Real-time RT-PCR for COVID-19 diagnosis: challenges and prospects

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Abstract

COVID-19 impacts global public health, economy, education, tourism/hospitality and sports; rapid and accurate testing of clinical samples dictate effective response. So far, the real-time reverse transcriptase-polymerase chain reaction (RT-qPCR) is the assay of choice for COVID-19 diagnosis considering its rapidity and accuracy in informing on active coronavirus (CoV) infection. Presently, several RT-qPCR protocols with differing sensitivity/specificity are used for performing this assay; some of them are known to have generated debatable test results to constitute challenges worthy of consideration. This review provides a critical assessment of various published works on RT-qPCR assays used for COVID-19 diagnosis with their different indicators of positivity i.e., cycle threshold (Ct) cut-off values. Knowledge of diagnostic tests for COVID-19 is still evolving and, as a prospect, underscores the need for local validation of positive-negative Ct cut-off values when establishing RT-qPCR assays for SARS-CoV-2 detection.

Commentary

Following the outbreak of a severe acute respiratory disease caused by a newly emerged virus among humans in Wuhan city, Hubei Province, China in December, 2019, the causal agent was initially called 2019-novel coronavirus (2019-nCoV) while the associated respiratory illness was officially designated Coronavirus Disease 2019 (COVID-19). However, with the availability of the complete genome sequence of this virus, the Coronavirus Study Group (CSG) of the International Committee on Taxonomy of Viruses renamed it severe acute respiratory syndrome-coronavirus type 2 (SARS-CoV-2) based on comparative analysis with the 2002 SARS-CoV [1]. The virus is a positive-sense, single-stranded RNA virus in the genus Betacoronavirus, family Coronaviridae. As of 3 June, 2020, the number of laboratory-confirmed cases of COVID-19 reported worldwide was 6,432,370 with 385,991 deaths [2]. According to Sharfstein et al. [3], the pivot of an effective human response to this COVID-19 pandemic is early, rapid and accurate testing of clinical samples from suspected and probable cases. The diagnostic test is strategic to interrupting transmission of SARS-CoV-2 as it identifies infected persons for appropriate clinical management in isolation facilities, contact-tracing and guides provision of real-time epidemiologic/surveillance information to the public for infection prevention and control (IPC) purposes. There are two major approaches to diagnosis of COVID-19: (1) laboratory tests that detect the SARS-CoV-2 (or its RNA or protein) in clinical samples of infected persons, including nasal/nasopharyngeal swabs, sputum, bronchoalveolar lavage fluid, faeces, and saliva; (2) laboratory tests that detect evidence of host immune response to the virus [4].

The real-time reverse transcriptase-polymerase chain reaction (RT-qPCR) is the molecular-based assay used globally to detect SARS-CoV-2 RNA in clinical samples of patients manifesting COVID-19 compatible signs and symptoms (fever, fatigue, chills, dry cough, sneezing, dyspnea, myalgia, lymphopenia and radiographic findings of pneumonia) [5]. This assay has the capacity to detect and measure minute amounts of nucleic acids in different sample types from various sources (environmental or...
It is also noteworthy that different RT-qPCR kits for SARS-CoV-2 detection have different reagents, and when the same, their concentrations in the reaction mixture may be different as seen with the assays/protocols developed by the US CDC, China CDC, Charité-Universitätsmedizin Berlin, Germany and Hong Kong University [5,9,10]. As observed by Vogels et al. [7] who critically compared the analytical efficiencies and sensitivities of these four RT-qPCR assays, each of them is likely to have different sensitivities and, possibly, different accuracies for the reasons that all the primer-probe sets for these four assays (developed by different scientists and listed by the WHO) can be used to detect SARS-CoV-2 as long as the limitations of each assay are recognized. However, they noted that the different assays have clear variations in their abilities to differentiate between true negatives and positives when low titer of SARS-CoV-2 is present in a given sample. This becomes crucial when samples from asymptomatic COVID-19 suspects are tested, in which case, the CoV RNA may be quite low to indicate early viral replication.

Although the RT-qPCR detection of SARS-CoV-2 RNA does not necessarily mean viable virus is present in the samples, appropriate interpretation of the diagnostic test results is guided by the clinical manifestations and epidemiologic history of the patient. A positive laboratory result therefore means the tested person has active SARS-CoV-2 infection (even if clinical signs/symptoms are absent) and hence, considered infectious to susceptible human contacts [3]. Conversely, two consecutive negative test results indicate that the individual has no detectable SARS-CoV-2 RNA as at the time of sampling and is considered not infected or infectious. However, when history of the patient points to probable exposure to the CoV, self-isolation for a period of 14 days is recommended for manifestation of pertinent signs and symptoms, otherwise, the patient is discharged as uninfected [8]. It has however been suggested that negative results do not exclude SARS-CoV-2 infection [3]. Any positive/infected person in isolation facility undergoing clinical management can only be discharged as having recovered from COVID-19 when RT-qPCR returns negative results twice from two or more clinical samples collected, at least, 24 hours apart [4,8]. This further highlights the importance of the assay. With respect to sample types, we observed in this review that different clinical samples displayed different sensitivities for SARS-CoV-2 detection. For instance, samples from the lower respiratory tract that detect the SARS-CoV-2 N1, N2 and RdRP genes, and human RNase P gene for each COVID-19 clinical sample. However, many centers and referral laboratories, guided by the US CDC, require three RT-qPCRs (that detect N1, N2 and RNase P genes) per sample. Nevertheless, diagnostic laboratories all over the world are caught between which of the existing RT-qPCR assays to adopt [7]. Other factors that may impact accuracy/reliability of RT-qPCR include human errors in sample processing/preparation and testing procedure, presence of RT and PCR inhibitors in reaction mixture, and contamination of sample by RNase. These can be overcome by ensuring that sample/assay transport are done by trained healthcare workers, assays are conducted by personnel with demonstrable expertise and experience in RT-qPCR, and inclusion of RNase inhibitor in the assay. As an RNA virus with high propensity for mutational changes in the genome, evolution of SARS-CoV-2 with the emergence of strains having diverse replication efficiencies and clinicopathological manifestations over the course of the pandemic, could be another reason accounts for the divergent in analytical sensitivity of available RT-qPCR assays and consequently, the divergent test results obtained. So far, different strains of the virus have been identified. Although at the moment, several RT-qPCR assays for COVID-19 diagnosis are available, none is perfect yet. It is advocated that positive-negative Ct cut-off values should be locally validated when establishing RT-qPCR assays for SARS-CoV-2 detection [7].

### Competing interests

The authors declare no competing interests.
### Authors’ contributions

Both authors have read and agreed to the final manuscript.

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