

Available online at www.mchandaids.orgINTERNATIONAL JOURNAL of
MATERNAL and CHILD HEALTH and AIDS
ISSN: 2161-864X (Online)
ISSN: 2161-8674 (Print)
DOI: 10.21106/ijma.571**LETTER TO THE EDITOR | COVID-19 TEST****Considerations about Reducing False-Negative PCR Test for COVID-19****Alireza Ghannad Sabzevari, PhD¹; Mohsen Nikbakht, PhD^{2✉}**¹Department of Life Science Engineering, Faculty of New Sciences & Technologies, University of Tehran, Tehran, Iran; ²Hematology Oncology and Stem Cell Transplantation Research Center, Shariati Hospital, North Karegar Ave., Tehran, Iran✉ **Corresponding author email:** m-nikbakht@sina.tums.ac.ir**ABSTRACT**

Polymerase chain reaction (PCR) for the detection of nucleic acids is the gold standard test for the diagnosis of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). However, there is the probability of false-negative results with this test, which poses a threat to public health. Here, we highlight some important factors that should be considered for reducing the false-negative results of the SARS-CoV-2 PCR test.

Keywords: • Polymerase Chain Reaction • PCR Test • SARS-CoV-2 • COVID-19 • False-Negative • Diagnosis

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To the Editors

We are writing about the article by Bahreini et al.¹ The authors discussed several probable factors associated with the false-negative results of polymerase chain reaction (PCR) test for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), including genetic diversity, sampling error, sample type, viral load, and sampling time. However, some factors were not considered by the authors. Here, we discuss other important factors that may be responsible for the false-negative results of the SARS-CoV-2 PCR test.

Thermal Inactivation of SARS-CoV-2

Heat treatment of specimens at 56 °C for 30 minutes has been recommended to inactivate SARS-CoV-2 before nucleic acid testing. However, this treatment process can damage the single-stranded RNA, thereby reducing the detectable amount of SARS-CoV-2 in the PCR assay. Pan et al.² reported that 46.7% of weak-positive specimens

tested PCR-negative after thermal inactivation. In comparison, chemical inactivation by guanidinium had less effect on PCR results, with 13.3% false-negatives. Therefore, thermal inactivation of SARS-CoV-2 may dramatically reverse PCR test results from positive to negative when the viral load is low.

Targets of SARS-CoV-2 Genome

PCR assays target different regions of the SARS-CoV-2 genome, including ORF1ab (Open Reading Frames), N (Nucleocapsid), S (Spike), E (Envelope), and RdRp (RNA depended-RNA polymerase) genes. This target difference can affect the accuracy of the SARS-CoV-2 PCR test. Niu et al.³ performed PCR assays targeting the ORF1ab, N, E, and RdRp genes of SARS-CoV-2. The primer sets of the E and RdRp genes showed cross-reactions with SARS-CoV, while the primer sets of the ORF1ab and N genes showed no positive reactions. Mollaei et al.⁴ performed PCR assays targeting the ORF1ab, N, S, E, and RdRp genes

of SARS-CoV-2. The primer sets of the ORF1ab and N genes showed the highest sensitivity and specificity and the least false-negatives. In contrast, the primer sets of the E gene showed the lowest sensitivity and the most false-negatives. Therefore, the ORF1ab and N targets seem to be more reliable than the E, RdRp, and S targets. Besides, dual-target assays can further reduce the false-negative results originating from the gene targets.

PCR Method Sensitivity

PCR method sensitivity is of high importance when the viral load is low. Lu et al.⁵ used a digital PCR (dPCR) instrument, DropX-2000, and assay kits to detect SARS-CoV-2 in samples with low viral load. This dPCR assay with a 10-fold lower detection limit allowed a more accurate diagnosis of SARS-CoV-2 with fewer false-negatives compared to the official PCR assay. Huang et al.⁶ utilized a fluorescent probe and a custom CRISPR Cas12a/gRNA complex to detect SARS-CoV-2 target sequences amplified by standard PCR. As a result, the detection sensitivity was improved from 5 to 2 target copies per sample, and the invalid results were eliminated when compared to conventional PCR. Garg⁷ reported that six coronavirus disease 2019 (COVID-19) patients consistently tested negative by conventional PCR, while they tested positive by multiplex PCR with a BioFire Respiratory Panel 2.1. Therefore, highly-sensitive PCR methods can significantly reduce the false-negative results caused by insufficient SARS-CoV-2 load.

Co-Infection with Other Viruses

There are reports indicating that co-infection with other viruses may cause the false-negative results of the SARS-CoV-2 PCR test. Lai et al.⁸ stated that co-infection with influenza A, one of the most common co-infective viruses among COVID-19 patients, may cause initial false-negative PCR results for SARS-CoV-2. In addition, Zhao et al.⁹ reported that a COVID-19 patient with a history of hepatitis C virus (HCV) and human immunodeficiency virus type 1 (HIV-1) co-infection continuously tested negative by PCR on different specimens at various times and

showed delayed antibody responses against SARS-CoV-2. Therefore, SARS-CoV-2 infection cannot be ruled out based on a negative PCR test when a patient is co-infected with other viruses, such as influenza A, HCV, and HIV.

Conclusion and Global Health Implications

In this letter, we offer some considerations to minimize the false-negative results of the SARS-CoV-2 PCR test with the following suggestions: (1) avoid thermal inactivation of SARS-CoV-2; (2) dual-target the ORF1ab and N regions of the SARS-CoV-2 genome; (3) apply highly-sensitive PCR methods; and (4) further evaluate SARS-CoV-2 PCR-negative patients co-infected with other viruses.

Compliance with Ethical Standards

Conflicts of Interest: The authors declare no competing interests; **Financial Disclosure:** Nothing to declare. **Funding/Support:** There was no funding for this study. **Ethics Approval:** Not applicable. **Acknowledgments:** None. **Disclaimer:** None.

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