Can Quantitative RT-PCR for SARS-CoV-2 Help in Better Management of Patients and Control of Coronavirus Disease 2019 Pandemic

Ashok Rattan, Hafiz Ahmad[1,2]

Advisor - Quality, Research and Development, Pathkind Labs, Gurgaon, Haryana, India, [1]Department of Medical Microbiology and Immunology, RAK College of Medical Sciences, RAK Medical and Health Sciences University, [2]Molecular Microbiology and COVID Lab In-Charge, RAK Hospital, Ras Al Khaimah, UAE

Abstract

The emergence of SARS-CoV-2, the causative agent of coronavirus disease 2019 (COVID-19), represents a public health emergency of unprecedented proportion. The global containment efforts have been focused on testing, tracing of contacts and treatment (isolation) of those found COVID-19 positive. Since the whole genome sequences of a number of strains of this novel RNA virus were available in the public domain by early January 2020, a number of real-time polymerase chain reaction (RT-PCR) protocols were designed and used for diagnosis of this infection. Most RT-PCRs are designed for qualitative COVID-19 reporting (SARS-CoV-2 detected or not detected), but have been used for semi-quantitative estimation of viral load based on cycle threshold value. Our manuscript discusses the utility of quantitative PCR testing for COVID-19 and its patient management benefits.

Keywords: Coronavirus disease 2019, cycle threshold, patient management, quantitative RT-PCR, SARS-CoV-2

INTRODUCTION

The emergence of SARS-CoV-2, the causative agent of coronavirus disease 2019 (COVID-19), represents a public health emergency of unprecedented proportion. The global containment efforts have been focused on testing, tracing of contacts and treatment (isolation) of those found COVID-19 positive. Since the whole genome sequences of a number of strains of this novel RNA virus were available in the public domain by early January 2020, a number of real-time polymerase chain reaction (RT-PCR) protocols were designed and used for diagnosis of this infection. The term RT-PCR is also used synonymously for reverse transcriptase PCR for RNA viruses which require additional step of converting RNA to complimentary (cDNA) using reverse transcription and before cDNA is converted to DNA as in the case of SARS-CoV-2.

Consequent on the explosive nature of spread of infection in different parts of the world, different RT-PCR protocols were made available for clinical use without first ascertaining the sensitivity of detection. In order to make diagnostic kits easily available, USA Food And Drug Administration permitted emergency use authorization of both RT-PCR and serology kits and regulatory authorities worldwide followed suit making unvalidated and not fully characterized kits available for clinical use.

RT-PCR emerged as the gold standard for the diagnosis of COVID-19 infection. Reports on viral dynamics indicated that viral shedding peaked on or before symptom onset and a substantial proportion of transmission probably occurred before first symptoms in the index case. After symptoms onset, viral loads decreased monotonically. Virus was detected for a medium of 20 days after symptom onset, but infectiousness declined significantly 8 days after symptom onset.[1]

Address for correspondence: Dr. Hafiz Ahmad, Department of Medical Microbiology and Immunology, RAK College of Medical Sciences, RAK Medical and Health Sciences University, Molecular Microbiology and COVID Lab In-Charge RAK Hospital, Ras Al Khaimah, UAE. E-mail: hafeezaiims@gmail.com

How to cite this article: Rattan A, Ahmad H. Can quantitative RT-PCR for SARS-CoV-2 help in better management of patients and control of coronavirus disease 2019 pandemic. Indian J Med Microbiol 2020;38:284-7.

Access this article online

Quick Response Code:

Website:
www.ijmm.org

DOI:
10.4103/ijmm.IJMM_20_380

Since accurate testing for SARS-CoV-2 followed by appropriate preventive measures are paramount in health-care setting to prevent both nosocomial and community transmission, it is essential to characterize the sensitivity and specificity as well as predictive value of the results knowing well that the 'window period' after acquisition could produce false-negative results. Since RT-PCR tests are being used not only to diagnose infection but also to 'rule out' infection to conserve scarce personal protective equipment and preserve the workforce, it is essential to understand how the predictive value of the test varied with time from exposure and symptom onset to avoid being falsely reassured by negative results from tests done early in the course of infection.

