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COMMENTARY

False negative results and tolerance limits of SARS-CoV-2 laboratory tests

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Commentary

Early treatment, patient isolation, and contact tracing to decrease SARS-CoV-2 transmission depend on timely and accurate detection of cases [1]. As such, the quality and diagnostic accuracy of laboratory tests are crucial for the management of this pandemic. It has been reported that 79% of detected cases originate from unidentified cases [2]. False-negative test results and the general tolerance paradigm for diagnostic test performance constitute two major hurdles that are disrupting the contribution of laboratory tests to SARS CoV-2 diagnosis.

False-negative results from real-time polymerase chain reaction (RT-PCR) testing arise mostly due errors occurring in the pre-analytical phase, such as: misidentification, too early or too late sampling, inadequate sample quality, specimen contamination, improper handling and transportation of specimens, sampling site, low viral load, and the existence of PCR inhibitors [3–5]. Thermal inactivation is used to inactivate SARS-CoV-2 before nucleic acid testing to protect laboratory staff from contamination. However, this pretreatment can increase the cycle threshold (Ct) and cause false-negative test results. Thus, novel safe methods to protect laboratory staff need to be established [6]. On the other hand, laboratory scientists should be alert to probable mutations that may affect the detection of SARS-CoV-2 RNA [5]. Antiviral drugs may also keep the viral load at low levels and cause false-negative test results [7]. Nevertheless, the sampling site and sampling time seem to be two primary sources of pre-analytical errors.

A recent meta-analysis revealed that the sample positivity rates of the upper and lower respiratory tract with RT-PCR were 40.6% and 71.3%, respectively [8]. False-negative result rates of RT-PCR were reported as 38%, 20%, 21%, and 66% for 0, 8, 9, and 21 days respectively, from symptoms onset [9]. The viral load can also be lower than the limit of quantitation (LoQ) at the early [10] and final stage of infection [5]. Moreover, Padoan et al. showed that the serological test results (IgG and IgM) of Covid-19 patients were negative in the first five days from fever onset. The positivity of the serological tests could reach the highest rate at the 12th day [11].

While analytical sensitivities of commercial tests are well defined in emergency use authorization documents, there is a gap in knowledge of clinical sensitivities [12]. Basu et al. reported discordant results of analytical performance between package insert and laboratory evaluations [,13]. In a systematic review and meta-analysis, pooled sensitivities of chemiluminescent immunoassays (CLIAs)), lateral flow immunoassays (LFIAs), and enzyme-linked immunosorbent assays (ELISAs) were reported as 97.8%, 66%, and 84.3%, respectively [14]. Furthermore, in an analytical sensitivity study, an RT-PCR primer-probe set used for confirmation of diagnosis could not detect SARS-CoV-2 RNA lower than 1,000 copies per microliter, contrary to other products [15]. Therefore, laboratories should verify the accuracy of SARS-CoV-2 tests before usage [11].

While tests with high sensitivity are more suitable for population screening, more specific tests are essential to confirm the diagnosis. SARS-CoV-2 laboratory tests' performances seem to have higher specificity values than sensitivity [9]. However, higher sensitivity values are needed in pandemics to obviate false-negative results that can halt contact tracing, treatment, and isolation of COVID-19 patients. The classical statistical approach of 95% as a good indicator should not be accepted in pandemics. For example, in one million tests, 5% and 1% tolerance correspond to 50,000 and 10,000 false-negative patients, respectively. Thus, reducing the tolerance limit from 5% to 1% leads to the detection of additional 40,000 cases. In contrast, the industry's world-class acceptable error or defect rate is 6 sigma, which means less than four defects

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List of Abbreviations: SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2, LoD: the limits of detection, RT-PCR: Real-time polymerase chain reaction, Ct: cycle threshold, LoQ: limit of quantitation, CLIA: chemiluminescent immunoassay, LFIA: Lateral flow immunoassay, ELISA: Enzyme-linked immunosorbent assay.

per million opportunities [16]. Additionally, The Clinical Laboratory Standards Institute document EP17-A2 accepts the LoD as the lowest concentration level with a detection rate of 95% for positive results [17]; however, this LoD is not acceptable for diseases such as those caused by SARS-CoV-2.

The classical statistical tolerance or acceptable rates mentioned in some national or international standards may impede scientists from finding effective solutions to pandemics because manufacturers usually consider such rates as 'acceptable' and manufacture laboratory tests accordingly. We should be aware that laboratory tests' diagnostic accuracy, including RT-PCR used for diagnosing SARS-CoV-2, is not at the desirable level. Generally, 3 sigma is regarded as the minimum quality threshold for manufacturing processes, which corresponds to 66,800 defects per million and 93.3 percent. However, 4 sigma corresponding to 6,200 defects per million, and 99.4 percent seems to be a more reliable level for tests of SARS-CoV-2. While higher levels of sigma metric values represent better performance, 4 sigma is a more achievable goal for the current in vitro diagnostic technology [16].

Statistics is the main guide for physicians, particularly those managing pandemics. The acceptable tolerance limits of tests used in the diagnosis and management of infectious and noninfectious diseases should not be the same. In noninfectious diseases, the false-negative test results mostly affect the patients. However, in infectious diseases such as COVID-19, false-negative test results lead to catastrophic outcomes by affecting the whole population and the control of the pandemic. Therefore, the revision of tolerance limits by regulatory bodies is needed promptly.

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