

Cycle Threshold to Test Positivity in COVID-19 for Return to Work Clearance in Health Care Workers

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Abstract

Objective: To ascertain whether reverse transcriptase polymerase chain reaction (RT-PCR) cycle amplifications until detection, the cycle threshold (Ct), could help inform return to work (RTW) strategies for health care workers (HCWs) recovering from COVID-19 infection.

Methods: Sequential Ct data from COVID-19 nasal pharyngeal (NP) RT-PCR testing in all COVID-19 positive HCWs at a single institution. Analysis of Ct in relation to time until negative testing for RTW clearance.

Results: Data for 12 employees showed that time elapsed until RT-PCR test-based RTW clearance ranged from 7 to 57 days (median, 34.5 days). Lower initial Ct correlated with the total time elapsed until clearance ($r = -0.80$; $p = 0.002$).

Conclusion: Considering the RT-PCR Ct, which correlates with the estimated viral load, may help inform RTW planning and decision making beyond solely relying on dichotomized positive/negative results.

Key words: Return to work, COVID-19, health care workers, RT-PCR, cycle threshold number

Introduction

Infection with COVID-19 among health care workers (HCWs) has been a focus of public health concerns since the onset of the pandemic.¹ Return to work (RTW) after recovery from COVID-19 infection among HCWs is central to employee health protection, but has proved to be challenging. The initial recommendation by the Centers for Disease Control and Prevention (CDC) was reverse transcriptase-polymerase chain reaction (RT-PCR) testing to demonstrate two sequentially negative viral nasopharyngeal (NP) swabs prior to RTW in HCWs. Test-based clearance was also predicated on fever resolution for at least 72 hours and improvement in respiratory symptoms. Subsequently, the CDC expanded its recommendations to allow for a symptom (non-test) based protocol for RTW relying on symptom improvement and time elapsed from diagnosis (or time-based alone in asymptomatic COVID-19 infection). This was in lieu of RT-PCR testing, although the latter protocol has remained an acceptable option as well.²

Early experience, initially anecdotal and later confirmed in published data, established that continued NP RT-PCR viral positivity was frequently observed weeks after the onset of COVID-19 illness.^{3,4} For HCWs in particular, lack of unequivocal data on the potential risk of continued viral shedding, juxtaposed with concern over potentially unnecessary prolonged work

absence due to continued detection, has led to heterogeneity in RTW protocols, consistent with the very different options sanctioned by the CDC.

Although RT-PCR test results for COVID-19 typically are dichotomized (detected vs. not detected), we wished to ascertain whether RT-PCR amplification cycle threshold (Ct) could help inform such RTW strategies. Specifically, by providing an indication of viral load, based on the test's calibration curve, we hypothesized these data might better characterize RTW trajectories. We report RT-PCR cycle amplification data for sequential COVID-19 test results in a cohort of COVID-19 positive HCWs undergoing viral NP swabbing for RTW clearance.

Methods

We collected data from COVID-19 NP RT-PCR testing in all COVID-19 positive employees at the San Francisco Veterans Affairs Health Care System (SFVAHCS) as part of clinical quality improvement efforts for a standardized RTW protocol. All employees were originally tested due to symptoms consistent with COVID-19. We initially retested employees seven days after their initial positive detection of COVID-19. Because NP swab specimens from all of the employees retested at seven days were still RT-PCR test positive and due to national limitations of testing supplies at the time, we quickly modified the protocol to perform the first RTW test no sooner than 14 days after initial (baseline) COVID-19 detection. The protocol retested every seven days thereafter until the first negative (not detectable) result. This was followed by an additional test at least 24 hours later, with two sequentially negative tests meeting criteria for RTW clearance.

Based on our initial experience with employees who tested negative and then positive again, albeit with consistently higher RT-PCR Ct values reflecting lower viral loads, we modified the initial protocol. That modification used a Ct of ≥ 24 (consistent with an estimated viral level of < 1000 copies per ml) on two previous clearance tests to allow a single negative test to suffice for clearance, rather than two sequentially (in all cases this lower level detection was at 21 days or longer since diagnosis).