After exposure to an infected patient, over the 4 days of infection before the typical time of symptom onset (day 5), the probability of a false-negative result in an infected person decreased from 100% on day 1%–67% on day 4. On the day of symptom onset, the median false-negative rate was 38%, this decreased to 20% on day 8 (3 days after symptom onset) then began to increase again from 21% on day 9%–66% on day 21. Serial testing in symptomatic patients would almost certainly reduce false-negative rate.[2]

In a recent study by Wolfel *et al.* in 2020, seroconversion was detected by IgG and IgM immunofluorescence using cells that expressed the spike protein of SARS-CoV-2 and a virus neutralization assay using SARS-CoV-2 in 50% of patients by day 7 and in all patients by day 14. All patients showed detectable neutralizing antibodies, the titters of which did not suggest close correlation with clinical course. Whereas, the virus was readily isolated during the 1st week of symptoms from considerable fraction of samples and no isolates were obtained from samples after day 8 in spite of ongoing high viral load.[3]

Simultaneously, performance of RT-PCR and virus isolation in cell culture in a study from Canada demonstrated that infectivity (as defined by growth in cell culture), is significantly reduced when RT-PCR cycle threshold (Ct) values were >24. For every unit increase in Ct, the odds ratio for infectivity decreased by 32%. The high specificity of Ct and symptom onset to test suggested that Ct values >24, along with duration of symptoms >8 days may be used in combination to determine duration of infectivity in patients.[4] It is also noteworthy that the Ct value for detecting live virus may differ based on the context of testing: setting (hospital vs. community); depending on COVID-19 symptoms (asymptomatic vs. symptomatic); severity of infection and duration of symptoms as well as the quality of the testing.

Similar observation had been made earlier from China by Zou *et al.*, who detected COVID-19 infection by RT-PCR for *N* and *Orf 1b* genes in an asymptomatic patient with Ct values of 30-32, when tested on days 7, 10 and 11 after contact. In their study, higher viral load (inversely related to Ct value) were detected soon after symptom onset, with higher viral loads detected in the nose than in the throat.[5]

In another similar study by La Scola *et al.*, they observed a strong correlation between Ct value and sample infectivity in a cell culture model. Based on their data they inferred that with their, patients with Ct values equal or above 34 did not excrete infectious viral particles. It was observed that SARS-CoV-2 was detected up to 20 days after onset of symptoms by PCR in infected patients but that the virus could not be isolated after day 8 in spite of ongoing high viral loads of approximately 10^5 RNA copies/mL, using the RT-PCR system.[6]

In view of different cut-off of Ct reported by Bullad and La Scola *et al.* Binnicker[7] in an invited editorial to caution that though data indicate that PCR positivity was not a reliable surrogate marker for determining the infectious status of COVID-19 patients, the fact that SARS-CoV-2 culture positivity declined with increasing PCR Ct values and SARS-Co-V was not isolated in culture from any sample that had a PCR Ct value >34 (different from the Ct value of 24 reported from Canada). 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, the author suggests that Ct value criteria must be established by each healthcare institution. The common thread in all the studies was that no replicative virus was isolated after 8 days of appearance of symptoms. Thus, it is important to know that the viral load at the end of PCR cycle (>34 Ct) may not represent infectious virus replication. This is an inherent limitation of PCR technology and therefore determination of viral load especially in the late PCR cycles may be subgenomic RNA or parts of the viral genome and should be interpreted with caution and clinically correlated.[4,7]

Another important point with respect to quantitative PCR is as cautioned by Han *et al.*, that quantitative RT-PCR was entirely different from qualitative RT-PCR. Ct values itself could not be directly interpreted as viral load without a standard curve using reference material. Hence, there is lack consensus on using Ct values as indicator of lack of infectivity, but all authors have reported similar results that replication efficient virus is not found after 8 days of appearance of symptoms.[8]

It was also demonstrated by Poon *et al.* that quantitative real-time RT-PCR assay was more sensitive than conventional RT-PCR for the detection of SARS-CoV-2 in samples collected early in the course of the disease. At days 1–3, the quantitative RT-PCR assay was able to detect SARS-CoV-2 in one half of nasopharyngeal samples, by contrast only one-third of these samples were positive by conventional RT-PCR. At days 7–10, the detection rates of the quantitative assay became comparable to those for conventional RT-PCR assay. These results indicated that the real-time quantitative assay was better diagnostic method for early SARS diagnosis.[9]

Multi-centric comparison of quantitative PCR based assays to detect different genes of SARS-CoV-2 has been carried out in seven laboratories and results indicate that most methods reliably detect the sample at 10^{-3} dilution, which was equivalent to ca 5 RNA copies for CDC N1, N2, N3 and E reactions based on the absolute quantifications by One-Step RT-digital droplet (dd) PCR.[10]

The need to determine viral load by quantitative assay was brought out by Pujadas *et al*. from New York who reported on 1145 SARS-CoV-2 positive hospitalised patients and were followed up for 66 days and only 807 (70.5%) patients were alive at the end of the study. The viral load in those who survived had a mean viral load of $\log_{10}$ 5.19 viral copies/ml, while the viral load in those who expired was 6.44 viral copies/ml. There was a statistically significant survival probability between those with a high (defined as greater than 5.557 log10/ml viral load) and those with lower viral load (<5.19 log10/ml). Thus, understanding who is at risk for worse outcomes early in their illness could help clinician, patients with higher viral load and closely monitor them, while those with lower viral load could safety convalesce at home.[11]

