LETTER TO THE EDITOR | COVID-19 TEST

Reducing False Negative PCR Test for COVID-19

Fatemeh Bahreini, PhD;[1]✉ **Rezvan Najafi,** PhD[2]; **Razieh Amini,** PhD[2]; **Salman Khazaei,** PhD[3]; **Saeid Bashirian,** PhD[4]

[1]Department of Molecular Medicine and Genetics, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran; [2]Research Center for Molecular Medicine, Hamadan University of Medical Sciences, Hamadan, Iran; [3]Modeling of Non-communicable Diseases Research Center, Hamadan University of Medical Sciences, Hamadan, Iran; [4]Department of Public Health, School of Public Health, Hamadan University of Medical Sciences, Hamadan, Iran

✉Corresponding author email: f.bahreini@yahoo.com

ABSTRACT

As the SARS-CoV-2 (COVID-19) pandemic spreads rapidly, there is need for a diagnostic test with high accuracy to detect infected individuals especially those without symptoms. Real-time polymerase chain reaction (RT-PCR) is a common molecular test for diagnosing SARS-CoV-2. If some factors are not taken into consideration when performing this test, it can have a relatively large number of false negative results. In this article, we discuss important considerations that could lead to false negative test reduction.

Key words: • SARS-CoV-2 • COVID-19 • Real time polymerase chain reaction • RT-PCR test • Diagnosis • False negatives • Genetics • Emerging disease

To the Editors

As the SARS-CoV-2 (COVID-19) pandemic spreads rapidly, development of diagnostic test to detect infected individuals, especially asymptomatic patients is vital. The real time polymerase chain reaction (RT-PCR) is the suggested gold standard for viral detection. This method is rapid, sensitive and specific, although in the real-world the risk of false negative of Real time-PCR for some reasons should not be forgotten as reported in recent studies.[1-4] A false negative result means someone who is actually infected has incorrect negative test. Researchers at John Hopkins University declared that the false negative ratio of RT-PCR test in patients infected with COVID-19 is approximately 1 in 5.[5] We discuss several probable factors associated with these false negative results. We hope it will be useful in prevention and improvement of COVID-19 diagnosis.

Genetic Diversity

Genetic diversity refers to the diversity within species, in this case SARS-CoV2. Evolution affects viral RNA genomes by creating variation in viral sequences;[6, 7] it is therefore vital that conserved regions of viral genomes be used for primer or probe designing. In spite of attempting to achieve this goal, due to rapid evolution, mismatches between target regions and primers can result in false negatives. Therefore, simultaneous targeting of multiple conserved regions of viral genome should be considered.

Sampling Errors

These are errors that can occur during sample collecting, transporting, and handling of RNA. Care in taking throat and nasal swabs samples can significantly improve test accuracy. At times sample collection is inadequate or health workers do not insert nose swabs deep enough to collect a sample with a sufficient viral load. In order to improve testing accuracy, sample collection should be carried out by an experienced laboratory technologist or trained healthcare professional. Swabs should be placed in transport medium immediately after collection. In addition, the time between sample collection and test performance should not be too long. The sample should be stored at 2-8°C for maximum of 72 hours. If transporting is not possible during 72 hours, they should be stored at -70°C to prevent viral RNA degradation.

Sample Types

This refers to the types of samples that can be used to diagnose of COVID-19. Finding the best sample type in the best time during infection can lead to the best result with minimum false negatives. One study found that sputum is accurate for diagnosis, followed by nasal swabs.[8] Another study suggested using sputum, nasal swabs and throat swabs in early stages.[1] A previous study showed minimal or no viral replication in stool sample.[9] This topic is an evolving topic; the most accurate sample type will become more apparent as our knowledge of COVID-19 improves. Viruses can migrate to the lower respiratory tract over time. Therefore, nasal swab specimens may be negative in such conditions. Considering time of exposure and symptom onset can guide a healthcare worker to target correct anatomical site for choosing the best sample. Therefore, it can be suggested that nasopharyngeal and oropharyngeal swabs are appropriate to detect early infection in early stages.

Viral Load

This refers to the amount of virus in an infected person's nasal swab. It is important to know when an infected individual has an optimum viral load. Wolfel et al. reported that maximum COVID-19 replication

in the throat is five days after symptom onset.[9] A recent study reported that the viral load between asymptomatic and severe cases is similar,[10] thus suggesting the potential of infection transmission by both groups.

Optimal Time

RT-PCR false negatives are related to the viral load and virus exposure time. A study has reported this issue in a literature review and pooled analysis,[5] showing that the probability of a false negative RT-PCR test on day 1 after infection is 100% and it decreased respectively to 67% on day 4, 20 % on day 8, and increased again to 66% on day 21 after infection. In other words, the risk of false negative will be highest for RT-PCR when the test was used too early. In this situation, researchers suggest consecutive testing may be needed to improve adequate performance of the test.[5] A study from John Hopkins University reported that the incubation time for COVID-19 is about a week from exposure.[11] According to the finding of this study, time of sampling is a major factor that can reduce false negative ratio. We cannot rule out infection according to a negative test when a patient has symptom. In such cases, repeating the test increases our confidence.

Conclusion and Global Health Implications

Choosing sample type based on exposure time, the appropriate sampling time, and staff experience can be important in reducing the false negative RT-PCR test.

Compliance with Ethical Standards

Conflicts of Interest: *The authors declare no competing interest.* **Acknowledgement:** *We sincerely thank the healthcare staff.*

References

1. Tahamtan A, Ardebili A. Real-time RT-PCR in COVID-19 detection: issues affecting the results. *Expert Rev Mol Diagn.* 2020 May;20(5):453-454.

2. Shen M, Zhou Y, Ye J, Ahmed A, AL-maskri A, Kang Y, et al. Recent advances and perspectives of nucleic acid detection for coronavirus. *J Pharm Anal.* 2020 Apr;10(2):97-101.

3. Wan Z, Zhang Y, He Z, et al. A melting curve-based

multiplex RT-qPCR assay for simultaneous detection of four human corona-viruses. *Int J Mol Sci.* 2016 Nov 23;17(11):1880.

4. Noh J, Yoon S, Kim D, Lee M, Kim J, Na W, et al. Simultaneous detection of severe acute respiratory syndrome, Middle East respiratory syndrome, and related bat coronaviruses by real-time reverse transcription PCR. *Arch Virol.* 2017 Jun;162(6):1617-1623.

5. Kucirka L, Lauer S, 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 Aug 18;173(4):262-267.

6. Phan T. Genetic diversity and evolution of SARS-CoV-2. *Infect Genet Evol.* 2020 Jul;81:104260.

7. Shen Z, Xiao Y, Kang L, et al. Genomic diversity of severe acute respiratory syndrome-coronavirus 2 in patients with coronavirus disease 2019. *Clin Infect Dis.* 2020 Jul 28;71(15):713-720. doi:10.1093/cid/ciaa203.

8. 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:[preprint].

9. Wolfel R, Corman V, Guggemos W, Seilmaier M,

Zange S, Müller M, et al. Virological assessment of hospitalized patients with COVID-2019. *Nature.* 2020 May;581(7809):465-469.

10. 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 Mar 19;382(12):1177-1179.

11. Lauer SA, Grantz KH, Bi Q, et al. The incubation period of coronavirus disease 2019 (COVID-19) from publicly reported confirmed cases: estimation and application. *Ann Intern Med.* 2020 May 5;172(9):577-582. doi:10.7326/M20-0504.