All NP RT-PCR testing was performed using the same NP swab protocol and analyzed at SFVAHCS molecular core laboratory. Testing was performed with the Abbott m2000 molecular platform, which was approved by the FDA under Emergency Use Authorization and validated by the molecular core laboratory. Although the test was approved only for the qualitative detection of nucleic acid from SARS-CoV-2, the laboratory established a calibration curve to help estimate the viral load (copies/mL) in swab transport media based on the reported RT PCR cycles Ct.

The target sequences for the Abbott PCR assay are the SARS-CoV-2 RdRp and N genes of the viral genome. If the positive, negative and internal controls are acceptable and there is no evidence of amplification of the target sequences, the test is reported as negative (or “not detected”). Although not officially published by Abbott Diagnostics, a negative sample typically shows no amplification of target sequences at a Ct number of 31.5 or higher. The lower limit of detection (LOD) of the Abbott SARS-CoV-2 PCR assay is about 100 copies/mL. Although RT-PCR cycles until detection (Ct number) may not precisely equate with the viral load (copies/mL), a lower Ct number does indicate a higher viral level. Our laboratory’s in-house validation established a calibration equation: $\text{Log copies/mL} = -0.26 \times \text{cycle number} + 9.11$ (Figure 1). This calibration, however, should not be extrapolated when the viral level is below 80 copies/mL. We did not otherwise directly consider estimated viral level in copies/mL in this clinical analysis.

We qualitatively assessed the pattern of RT-PCR Ct number until detection to a maximum of 31.5 and higher (not detected) over time for each of the employees included in the analysis. Most, but not all employees included originally tested positive at the SFVAHCS. For those, we considered initial detection as day zero. For those tested in our laboratory at follow-up only (e.g., initially diagnosed through a test performed at another facility), we assigned the days elapsed since outside testing as their date of entry into tracking. We quantified time elapsed until RTW clearance from the date of first COVID-19 RT-PCR diagnosis.

We used Spearman rank correlation to test the association between the days elapsed from the first available Ct number (thus, an NP RT-PCR test result from our laboratory) until RTW clearance. The latter was based on either two negative tests or a single test per protocol, as aforementioned. In a sensitivity analysis we re-estimated the correlation excluding those for whom the first value was ≥ 14 days since first testing positive.

Results

We analyzed data for 12 employees (two male; ten female). None of these cases was critically ill, and only one was briefly hospitalized. Data for Ct over time for the 12 employees are displayed in Figure 2. The time elapsed until RTW clearance ranged from 7 to 57 days (median, 34.5 days). Six of the 12 (50%) had multiple positive test results reaching a $\text{Ct} \geq 24$ with ≥ 28 days elapsed follow-up, allowing RTW clearance with a single negative result. The remaining six employees were cleared for RTW following two sequential negative tests. There were three persons who tested positive again after their first negative result; one of these reverted

twice before ultimately being cleared. Of note, these three did have varying degrees of recrudescence symptoms during follow-up.

All the individual trajectories are generally in the direction of an increasing Ct, albeit without any consistent slope and with substantial fluctuations for some. One such case in particular, who was first detected at a relatively low Ct, continued to have complaints of feeling generally unwell (cough, sore throat, anosmia) for a number of weeks, although without objective fever or dyspnea.

Lower initial Ct number correlated with the total time elapsed until clearance ($r = -0.80$, 95% CI -0.94 to -0.41 , $p=0.002$). Re-estimated among nine of the twelve cases after excluding three with initial RT-PCR Ct data 14 days or more since original detection, the correlation remained similar ($r = -0.78$, 95% CI -0.95 to -0.24 , $p=0.01$).

Discussion

These data document the extended time frame of COVID-19 viral detection by RT-PCR in persons recovering from illness and indicate that RT-PCR Ct at initial surveillance correlates with the duration until clearance. Our findings suggest that considering the RT PCR Ct number, which correlates with the estimated viral load, may help inform RTW planning and decision making beyond solely relying on dichotomized positive/negative results. We already took these data into consideration in implementing a modified protocol for clearance with one negative result following two positive tests at Ct number ≥ 24 with at least four weeks elapsed since symptom onset, which allowed us to clear half the group after one negative test.

