assays (6, 7). Interestingly, a recent report by La Scola *et al* (8) assessed the correlation of SARS-CoV-2 isolation in cell culture with real-time PCR Ct values. In this study, inoculation of 183 respiratory samples (nasopharyngeal swabs [n=174], sputum [n=9]) into a Vero E6 cell line yielded detectable cytopathic effect in 129 (70.5%) cases. As expected, culture positivity declined with increasing PCR Ct values, and SARS-CoV-2 was not isolated in culture from any sample that had a PCR Ct value ≥34. These data highlight that despite the same PCR gene target being used, the Ct value threshold correlating with SARS-CoV-2 culture positivity may vary significantly between tests. Therefore, Ct value criteria must be established by each healthcare institution.

A second key consideration when interpreting the results from Bullard *et al* is that samples were stored at -80°C for up to 4 weeks prior to being inoculated in cell culture. Although storage of clinical samples at temperatures ≤-70°C has been shown to maintain infectivity of other human coronaviruses (8), it is important to emphasize that we do not yet fully appreciate all of the factors that may influence the recovery of SARS-CoV-2 in cell culture. Furthermore, we do not yet know what percentage of COVID-19 patients are culture positive, since the overall number of patients with confirmed disease who have had viral culture performed is exceedingly small. Due to its low sensitivity for recovery of other respiratory viruses (e.g., influenza), cell culture has been discontinued in most clinical laboratories and replaced by molecular testing. Therefore, we should be hesitant to conclude that a negative viral culture equates to a lack of SARS-CoV-2 infectivity. In the study by La Scola *et al*, growth of SARS-CoV-2 in 5 cases was only accomplished following blind subculture, suggesting there was a low amount of replicating virus in these samples, which may have been missed if blind passage had not been performed. Due to these challenges, future studies incorporating viral culture should be designed to optimize the recovery of SARS-CoV-2 by inoculating cell lines as soon as possible following collection. If prolonged specimen storage is required, positive controls containing low viral titers (i.e., SARS-CoV-2 whole virus spiked into representative specimen matrices) should be included and stored under the same conditions as clinical samples prior to culture. This will help control for the potential impact of prolonged storage on the recovery of SARS-CoV-2.

Despite these limitations, the study by Bullard *et al* provides informative data on the period of infectivity of SARS-CoV-2, and helps to confirm that PCR positivity is likely not a reliable surrogate marker for determining the infectious status of COVID-19 patients. Along with recent data emerging from South Korea (9) and Singapore (10), these results suggest that the period of SARS-CoV-2 infectivity is likely highest during the first week of illness,

and subsequently declines between days 8 and 11 post onset of symptoms. Together, these data should help inform testing guidelines and isolation policies. Future studies are needed to confirm these observations, and should include a combination of testing by multiple real-time PCR assays, carefully controlled viral culture studies and contact tracing to define which factors can be used to reliably predict the infectious status of patients with COVID-19.

Potential conflicts of interest:

M. J. B. reports personal fees from DiaSorin Molecular, Roche Molecular, and Abbott Molecular.

References

1. Centers for Disease Control and Prevention. Discontinuation of transmission-based precautions and disposition of patients with COVID-19 in healthcare settings (interim guidance). Available at https://www.cdc.gov/coronavirus/2019-ncov/hcp/disposition-hospitalized-patients.html. Accessed 27 May 2020.
2. Zhou F, Yu T, Ronghui D, *et al*. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: A retrospective cohort study. Lancet. **2020** Mar 28;395(10229):1054-62.
3. Cao H, Ruan L, Liu J, *et al*. The clinical characteristic of eight patients of COVID-19 with positive RT-PCR test after discharge. J Med Virol. **2020** May 15. doi:10.1002/jmv.26017.
4. Bullard J, Dust K, Funk D, *et al*. Predicting infectious SARS-CoV-2 from diagnostic samples. Clin Infect Dis. **2020** May 22. doi:10.1093/cid/ciaa638.
5. Corman VM, Landt O, Kaiser M, *et al*. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill. **2020** Jan; 25(3). doi:10.2807/1560-7917.ES.2020.25.3.2000045.
6. Nalla AK, Casto AM, Huang MW, *et al*. Comparative performance of SARS-CoV-2 detection assays using seven different primer-probe sets and one assay kit. J Clin Microbiol. **2020** May 26;58(6)e00557-20.
7. Rodino KG, Espy MJ, Buckwalter SP, *et al*. Evaluation of saline, phosphate-buffered saline, and minimum essential medium as potential alternatives to viral transport media for SARS-CoV-2 testing. J Clin Microbiol. **2020** June;58(6)e00590-20.
8. Lamarre A, Talbot PJ. Effect of pH and temperature on the infectivity of human coronavirus 229E. Can J Microbiol. **1989** June;35:972-74.

9. Korea Centers for Disease Control and Prevention. Findings from investigation and analysis of re-positive cases. Available at https://www.cdc.go.kr/board/board.es?mid=a30402000000&bid=0030. Accessed 28 May 2020.
10. National Centre for Infectious Diseases and the Chapter of Infectious Disease Physicians, Academy of Medicine, Singapore. Period of infectivity to inform strategies for de-isolation of COVID-19 patients. Available at https://www.ams.edu.sg/view-pdf.aspx?file=media%5c5556_fi_331.pdf&ofile=Period+of+Infectivity+Position+Statement+(final)+23-5-20+(logos).pdf. Accessed 28 May 2020.