Clinical and Epidemiologic Evaluation of Inconclusive COVID-19 PCR Results Using a Quantitative Algorithm

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ABSTRACT

Objectives: *The inconclusive severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) polymerase chain reaction (PCR) result causes confusion and delay for infection prevention precautions and patient management. We aimed to develop a quantitative algorithm to assess and interpret these inconclusive results.*

Methods: *We created a score-based algorithm by combining laboratory, clinical, and epidemiologic data to evaluate 69 cases with inconclusive coronavirus disease 2019 (COVID-19) PCR results from the Centers for Disease Control and Prevention (CDC) assay (18 cases) and the TaqPath assay (51 cases).*

Results: *We determined 5 (28%) of 18 (CDC assay) and 20 (39%) of 51 (TaqPath assay) cases to be false positive. Lowering the cycle threshold cutoff from 40 to 37 in the TaqPath assay resulted in a dramatic reduction of the false-positive rate to 14%. We also showed testing of asymptomatic individuals is associated with a significantly higher probability of having a false-positive result.*

Conclusions: *A substantial percentage of inconclusive SARS-CoV-2 PCR results can be false positive, especially among asymptomatic patients. The quantitative algorithm we created was shown to be effective and could provide a useful tool for clinicians and hospital epidemiologists to interpret inconclusive COVID-19 PCR results and provide clinical guidance when additional PCR or antibody test results are available.*

Key Points

- Between 14% and 39% of inconclusive SARS-CoV-2 PCR results can be false positive.
- Asymptomatic testing increases the probability of having a false-positive result.
- A tool combining clinical, epidemiologic, and laboratory data can be used to help interpret these inconclusive results.

Early diagnosis of coronavirus disease 2019 (COVID-19) is important for both infection prevention and patient management.[1] In February 2020, the US Centers for Disease Control and Prevention (CDC) developed and distributed for use the 2019-nCOV polymerase chain reaction (PCR) assay to both public health and clinical laboratories.[2] This assay is designed to detect two specific target regions in the nucleocapsid (N) gene of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus (N1 and N2).[3] A positive result requires both targets to be detected with the cycle threshold (Ct) cutoff as 40. However, an inconclusive result can occur when only one of the targets (N1 or N2) is detected. Similarly, another widely used assay, the TaqPath COVID-19 Combo Kit (Thermo Fisher Scientific), also reports an inconclusive result when only one of three targets (ORF1ab, N, S) is detected.

The inconclusive result causes confusion among clinicians and can delay appropriate infection prevention precautions and patient management. Current practice is for institutions to recollect and retest for COVID-19, but that

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may be problematic for outpatients due to various reasons such as a long waiting time for another appointment. It is even more challenging for clinicians to interpret the first inconclusive result when a repeat test is negative while there is still clinical suspicion. Therefore, it is important to have a reliable mechanism for providers and institutions to be able to assess and interpret these inconclusive results.

Materials and Methods

Cases and Specimens

In our institution, from March 10, 2020, to June 18, 2020, a total of 3,412 specimens from 3,247 patients were tested by the CDC 2019-nCOV PCR assay; 183 (5.4%) specimens tested positive, 3,210 (94.1%) specimens tested negative, and 19 (0.56%) specimens tested inconclusive. Among these specimens, 3,103 (90.9%) were nasopharyngeal (NP) swabs, 189 (5.5%) were bronchoalveolar lavage (BAL), 58 (1.7%) were sputum, and 62 (1.8%) were miscellaneous. The 18 specimens with inconclusive results were NP swabs (n = 14), BAL (n = 3), and lung swab (n = 1) from 18 unique patients.

From April 4, 2020, to June 14, 2020, a total of 16,543 specimens (all NP swabs) from 15,017 unique patients were tested by the TaqPath assay. Of these, 346 (2.1%) specimens tested positive and 54 (0.33%) specimens tested inconclusive from 51 (0.34%) unique patients.

All inconclusive results were reported, and re-collection of specimens for repeat testing was requested for these cases. All the additional PCR tests in this study were performed on the NP swabs, except for one patient (CDC-8) (Supplemental Table S1; all supplemental materials can be found at *American Journal of Clinical Pathology* online) who had a different collection of BAL and tracheal aspirate, both of which tested positive.

Laboratory Investigation

All the specimens with inconclusive results were not adjacent to another highly positive specimen (defined as Ct <20), and the run quality control results were valid, suggesting cross-contamination was unlikely. The amplification curves were visually checked, and all appeared correct with a typical smooth "S"-shape curve above the threshold, suggesting the amplified signals were true. The cycle threshold (Ct) values and the case review information are summarized in Supplemental Tables S1 and S2 for the CDC assay and TaqPath assay, respectively.