The median of 34.5 days until RTW clearance we observed is longer than the 23 median days for “transition to negative” (but not RTW) reported among 63 HCWs.⁴ More saliently, RT-PCR Ct numbers were not presented in that analysis. Indeed, RT-PCR Ct data have not been examined extensively in follow-up testing. A position statement from the National Centre for Infectious Diseases in Singapore reported data from a local cohort of 73 COVID-19 patients who were followed using a amplification Ct of 30 or higher finding no viable virus by culture at that point although they also reported that no virus could be cultured after 11 days.⁵ Thus, the Ct threshold of 24 at 21 days or more we used to truncate the protocol to a single subsequent negative test would be unlikely to be associated with infectivity. It also should be noted that the Ct number is assay and laboratory specific requiring appropriate internal calibration if RTW decision making were to be guided by such data.

Continued viral detection after initial illness may not equate with infectivity, in particular nine days or more after first positivity.⁶⁻⁸ This informed the initial CDC symptom-based (no-test) strategy for RTW in HCWs which stated “Note that detecting viral RNA via PCR does not necessarily mean that infectious virus is present.”² Subsequent CDC recommendations for return to work in HCWs, released after the collection of the observations we report here, abandon the option of RT-PCR viral testing “except to rare situations.”⁹ The newer CDC time- and symptom-based recommendations allow for RTW as early as 10 days, extended to 20 days for severe illness or immunocompromise in the HCW.

These recommendations do not consider explicitly immunocompromised or other vulnerable patients with whom a post-COVID HCW may come into contact. Of note, a study of RT-PCR surveillance in a group of nursing home workers noted that some had infectious virus for multiple consecutive weeks, extending beyond 20 days.¹⁰ That report, along with our observation of a RT-PCR Ct pattern in which, for one of our patients, there was a substantial rebound to a relatively lower Ct number nearly one month into infection, raises potential concerns about relying on time and symptoms alone.

Conclusions

Time- and symptom-based (non-test) protocols for RTW are attractive because they are simple, do not require repeated testing and follow-up, and invariably lead to shorter work absences. Our data suggest that the story may not be so simple and that occupational health practitioners should proceed with caution. Additional experience in test-based RTW among HCWs, especially those caring for the most vulnerable patients, should assess whether Ct data from repeated testing correlate with symptom fluctuation, antibody development, viral infectivity, and any delayed adverse outcomes. Ultimately, such information can better inform RTW protocols.

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Figure 1. Our laboratory's in-house validation calibration equation for the relationship between cycle number and viral copies per ml.

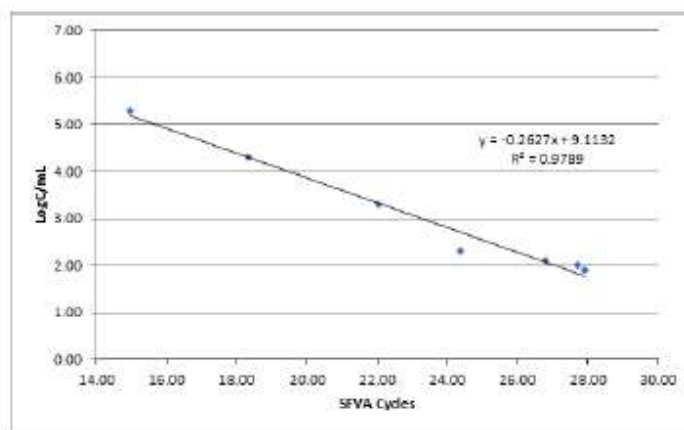


Figure 2. The RT-PCR cycle threshold (Ct) number until COVID-19 viral detection among 12 employees with repeated testing as part of protocol for clearance prior to return to work. Results with Ct numbers shown as 31.5 are non-detectable.

