CORRESPONDENCE

The Prognostic Value of an RT-PCR Test for Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Is Contingent on Timing across Disease Time Course in addition to Assay Sensitivity

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To the Editor-in-Chief:

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We read with great interest the article by Tian et al[1] in a recent issue of the *Journal of Molecular Diagnostics* that showed a significant overlap in RT-PCR cycle threshold (Ct) value among spreader and non-spreader individuals. The study also discussed the limited potential to identify individuals as spreaders based on the viral load as detected from a nasal swab, showing that single Ct values obtained from serial surveillance testing at the individual level provides little diagnostic value for differential case management. The study also comments that "instead, a sensitive method to detect the presence of virus is needed to identify asymptomatic individuals who may carry a low viral load but can still be infectious."[1, pp.1078] However, RT-PCR Ct value is a sensitive method to detect and quantify the presence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus.[2–4] The reason that single Ct values obtained from serial surveillance testing provide little diagnostic value for differential case management is that the Ct value obtained depends critically on when during the disease time course the measurement is taken,[3,4] much more than the spreader or non-spreader status of the subject.[1] Therefore, a more sensitive test than RT-PCR may not address the issue of overlap in Ct among spreaders and nonspreaders and among symptomatic patients and non-symptomatic patients.

A single measurement of viral load at a single time point in an asymptomatic individual provides very little insight into prospective transmission because of the dynamic changes in viral load that are characteristic of the SARS-CoV-2 disease time course[3,5,6] and consequent RT-PCR diagnostic specificity (Figure 1).[1,3,5,7–9] During the latent period immediately following exposure, viral load is very low and likely localized, and nasal swabs are unlikely to recover any virus: an RT-PCR test may yield a negative result. Viral load increases steeply later in incubation, when both symptomatic and asymptomatic cases become infectious and the RT-PCR test becomes sensitive enough to detect virus.[3,5,7,8,10]

In the college population studied by Tian et al,[1] currently asymptomatic students were tested twice a week (approximately once per 3.5 days). Because of the distinct temporal sensitivity of RT-PCR (or indeed, any conceivable sample-based test), a consequence of testing every approximately 3.5 days is that positive tests will typically have a uniform probability of identifying infected students across a biweekly window starting at the point when swabs begin to sample virus and RT-PCR becomes sensitive. Because RT-PCR is a sensitive test for quantitative detection of specific RNA,[2–4] this 3.5-day window spans an extremely dynamic range (many orders of magnitude) of viral load (Figure 1).[3,5,6] Most of the variance in these measurements corresponds to the uniformly distributed variate (happenstance) of when during their infection the student was sampled, and has much less to do with peak viral load. Peak

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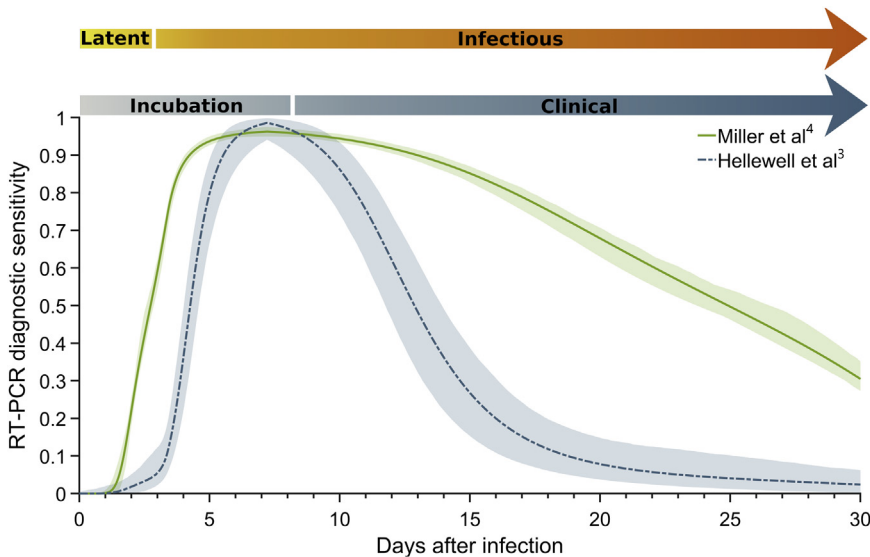
Figure 1 Sensitivity of RT-PCR for detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) across the COVID-19 disease time course from infection. The approximately 3.5-day period during which students surveilled in the study by Tian et al[1] are likely to first test positive spans a rapid rise in viral load early in disease. Sensitivity when sampling only symptomatic cases (green) is based on Miller et al,[4] He et al,[7,8] and Qin et al[9] using the approach of Wells et al.[5] Sensitivity when sampling currently asymptomatic cases (blue) is based on data from Hellewell et al,[3] He et al,[7,8] and Qin et al[9] using the approach of Wells et al.[5]

