Short Report

Journal of Iranian Medical Council, Volume 4, Issue no. 2
http://dx.doi.org/10.18502/jimc.v4i2.6465

Factors Associated with False Negative and False Positive RT-PCR Test Results for COVID-19 Detection

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Received: Jan 23 2021
Accepted: Mar 23 2021

Citation to this article:

Hakimi Sh. Factors Associated with False Negative and False Positive RT-PCR Test Results for COVID-19 Detection. *J Iran Med Counc*. 2021;4(2):110-2.

Abstract

The Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) has spread rapidly and developed the current pandemic and the stressful lifestyle in addition with extreme pressure on people was the consequence of its increasing mortality rate. Since COVID-19 is highly infectious, it is crucial to diagnose the disease timely and initiate preventive measures to control the epidemic. Therefore, the need for accurate detection of this virus has been increased dramatically. Real-Time reverse-transcription Polymerase Chain Reaction (RT-PCR) tests are considered a gold standard to detect SARS-CoV-2 RNA. Besides, the recent pandemic has posed the most serious challenge in PCR applications to date. Although RT-PCR has great accuracy, some factors can reduce the efficiency of this test. Time of testing and type of sample are typical elements that may cause false negative results. Furthermore, false positive cases would be the result of contamination and unoptimized primers. In this paper, the relevant factors creating false positive and false negative results have been investigated in depth to increase the awareness of clinicians.

Keywords: COVID-19, False negatives, False positives, Pandemic, RT-PCR test, SARS-CoV-2

Introduction

In the current pandemic, there is a necessity to rule out infection, identify people in need of care escalation, or to test for past infection and immune response (1). The availability of accurate laboratory tools for COVID-19 is essential for case identification, contact tracing, and optimization of infection control measures (2). Even though the Real-Time reverse-transcription Polymerase Chain Reaction (RT-PCR) test has become the standard method for the diagnosis of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) infection, false-negative and false-positive rates have been reported (3).The accuracy of this test is vital because accurate testing allows suspected patients to be detected which assists people who might need treatment or who need to isolate themselves to prevent the spread of infection. Besides, correct identification of people who have previously had COVID-19 is vital in measuring transmission potential, evaluating the success of public health interventions (like isolation), and potentially in identifying individuals' immunity (the future possibility of providing immunity by antibodies) (4). It is noteworthy to mention that there is no diagnostic technique with 100% sensitivity and specificity (5); therefore, in this article, for improving the accuracy of COVID-19 tests, an attempt was made to investigate the possible factors that may cause false negatives and false positives leading to over- or under-diagnosis.

Testing at an inappropriate time and false negatives

Time of testing could be a potential source of false negative results in real-time PCR since viral load and exposure time are associated to false negative rate for RT-PCR. The fact of a matter is that time of exposure is important and testing is often done on the basis of time of symptom onset. However, roughly 8 days after exposure and 3 days after onset of symptoms, viral load is enough to be detected and testing sooner may lead to false negative results. Thus, clinicians should consider this factor to reduce the probability of false negative results (6).

Improper type of sample and false negative results

Rapid spread of coronavirus disease 2019 (COVID-19) which caused the current pandemic has put pressure on clinical board to be meticulous about type of sample for detection of this virus. Among nasal swabs, throat swabs, sputum and Bronchoalveolar Lavage Fluid (BALF), the most accurate sample for laboratory diagnosis is sputum followed by nasal swabs, whilst throat swabs are not recommended for the diagnosis. In addition, detection of viral RNAs in BLAF is essential for the diagnosis and monitoring of viruses in severe cases (7). Furthermore, the stability of nasal swab sample containing the virus is more than blood and saliva samples and it provides higher accuracy of detection (8).Therefore, the proper sample plays pivotal role to indicate virus and minimize false negative results.

Contamination and false positive results

False positive results represent people being isolated when they are well, indicating that ultimately all contact tracing efforts were futile. The common false positives are associated with the central source of contamination. Technical problems such as contamination during sampling (*e.g.*, a swab accidentally touches a contaminated gloves or surface). Furthermore, contamination by PCR amplicons would be another probable reason of false positives because the PCR amplification process produces millions of copies of the DNA target (amplicon) that create false positives in subsequent PCR reactions. If a testing lab is accidently contaminated with amplicon, it may lead to sporadic false positives. Besides, sample cross contamination (samples can be contaminated by a positive sample analyzed at the same time), contamination of reagents, and cross-reactions with other viruses or genetic material could also be responsible for false positive results (9,10).

Unoptimized primers and false positive results

There is a possibility of obtaining a false-positive result when primers are not verified during the development of primer sets using real-time PCR assays to detect SARS-CoV-2. Hence, this is a very crucial issue when the sensitivity relies on the primer specificity (11). It should be pointed out that the final concentration of the primer set in the PCR mixture is critical for target-specific PCR. At concentrations greater than the optimal concentration, primers can form dimers, primer-dimers, as the by-product of PCR and interfere

with target-specific PCR. When confirming the PCR result with electrophoresis, the efficacy of the reaction needs to be checked based on whether the band size of the amplicon and the amount of template added are appropriate or not. Therefore, the absence of a primer–dimer band indicates that the primer set itself is an optimal primer set and there would not be any false positive reports (12).

References

1. Tahamtan A, Ardebili A. Real-time RT-PCR in COVID-19 detection: issues affecting the results. Expert Rev Mol Diagn 2020;20(5):453-4.

2. Gostin LO. Public health emergency preparedness: globalizing risk, localizing threats. JAMA 2018;320(17):1743-4.

3. Jia X, Xiao L, Liu Y. False negative RT-PCR and false positive antibody tests-Concern and solutions in the diagnosis of COVID-19. J Infect 2021 Mar;82(3):414-51.

4. Deeks JJ, Dinnes J, Takwoingi Y, Davenport C, Spijker R, Taylor-Phillips S, et al. Antibody tests for identification of current and past infection with SARS-CoV-2. Cochrane Database Syst Rev 2020;6(6):Cd013652.

5. Watson J, Whiting PF, Brush JE. Interpreting a covid-19 test result. BMJ 2020;369:m1808.

6. 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(4):262-7.

7. Yang Y, Yang M, Shen C, Wang F, Yuan J, Li J, et al. Laboratory diagnosis and monitoring the viral shedding of 2019-nCoV infections. MedRxiv. 2020.

8. Fang X, Mei Q, Yang T, Li L, Wang Y, Tong F, et al. Low-dose corticosteroid therapy does not delay viral clearance in patients with COVID-19. J Infect 2020;81(1):147-78.

9. Surkova E, Nikolayevskyy V, Drobniewski F. False-positive COVID-19 results: hidden problems and costs. Lancet Respir Med 2020;8(12):1167-8.

10. Cohen AN, Kessel B. False positives in reverse transcription PCR testing for SARS-CoV-2. medRxiv. 2020.

11. Won J, Lee S, Park M, Kim TY, Park MG, Choi BY, et al. Development of a Laboratory-safe and Low-cost Detection Protocol for SARS-CoV-2 of the Coronavirus Disease 2019 (COVID-19). Exp Neurobiol 2020;29(2):107-19.

12. Park M, Won J, Choi BY, Lee CJ. Optimization of primer sets and detection protocols for SARS-CoV-2 of coronavirus disease 2019 (COVID-19) using PCR and real-time PCR. Exp Mol Med 2020;52(6):963-77.