Clinical Sensitivity of SARS-CoV-2 Nucleic Acid Amplification Tests for Diagnosing COVID-19

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Abstract

Utilizing 34,348 SARS CoV-2 NAAT results from two health systems, we estimated the clinical sensitivity of a single SARS CoV-2 NAAT. We found that SARS CoV-2 NAAT has 82-97% sensitivity for diagnosing COVID-19 among symptomatic patients.

Key Words: COVID-19, SARS-CoV-2

Introduction

In response to the COVID-19 pandemic, the Food and Drug Administration issued emergency authorization for use of SARS-CoV-2 nucleic acid amplification tests (NAAT) to diagnose COVID-19 [1]. Despite widespread use of SARS-CoV-2 molecular testing, its clinical sensitivity remains uncertain. Reports of false negative results among patients with COVID-19 underscore the need for systematic study of the test's sensitivity in a real-world setting [2, 3].

Methods

We utilized two methods to calculate the clinical sensitivity of a single SARS-CoV-2 NAAT.

Sensitivity Calculation Method 1

We collected test results for all symptomatic patients tested with SARS-CoV-2 NAAT via nasopharyngeal swab at University of Chicago Medicine (UCM) and Providence St. Joseph Health between January 22, 2020 and April 23, 2020. During the study period, UCM only tested patients with symptoms consistent with COVID-19. Because Providence includes 51 hospitals with different policies around COVID-19 testing (including some that were screening asymptomatic patients), we only included Providence test data for patients with at least two of the following three symptoms: fever, cough, and shortness of breath. Symptom data were extracted from the electronic medical record using natural language processing of clinical notes.

Several different SARS-CoV-2 NAATs were utilized during the study period. Tests utilized at UCM were Cepheid Xpert Xpress SARS-CoV-2 and Roche cobas SARS-CoV-2. Tests utilized at Providence included Abbott ID Now COVID-19, BD SARS-CoV-2 Reagents for BD Max System,

BioFire COVID-19, BioGX SARS-CoV-2 reagents for BD Max System, CDC 2019-nCoV RT-PCR Diagnostic Panel, Cepheid Xpert Xpress SARS-CoV-2, LabCorp COVID-19 RT-PCR, Panther Fusion SARS-CoV-2, Roche cobas SARS-CoV-2, Quest SARS-CoV-2 rRT-PCR, and Simplexa COVID-19 Direct.

To calculate sensitivity, we made several assumptions. We assumed all positive SARS-CoV-2 NAATs represented true positive (TP) results. Among patients testing negative who were subsequently re-tested within 7 days of their first test, we assumed that an initial negative test followed by a positive test represented a false negative (FN) result, and that an initial negative test followed by a second negative test represented a true negative (TN) result. Because many patients were tested only once for SARS-CoV-2, we assumed that the false negative rate (FNR, calculated as FN/(FN+TN)) of a single negative NAAT was the same among patients tested once as it was among patients tested multiple times. Based on this assumption, the number of false negative results in the entire population of tested patients was calculated as FN = # initial negative tests * FNR. Sensitivity was calculated as TP/(TP+FN).

Sensitivity Calculation Method 2

For the second sensitivity calculation, we limited our analysis to patients hospitalized at UCM during the study period. UCM requires that inpatients with a negative test for SARS-CoV-2 undergo repeat testing at least 48 hours after their initial test. This two-test policy is in place to ensure that patients with possible COVID-19 remain on appropriate infection control precautions in case of a false negative result. We made the same assumptions regarding TP and FN as in Method 1. However, because Method 2 only included test results from a population of inpatients routinely tested twice for SARS-CoV-2, we were able to accurately identify false negative test results without further assumptions. Sensitivity was calculated as TP/(TP+FN).

Study Ethical Approval

This study was approved by Institutional Review Boards at University of Chicago and Providence St. Joseph Health. Informed consent was not obtained as a waiver of consent was granted by the IRBs.

Results

Sensitivity Calculation #1

Over the study period, 34,348 SARS CoV-2 NAATs were performed (Figure 1). 11.8% (4,037/34,348) were positive. Of the 30,311 negative SARS-CoV-2 NAATs, 5.9% (1,774/30,311) were followed by a subsequent SARS-CoV-2 NAAT within 7 days. Of these subsequent tests, 53 were positive, indicating a false negative rate of 3.0% (53/1,774). Estimated false negative results in the entire tested population was calculated as 30,311*(53/1,774) = 906. Sensitivity was calculated as 4,037/(4,037+906) = 82% (Table 1).