viral load is very poorly measured by randomly sampling one time point across 3.5 days that includes the viral exponential growth phase, and it is the eventual peak viral load that is most likely to correspond with future symptomatic versus asymptomatic status.[6]

The high variance in viral load during late incubation due to rapid viral growth across this period explains why Ct measurements by Tian et al[1] spanned such a wide range of Ct values (Figure 3E in Tian et al[1]), with such a slight difference in cumulative frequency toward lower Ct values (higher viral load) in symptomatic individuals compared to asymptomatic cases. Sampling the RT-PCR cycle threshold of many infected individuals in the population enables detection of that shift because of the law of large numbers: a very high sample size addresses the very high inter-individual variance, enabling Ct values to be useful for estimating epidemiological dynamics from cross-sectional viral load distributions.[11]

The interesting and useful results published by Tian et al suggest that a sensitive method to detect the presence of virus is needed. An alternative conclusion could be that consideration of the timing of measurements of viral load across the disease time course is vital to their prognostic value. A high prognostic value test would not come from increased sensitivity, but instead from many fine-scale or exquisitely timed measurements that capture dynamic changes in viral load that may be statistically associated with the peak levels experienced by subjects when they become most infectious.

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Authors' Reply

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We read with great interest the letter by Townsend and Wells[1] that has provided a robust discussion on the viral dynamics and its influence on the detection of virus in clinical samples. The major conclusion from our research is that the cycle threshold (Ct) values cannot predict the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) transmissibility. The need for a sensitive detection method is a secondary conclusion aiming to control the virus spread at the early stage, with no differential consideration on the Ct values. We agree that a more frequent and timed detection would be a better approach for Ct values to have a diagnostic value. However, it is unlikely that Ct values obtained from any more sensitive methods and/or from any more refined testing regimes have a clinical value in differentiating the spreaders from the nonspreaders because viral transmissibility is affected by many factors other than viral load at any given time. Other major impacting factors would include viral virulence, individual susceptibility, and human behaviors, to name just a few. This notion has been well expressed in our paper.

A diagnostic test for SARS-CoV-2 with limited transmission predictability may still be useful in detecting the virus earlier to help limit potential virus spread. Frequent testing, such as daily testing, may compensate for the low sensitivity of some assays as Townsend and Wells proposed on the understanding of viral dynamics. However, this approach may not be cost-effective, compliant, or may not alleviate the need for a sensitive detection method. A more sensitive method could certainly detect the presence of virus at the earlier phase of its expansion without the need to repeat the assay in subsequent days when a less sensitive method is employed. For example, our studies show that 13.8% of the spreaders had a Ct value above 32. These samples would most likely be tested negative using a less sensitive method. Currently there are many SARS-CoV-2 assays on the market that have a limit of detection ranging from 100 copies/mL to 10,000 copies/mL. A more sensitive method is more useful. Though the use of a more sensitive method and/or a more frequent testing regime may help to control viral spread through early detection, they do not negate the fact that Ct values alone could not predict the viral transmissibility.

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