Quantitative Algorithm

We reviewed the patients' charts for documentation of symptoms and exposures. The reason for ordering a test was routinely documented in the note when a test was ordered as well as when a patient went to the testing site for their test. A quantitative algorithm was developed to assess whether the inconclusive result was a "confirmed positive," "most likely true positive," or "most likely false positive." Five parameters were included in the algorithm: (1) symptoms consistent with COVID-19, including fever, cough, sore throat, upper respiratory symptoms, chest tightness, headache, fatigue, or diarrhea; (2) symptoms highly suspected for COVID-19, including shortness of breath, hypoxia, anosmia, or loss of taste; (3) history of sick contact or exposure to confirmed cases; (4) additional COVID-19 PCR results; and (5) COVID-19 antibody test results. Different scores (0-5) were assigned to each circumstance, as displayed in **Table 1**. The scores were based on the strength of

Table 1
Scoring Scheme for Case Review[a]

Parameter	Result	Score	Rationale
Symptoms consistent with COVID-19	Yes	1	Nonspecific symptom is a weak support for COVID-19.
	No or unclear	0	
Symptoms highly suspected for COVID-19	Yes	3	More specific symptom is a stronger support for COVID-19.
	No or unclear	0	
Sick contact	Yes	1	Sick contact alone is a weak support for COVID-19.
	No or unclear	0	
Additional PCR test results	Positive	5	An additional inconclusive PCR result is a moderate support for COVID-19.
	Inconclusive	2	
	Negative or NA	0	
Antibody (IgG) test results	Positive	4	A positive antibody result is a strong support for COVID-19.
	Negative or unclear	0	

COVID-19, coronavirus disease 2019; IgG, immunoglobulin G; NA, not available; PCR, polymerase chain reaction.
[a]See the definition of each parameter in the text. Score interpretation: >5: confirmed positive; 3-5: most likely true positive; <3: most likely false positive.

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the evidence that support the likelihood of COVID-19 infection. The sum of the scores (total score) is used as the basis for the result interpretation. The result is interpreted as "confirmed positive," "most likely true positive," or "most likely false positive" when the total score is more than 5, 3 to 5, or less than 3, respectively. This algorithm is designed this way so that a case deemed "confirmed positive" (total score >5) should have at least one positive PCR result (score = 5) plus any other positive parameters, one additional inconclusive PCR result (score = 2) plus having specific symptoms (score = 1 + 3), or one positive antibody result (score = 4) plus having both symptoms (score = 1 or 4) and/or sick contact (score = 1). A case highly suspected due to specific symptoms (score = 1 + 3) and/or exposure history (score = 1) but without any additional positive or inconclusive PCR or antibody results can be deemed "most likely true positive" (total score = 3-5). Other scenarios for "most likely true positive" include a case with nonspecific symptoms (score = 1) and/or exposure history (score = 1) plus an additional inconclusive PCR result (score = 2) or a positive antibody result (score = 4). A case with only nonspecific symptoms (score = 1) and/or exposure history (score = 1) or without either is deemed "most likely false positive" (total score < 3).

Statistical Analysis

All data were analyzed using Microsoft Excel and IBM SPSS. The Pearson χ^2 test was used to identify any association between two categorical variables. $P < .05$ was considered statistically significant.

This project was performed as a quality improvement project to better understand reporting of inconclusive COVID-19 results and was thus exempt from institutional review board approval.

Results

In the 23 inconclusive CDC assay results, N2 was more frequently detected (n = 14) than N1 (n = 9). The mean Ct values of N1 (35.5) and N2 (35.8) were nearly identical, but the Ct range of N1 (minimum = 34.1, maximum = 36.4) was much narrower than that of N2 (minimum = 31.0, maximum = 39.8). In the 54 inconclusive TaqPath assay results, N was most frequently detected (n = 34) with the mean Ct value of 36.4 (minimum = 33.9, maximum = 39.8); ORF1ab (n = 9) and S (n = 11) were detected less frequently with the mean Ct value of 35.5 (minimum = 26.0, maximum = 39.9) and 37.4 (minimum = 27.5, maximum = 39.4), respectively. Using 37 as the Ct cutoff, the frequencies of detection became 24, 5, and 1 for N, ORF1ab, and S, respectively.