Coronaviruses are known to contain one linear RNA but also many subgenomic RNA and these subgenomic RNA are closely associated with the membrane and thus very stable. It is likely that what is being detected for a protracted time after replicative virus has ceased, are the subgenomic RNA. The same also contributes to the fluctuation of negative and weak positive RT-PCR results that are seen later in the course of COVID-19 infections and to certain extend it's related to how samples were taken and treated. Not only are these subgenomic RNA responsible for extended period of PCR positivity but this may in part explain conflicting findings around reinfection as well as discrepancies among diagnostic PCRs detecting targets in different parts of the SARS-CoV-2 genome. Hence, clinical reporting of positive cases with low viremia is important as RNA from RT- PCR may continue to be detected in positive cases long after infection had resolved. This may be possible because inactivated RNA degrades slowly over time and it may still be detected months after infectiousness.[12,13]

It is based on these evidence that most global authorities have shifted from two RT-PCR negatives to make patients eligible for discharge from being asymptomatic for 3 days after 10 days have passed after first appearance of symptoms. Hence, a change from test based to a symptom-based discharge policy has been implemented by all authorities.[14,15]

Looking at the viral load dynamics of SARS-CoV-2, it has been projected that test sensitivity is secondary to frequency and turnaround time in COVID-19 surveillance since control of this pandemic is critically dependent on quickly identifying an infected person and isolating him to interrupt the spread of infection.[16] In this endeavour RT-PCR with its central laboratory positioning and slow turn round time has proved unequal to the task. If instead a rapid antigen test which may have lower sensitivity than RT-PCR, but would give results in minutes would help identify symptomatic patients with higher viral load (and thus more infectious) leading to immediate isolation. Symptomatic patients could be retested after a day or two if negative in the first test, as progression of the disease would have increased the viral load to detectable levels in the next couple of days.

Quantitative RT-PCR could be gainfully employed for risk stratification as well as for detection of asymptomatic patients with lower viral load, but they too need to be isolated to stop the spread of infection.

Clinical sensitivity of PCR decreased with days post symptom onset with >90% clinical sensitivity during the first 5 days after symptom onset, 70%–71% from days 9 to 11, 50% and 30% at day 21. In contrast, serological sensitivity increased with days post symptom onset with >50% of patients seropositive by at least one antibody isotype after day 7, >80% after day 12, and 100% by day 21. Therefore, although serology has no role in rapid diagnosis followed by isolation of infected persons as antibodies appear only after 7 days of symptoms, at a time when the patient maybe at the end of his infectious stage. However, many reports have indicated that if used after 14 days of infection, serology may help play an important complementary role in completing the diagnostic evaluation of an infected person.[17-19] PCR and serology are complimentary modalities that require time-dependent interpretation. Superimposition of sensitivities over time indicates that serology can function as a reliable diagnostic aid indicating recent or prior infection.

The construction of a pseudo virus which expresses spike protein on the surface but does not contain the RNA of SARS-CoV-2, should make the possibility of performing neutralizing antibody tests in clinical diagnostic laboratories a real possibility[21] and the growing understanding of the role of T cells may help us is devising an evidence-based best strategy to diagnose, treat and contain COVID-19 infection and stop the pandemic.[21,22]

In conclusion, quantitation of SARS-CoV-2 viral load by RT-PCR detection is helpful for patient management considering its merit and simultaneously limitation of detecting minute quantities of RNA. Thus, COVID-19 RT-PCR tests should be reported with caution after clinical correlation, as test may not detect infectious virus especially in asymptomatic cases with low viremia.

Financial support and sponsorship

Conflicts of interest
There are no conflicts of interest.

References

1. He Xi, Lau E, Wu P, Deng X, Wang J, Hao X, *et al*. Temporal dynamics in viral shedding and transmissibility of Covid 19. Nat Med 2020;26:672-5B.
2. Kucirka LM, Lauer SA, Laeyendecker O, Boon D, Lessler J. Variation in false-negative rate of reverse transcriptase polymerase chain reaction-based SARS-CoV-2 tests by time since exposure. Ann Intern Med 2020;173:262-7.
3. Wölfel R, Corman VM, Guggemos W, Seilmaier M, Zange S, Muller MA, *et al*. Virological assessment of hospitalized patients with Covid 19. Nature 2020;581:465-9.
4. Bullard J, Dust K, Funk D, Strong JE, Alexander D, Garnett L, *et al*. Predicting infectious SARS-CoV-2 from diagnostic samples. Clin Infect Dis. 2020 May 22:ciaa638. doi: 10.1093/cid/ciaa638. Epub ahead of print. PMID: 32442256; PMCID: PMC7314198.
5. Zou L, Ruan F, Huang M, Liang L, Huang H, Hong Z, *et al*. SARS-CoV-2 viral load in upper respiratory specimens of infected patients. N Engl J

Med 2020;382:1177-9.

