

To Interpret the SARS-CoV-2 Test, Consider the Cycle Threshold Value

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To prevent the spread of coronavirus disease 2019 (COVID-19), it is important to identify and isolate people who are infectious. It is especially important to ensure that those with a high viral load are isolated and not able to transmit to others. Currently, diagnosis, screening, and surveillance depend on a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) reverse transcriptase–quantitative polymerase chain reaction (RT-qPCR) test, and results are generally reported to the ordering physician as positive or negative. However, the test does provide a measure of the viral load in the sample, in what is called the cycle threshold (Ct) value. We suggest that reporting this Ct value, or a calculated viral load, can aid in interpretation and clinical decisions. We discuss the merits of PCR tests and other approaches, such as time-since-symptom-resolution-based approaches for removing individuals from isolation.

In this issue of *Clinical Infectious Diseases*, Xiao et al [1] report that SARS-CoV-2 RT-qPCR results can remain positive up to 5 weeks after onset of symptoms.

The authors studied RT-qPCR results from 56 hospitalized patients with mild to moderate COVID-19 disease. Each patient received 4 to 7 tests over several weeks after symptom onset. The percentage of positive results declined from 100% in week 1 to 89.3%, 66.1%, 32.1%, 5.4%, and 0% in weeks 2, 3, 4, 5, and 6, respectively. The median time from symptom onset to negative testing was 24 days. Prolonged positive test results were associated with older age and comorbid diabetes or hypertension. Additionally, there were 4 patients with 2 consecutive negative test results who later tested positive again. A limitation is that the test results did not report Ct values, which would have provided valuable information about the amount of viral RNA in the samples, particularly important later in the course of infection. Other case series and our own experience also suggest that patients with 2 consecutive negative tests and resolved symptoms can subsequently test positive [2, 3]. Xiao et al conclude that longer periods of follow-up and repeat testing are necessary to limit viral spread. The Centers for Disease Control and Prevention (CDC) suggests 1 of 2 approaches for discontinuing isolation, one based on time since symptom onset and resolution and the other centered on 2 negative tests at least 24 hours apart [4]. Generally no longer transmitting virus upon 10 days after symptom onset and 3 days after symptom resolution. This approach is test-sparing and is particularly

useful when resources are scarce or in the outpatient setting where repeat testing is onerous. When testing is available, decisions to lift isolation rely heavily on negative PCR tests to define a patient as no longer infectious. However, this may not be optimal because it assumes a positive PCR test is intended to mean infectivity. Closer examination of what the test results mean clinically, particularly when results are from RNA quantities near the lower limit of detection of the assay, could help guide clinical and public health strategies.

The SARS-CoV-2 RT-qPCR test provides real-time quantification by first reverse transcribing SARS-CoV-2 RNA into DNA (RT step), and then performing qPCR, during which a fluorescence signal increases proportionally to the amount of amplified nucleic acid, enabling accurate quantitation of the RNA in the sample. If the fluorescence reaches a specified threshold within a certain number of PCR cycles (Ct value), the sample is considered a positive result. The Ct value is inversely related to the viral load and every ~3.3 increase in the Ct value reflects a 10-fold reduction in starting material. Many qPCR assays involve a Ct cutoff of 40 to consider the test positive, allowing detection of very few starting RNA molecules.

This high sensitivity for viral RNA can be helpful for initial diagnosis. However, reporting as a binary positive or negative result removes useful information that could

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inform clinical decision making. Following complete resolution of symptoms, people can have prolonged positive SARS-CoV-2 RT-PCR test results, potentially for weeks, as Xiao et al report. At these late time points, the Ct value is often very high, representing the presence of very low copies of viral RNA [5–8]. In these cases, where viral RNA copies in the sample may be fewer than 100, results are reported to the clinician simply as positive. This leaves the clinician with little choice but to interpret the results no differently than for a sample from someone who is floridly positive and where RNA copies routinely reach 100 million or more.

A positive RT-qPCR result may not necessarily mean the person is still infectious or that he or she still has any meaningful disease. First, the RNA could be from nonviable or killed virus. Live virus is often isolable only during the first week of symptoms but not after day 8, even with positive RT-qPCR tests [9]. Second, there may need to be a minimum amount of viable virus for onward transmission. For infection control purposes, the utility of the assay is greatest when identifying people who are floridly positive and at risk of further transmission. Particularly when testing in the absence of symptoms for COVID-19, we believe that reporting the Ct value or range could help to better inform clinical decisions.

We propose that for inpatients whose symptoms have fully resolved and 2 tests over 24 hours apart are either negative or close to the Ct cutoff (ie, Ct >34), they likely do not have meaningful or transmissible disease, and thus do not need to be retested. This would conserve valuable testing capacity, reagents, and personal protective equipment (PPE). Furthermore, the clinician could take the Ct results in context and determine when the patient can discontinue isolation. This could shorten the duration of isolation and, for healthcare workers and other essential workers, would provide a more evidence-based, testing-informed pathway for more rapid return

to work. Taking the Ct value into account may also help justify symptom-based strategies recommended by the CDC including time-since-illness-onset-and-resolution-based approaches (ie, lifting of isolation after 10 days following onset of symptoms and 3 days following resolution of symptoms) [4]. Lastly, there may be implications for public health screening, enabling contact tracers to focus on persons most likely to be infectious. This will become increasingly important as asymptomatic screening expands.

The Ct value could be high as a result of early disease and the Ct value would have to be considered in clinical context. A person with a high Ct value tested early in the disease course might be or become infectious and this would present as a significant decrease in the Ct value 24 hours following the first test. A patient with resolved symptoms and 2 Ct values both close to the cutoff is likely recovering and no longer infectious. Evidence from both viral isolation [9] and contact tracing [8, 10] studies supports a short, early period of transmissibility. By accounting for the Ct value in context, RT-qPCR results can be used in a way that is personalized, highly sensitive, and more specific. To implement this, the actual Ct values could be reported along with reference ranges or converted to viral load and/or categorized as high, medium, or low.

Repeat testing over 24 hours is not always feasible and is always resource heavy when testing is limited. Time-since-symptom-onset- and time-since-symptom-resolution-based approaches may be as or more useful in many situations. As more detailed data emerge and provide increased certainty about the length of infectivity, there may be discussion about shifting entirely to these time-based criteria. These approaches, such as isolation until 10 days after symptom onset and 3 days after symptom resolution, are straightforward and can be performed at home, conserving medical resources and time [4]. As long as resource limitations in testing and PPE exist, we believe that

time-since-symptom-resolution- and test-based strategies should continue to coexist and complement one another. Healthcare workers, who may have easier access to testing and who may be most crucial to get back to work more quickly, might benefit by test-based clearance, particularly if the Ct value is considered.

For both of these approaches, it should be recognized that certain populations may tend to remain infectious for longer. Xiao et al [1] showed that older patients were more likely to have prolonged positive results and presumably longer infectivity. Severe disease is also a risk factor for longer viral shedding [11]. Larger studies that account for potential confounding are necessary to determine viral shedding dynamics in different populations, including those who are immunocompromised or treated with immunomodulatory agents. For now, test-based clearance may be preferred for these patients. Having both time- and test-based guidelines enables clinicians to select based on the patient and setting.

In summary, prolonged positive SARS-CoV-2 RT-qPCR results raise questions about the sufficiency and sustainability of current isolation guidelines. We suggest that the Ct value from positive test results, when interpreted in context, can help to refine clinical decision making.

Note

Potential conflicts of interest. The authors: No reported conflicts of interest. Both authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

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