In the 18 cases with inconclusive CDC assay results, 13 cases were deemed as "confirmed positive," with the total score ranging between 6 and 10 (Table 2 and Supplemental Table S1). All the 13 confirmed positive cases had either another positive or inconclusive PCR result or positive COVID-19 antibody results. The majority (11/13) of the confirmed positive cases had symptoms consistent with COVID-19. The five additional cases were deemed "most likely false positive" due to low scores (0 or 1). Two of them (CDC-15 and CDC-18) had

Table 2
Analysis of Cases With Inconclusive PCR Results[a]

Assay	Factor	Confirmed Positive, No. (%)	Most Likely True Positive, No. (%)	Confirmed and Most Likely True Positive, No. (%)	Most Likely False Positive, No. (%)	χ^2 Statistic	*P* Value
Combined cases	Total cases (*n* = *69*)	41 (60)	3 (4)	*44* (64)	*25* (36)		
	Asymptomatic (n = 16)	3	0	3 **[7]**	13 **[52]**	18.272	<.001[b]
CDC assay	Total cases (*n* = *18*)	13 (72)	0 (0)	***13*** (72)	***5*** (28)		
	Asymptomatic (n = 5)	2	0	2 **[15]**	3 **[60]**	3.583	.058
TaqPath assay	Total cases (*n* = *51*)	28 (55)	3 (6)	***31*** (61)	***20*** (39)		
(Ct cutoff = 40)	Asymptomatic (n = 11)	1	0	1 **[3]**	10 **[50]**	15.723	<.001[b]
	Preoperative (n = 12)	2	0	2 **[6]**	10 **[50]**	14.373	<.001[b]
	HCW (n = 13)	6	2	8 **[26]**	5 **[25]**	0.004	.949
TaqPath assay	Total cases (*n* = *28*)	21 (75)	3 (6)	***24*** (86)	***4*** (14)		
(Ct cutoff = 37)	Asymptomatic (n = 2)	0	0	0 **[0]**	2 **[50]**	12.923	.001[b]
	Preoperative (n = 5)	2	0	2 **[8]**	3 **[75]**	10.388	.001[b]
	HCW (n = 6)	3	2	5 **[21]**	1 **[25]**	0.035	.851

CDC, Centers for Disease Control and Prevention; HCW, health care worker.
[a]Percentages in parentheses (%) were calculated using the overall total case number (italicized) as the denominator. Percentages in brackets **[%]** were calculated using the categorial total case number (bolded) as the denominator.
[b]Statistically significant.

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2 days of subjective symptoms not specific to COVID-19 (Supplemental Table S1). The other three were asymptomatic, including one (CDC-2) whose only risk factor was exposure to a confirmed case, one (CDC-3) tested due to preadmission screening, and one (CDC-16) tested due to presurgical screening. Subsequent PCR or antibody test results were either negative or unavailable in these five cases (Supplemental Table S1).

In the 51 cases with inconclusive TaqPath assay results, 20 (including 10 symptomatic and 10 asymptomatic cases) were deemed most likely false positive (Table 2). All of these 20 cases had at least one other negative PCR result within 3 days of the inconclusive results, except for one case (TaqPath-48) in which the patient's only symptom was headache (Supplemental Table S2). Twenty-eight cases (including 27 with symptoms and only 1 without) were confirmed positive with either additional positive PCR results or antibody results. Three cases (TaqPath-3, TaqPath-18, and TaqPath-46) with symptoms consistent with COVID-19 were determined to be "most likely true positive," including one patient with an infected family member who had fevers, cough, and anosmia, and two health care workers (HCWs) who had symptoms consistent with COVID-19 and tested inconclusive repeatedly by PCR.

To determine which factors may correlate with increased chance of having the false-positive results, we performed a χ^2 test to examine several categorical variables, including (1) whether patient was asymptomatic, (2) whether the test was for presurgical screening, and (3) whether patient was a HCW. For the CDC assay, asymptomatic testing accounted for only 2 (15%) of 13 of the confirmed and most likely true-positive cases combined compared with 3 (60%) of 5 of the most likely false-positive cases, with a borderline statistical significance ($P = .058$) (Table 2). For the TaqPath assay, asymptomatic testing accounted for only 1 (3%) of 31 of the confirmed and most likely true-positive cases combined compared with 10 (50%) of 20 of the most likely false-positive cases, with a strong statistical significance ($P < .001$). When the CDC and the TaqPath data sets are combined, a strong statistically significant difference ($P < .001$) was also observed between the percentage of asymptomatic testing among the confirmed and most likely true-positive combined cases (3/44, 7%) and the most likely false-positive cases (13/25, 52%).