6. La Scola B, Le Bideau M, Andreani J, Hoang VT, Grimaldier C, Colson P, *et al*. Viral RNA load as determined by cell culture as a management tool for discharge of SARS-CoV-2 patients from infectious disease wards. Eur J Clin Microbiol Infect Dis 2020;39:1059-61.

7. Binnicker MJ. Can the SARS-CoV-2 PCR Cycle Threshold Value and Time from Symptom Onset to Testing Predict Infectivity? Clin Infect Dis. 2020 Jun 6:ciaa735. doi: 10.1093/cid/ciaa735. Epub ahead of print. PMID: 32504529; PMCID: PMC7314221.

8. Han MS, Byun JH, Cho Y, Rim JH. RT-PCR for SARS-CoV-2: quantitative versus qualitative. Lancet Infect Dis. 2020 May 20:S1473-3099(20)30424-2. doi: 10.1016/S1473-3099(20)30424-2. Epub ahead of print. PMID: 32445709; PMCID: PMC7239624.

9. Poon LL, Chan KH, Wong OK, Cheung TK, Ng I, Zheng B, *et al*. Detection of SARS coronavirus in patients with severe acute respiratory syndrome by conventional and real-time quantitative reverse transcription-PCR assays. Clin Chem 2004;50:67-72.

10. Muenchhoff M, Helga M, Hans N, Natascha GK, Dieter H, Annemarie B, *et al*. Multicentre comparison of quantitative PCR-based assays to detect SARS-CoV-2, Germany, March 2020. Euro Surveill.2020;25(24) https://doi.org/10.2807/1560-7917.ES.2020.25.24.2001057.

11. Pujadas E, Chaudhry F, McBride R, Richter F, Zhao S, Wajnberg, *et al*. SARS-CoV-2 viral load predicts COVID-19 mortality. medRxiv preprint, June 2020 medRxiv 2020.06.11.20128934; doi: https://doi.org/10.1101/2020.06.11.20128934.

12. 12. Alexandersen S, Chamings A and Bhatta TR. SARS-CoV-2 genomic and subgenomic RNA in diagnostic samples are not an indicator of active replication. medRxiv preprint doi: https://doi.org/10.1101/2020.06.01.20119750. 2020.

13. World Health Organization. Transmission of SARS-CoV-2: Implications for Infection Prevention Precautions. World Health Organization; 9 July, 2020.

14. Centre for Disease Control. Duration of Isolation and Precautions for Adults with COVID-19. Atlanta: Centre for Disease Control; 2020.

15. Ministry of Health, Government of India. Revised Discharge policy for COVID-19. Available at: https://www.mohfw.gov.in/pdf/Revised dischargePolicyforCOVID19.pd. [Last accessed on 2020 Aug 10].

16. Larremore DB, Wilder B, Lester E, Shehata S, Burke JM, Hay JA, *et al*. Test sensitivity is secondary to frequency and turnaround time for COVID-19 surveillance. Medrxiv : the Preprint Server for Health Sciences. 2020 Jun. DOI: 10.1101/2020.06.22.20136309.

17. Lan L, Xu D, Ye G, Xia C, Wang S, Li Y, *et al*. Positive RT-PCR test results in patients recovered from COVID-19. JAMA 2020;323:1502-3.

18. Guo L, Ren L, Yang S, Xiao M, Chang , Yang F, *et al*. Profiling early humoral response to diagnose novel coronavirus disease (COVID-19). Clin Infect Dis 2020;71:778-85.

19. Miller TE, Beltran W, Bard AZ, Gogakos T, Anahtar MN, Gerino M, *et al*. Clinical sensitivity and interpretation of PCR and serological COVID-19 diagnostics for patients presenting to the hospital. FASEB J. 2020 Aug 28:10.1096/fj.202001700RR. doi: 10.1096/fj.202001700RR. Epub ahead of print. PMID: 32856766; PMCID: PMC7461169.

20. Tan CW, Chia WN, Qin X, Liu P, Chen MI, Tiu C, *et al*. A SARS-CoV-2 surrogate virus neutralization test based on antibody-mediated blockage of ACE2–spike protein–protein interaction. Nat Biotechnol 2020;38:1073-8.

21. Le Bert N, Tan AT, Kunasegaran K, Tham CY, Hafezi M, Chia A, *et al*. SARS-CoV-2-specific T cell immunity in cases of COVID-19 and SARS, and uninfected controls. Nature 2020;584:457-62.

22. Odak I, Barros-Martins J, Bošnjak B, Stahl K, David S, Wiesner O, *et al*. Reappearance of effector T cells is associated with recovery from COVID-19. EBioMedicine 2020;57:102885.