Sensitivity Calculation #2

During the study period, 2,443 SARS CoV-2 NAATs were performed among patients hospitalized at UCM. 437 tests were initially positive (TP). 15 negative tests were followed by a positive test within 7 days (FN). Sensitivity was calculated as 437/(437+15) = 97%.

Discussion

In this multi-center U.S. study, we found that SARS CoV-2 NAAT has clinical sensitivity of 82 - 97% for diagnosing COVID-19 among symptomatic patients. While the test manufacturers have estimated clinical sensitivity using contrived clinical specimens, ours is the first large study to estimate SARS CoV-2 NAAT clinical sensitivity in a real-world setting [4].

In the absence of a gold standard for diagnosing COVID-19, we estimated sensitivity based on several assumptions. In our first sensitivity calculation, we assumed the same false negative rate among patients tested once and patients tested multiple times for SARS-CoV-2. In reality, patients with a single negative test result may have a lower likelihood of COVID-19 than patients who undergo repeat testing. Patients tested multiple times may have persistent symptoms consistent with COVID-19, prompting their medical providers to repeat testing. Therefore, our sensitivity estimate of 82% is best understood as the lower limit of sensitivity. If we assume that patients undergoing repeat testing have twice the likelihood of COVID-19 as patients who are not retested (i.e, repeat testers have twice the false negative rate as patients tested once), then the sensitivity estimate increases to 89% (4,037/4,516). If we assume that repeat testers have three times the likelihood of COVID-19, then the sensitivity estimate increases to 92% (4,037/4,374).

We also assumed that negative NAAT sfollowed by positive tests within 7 days were false negative results. In some cases patients may have acquired COVID-19 within the 7 days between tests. However, when we limited our analysis to tests repeated within 3 days, our results were similar (data not shown). Finally, we assumed all positive test results were true positive results. While it is possible that some of the positive SARS-CoV-2 NAAT results were false positives, it is unlikely that this would alter our results given the high analytical specificity of the SARS-CoV-2 NAATs used.

Beyond the assumptions made, our study had some additional limitations. Eleven different NAATs were utilized during the study period. Different tests have varying limits of detection, which

could result in different clinical sensitivity for each test. We were not able to determine the clinical sensitivity of each individual test used. Others have reported a lower sensitivity for isothermal application tests (e.g., Abbott ID Now COVID-19) compared to reverse-transcription polymerase chain reaction tests [5, 6]. In addition, we were not able to collect information regarding timing of symptom onset related to testing. We limited our analysis to symptomatic patients, and the clinical sensitivity of SARS-CoV-2 NAAT for diagnosis of COVID-19 among asymptomatic patients may be different. Our analysis only included nasopharyngeal samples tested for SARS-CoV-2. For patients with symptoms of COVID-19 with an initial negative nasopharyngeal SARS CoV-2 NAAT result, obtaining a bronchoalveolar lavage for SARS-CoV-2 testing may have a higher diagnostic yield compared to repeating another nasopharyngeal testx.

In conclusion, this study estimated clinical sensitivity of NAAT for clinical diagnosis of COVID-19 among symptomatic patients in a real-world setting among over 30,000 patients. Our findings of a relatively high sensitivity of a single SARS-CoV-2 NAAT have important implications for clinical diagnosis of COVID-19.

Notes

This work had no source of funding. The authors report no potential conflicts of interest.

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Table 1: Sensitivity Calculation for Method #1

Testing site	**Number of initial positive tests (TP)**	**Number of initial negative tests (NT)**	**False negative rate among patients retested (FNR)**	**Estimated false negatives in entire population (FNR * NT = FN)**	**Sensitivity TP/(TP+FN)**
UCM	1,526	7,116	2.57% (23/896)	183	89% (1526/1700)
Providence	2,511	23,195	3.42% (30/878)	793	76% (2511/3304)
Total	4,037	30,311	2.99% (53/1774)	906	82% (4037/4943)

Abbreviations: TP, True positive; NT, Initial negative tests; FNR, false negative rate; FN, false negative; UCM, University of Chicago Medicine

Figure 1: Flow Diagram of SARS-CoV-2 PCR Tests