In the CDC assay data set, since there were no HCW cases and only one presurgical case, no further analysis was performed for these two variables. In the TaqPath assay data set, sufficient cases associated with presurgical screening (n = 12) and HCWs (n = 13) allowed for further analyses. Presurgical screening accounted for only 2 (6%) of 31 in the confirmed and most likely true-positive combined cases but accounted for 50% (10/20) of the false-positive cases (Table 2). No correlation was found between HCWs and a higher chance of having false-positive results, since the percentage of HCWs in the confirmed and most likely true-positive combined cases (8/31, 26%) and in the most likely false-positive cases (5/20, 25%) was almost identical.

In late July 2020, the vendor of the TaqPath assay lowered the cycle threshold from 40 to 37[4] due to high frequencies of false-positive results reported by users (personal communication with clinical and public health laboratory directors, July 18, 2020). We therefore retrospectively reanalyzed our data set using the new cutoff. The lowered Ct cutoff eliminated 23 of 51 inconclusive TaqPath results and dramatically reduced the most likely false-positive cases to 4 (14%) of 28 compared with 20 (39%) of 51 originally: a reduction of 80% (16/20). To investigate whether lowering the Ct cutoff led to a loss of sensitivity, we found that 7 (25%) of 28 confirmed positive cases would be called negative instead of "inconclusive." Of those seven confirmed positive cases that would not have been detected with the new cutoff, all had previous positive PCR results, suggesting that they had a very low viral load at the time when they had an inconclusive test result. Therefore, the revised Ct cutoff resulted in an 80% improvement in the specificity and only 25% loss in the sensitivity. Importantly, even with the new Ct cutoff, asymptomatic ($P < .001$) testing and presurgical ($P = .001$) screening are still correlated with an increased chance of having a false-positive result (Table 1).

Discussion

Our study demonstrated that 28% to 39% of the inconclusive PCR results could be false positives. Asymptomatic testing was associated with much higher frequencies of false-positive results in one of the PCR targets. In a setting of low prevalence or positive rate, even a test with high specificity could generate a substantial percentage of false positives.[5] Although asymptomatic infection has been documented in previous studies,[6] the PCR-positive rate among asymptomatic individuals is not expected to be high, considering the overall PCR-positive rate in our institution ranged from 2.1% to 5.4%, and the presurgical screening positive rate is only 0.13%.[7] A massive scale of asymptomatic testing by PCR, such as for the purpose of preadmission and presurgical screening, will inevitably lead to false-positive results. Therefore, having multiple PCR targets in an assay and reporting an "inconclusive" result if only one target is detected is

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advantageous, especially in a setting of low pretest probability. Since clinical decisions including timing of elective surgery may be based in part on COVID-19 screening test results, it is important for clinicians to be able to understand the meaning of inconclusive tests. False-positive results can delay care for patients and have unintended consequences, including delayed surgery or delays in diagnosis, as has been reported in the literature.[8,9] Our analysis showed that lowering the Ct cutoff from 40 to 37 in the TaqPath assay resulted in a dramatic improvement of the specificity with only a slight loss of sensitivity. Of note, the manufacturer of the TaqPath assay had also updated its protocol adding more vigorous vortexing of the PCR Master Mix, which is viscous, to reduce the false amplification signals due to the uneven suspension of the fluorescent dyes. This protocol change, in addition to the lowered Ct cutoff, can also contribute to the reduction of false-positive results when implemented.

One of the limitations of our study is that the clinical and epidemiologic information was obtained by chart review and may have been incomplete. However, in most instances, exposures and symptoms were routinely documented. Second, false-positive and false-negative serology test results could be possible. However, the serology assay used in our institution (the DiaSorin LIAISON SARS-CoV 2 IgG assay) has high sensitivity (97.6%) and specificity (99.3%) (https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/eua-authorized-serology-test-performance). In a patient with an epidemiologic link or COVID-19 symptoms, the positive predictive value of a positive serology would presumably be high. In our study, a positive serology accounted for only 4 points in our algorithm, and more than 5 points were required to classify someone as a confirmed positive, meaning a patient with a positive antibody test but a negative PCR test needs to have both symptoms and an epidemiologic link or symptoms highly suspected for COVID-19 to be classified as positive.

It is also important to point out that the lower respiratory tract (LRT) specimens can have prolonged positivity and may be positive when the upper respiratory tract specimens test negative,[10,11] and therefore, testing LRT specimen would be helpful in cases with high clinical suspicion. However, this is not a major concern in this study since all patients with most likely false-positive results did not present with productive cough or severe respiratory symptoms to allow testing for LRT specimens, such as sputum or BAL.

In summary, the quantitative algorithm we created was shown to be effective and could provide a useful tool for clinicians and hospital epidemiologists to interpret inconclusive COVID-19 PCR results and provide clinical guidance if additional PCR or antibody test results are available.

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