Utilization and Results of Repeat SARS-CoV-2 RT-PCR Testing in a Presumptive Low Prevalence Population

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ABSTRACT

Introduction: Early reports have raised concerns regarding the clinical sensitivity of nasopharyngeal SARS-CoV-2 reverse transcriptase-polymerase chain reaction (RT-PCR) testing for patients with COVID-19 symptoms, which has led to requests for repeat testing at our institution. However, to our knowledge, there are no reports to date of the utilization or results of repeat testing to help guide this practice.

Methods: The authors searched the institutional laboratory information system for consecutive patients who were tested for SARS-CoV-2 by RT-PCR of a nasopharyngeal specimen over a 1-month period. Characteristics and results of patients who received a single or multiple tests were documented and analyzed.

Results: Six thousand three (6003) tests were performed on 5757 patients; 272 (4.7%) patients were positive based on their initial test results. Two hundred thirty-six (4%) patients were tested more than once, with 226 (96%) tested twice. The largest proportion of these patients (n=160, 71%) were those who had an initial negative test followed by a repeat test for persistent symptoms. This group included all 7 patients who had discordant positive results on their second test; the result concordance rate within this group was 96%.

Conclusion: In a population of patients with a low positive rate for SARS-CoV-2 by nasopharyngeal RT-PCR testing, repeat nasopharyngeal testing of negative patients who have persistent symptoms still yields a negative result in 96% of the cases.

INTRODUCTION

Since the first cases of corona virus disease 2019 (COVID-19) were diagnosed in Wisconsin in February 2020,[1] health systems within the state have focused on increasing their testing capacity. However, growth has been slow, and the demand for testing continues to outpace testing availability.[2] Under these conditions of scarcity, the patterns and utility of repeated patient testing become relevant in guiding ongoing refinement of best utilization practices.

Detection of SARS-CoV-2 nucleic acid from respiratory tract samples is currently the diagnostic strategy recommended by the Centers for Disease Control and Prevention (CDC) for symptomatic patients with suspected COVID-19.[3] Reverse transcriptase-polymerase chain reaction (RT-PCR) assays that use primer-probe sets targeting multiple SARS-CoV-2 genes have been developed by the CDC, World Health Organization, commercial vendors, and as laboratory-developed tests (LDT) by high complexity clinical laboratories. RT-PCR SARS-CoV-2 assays have very high *analytic* sensitivity and specificity, and their analytic performance are essentially equivalent to one another.[4,5] In contrast to traditional RT-PCR assays, several commercial vendors also have developed rapid cartridge-based assays to detect SARS-CoV-2 nucleic acid that can be performed outside the clinical laboratory environment. However, these assays have been shown to have lower analytical sensitivity, with positive percent agreement ranging from 88% to 98% when compared to traditional RT-PCR.[6] Likewise, SARS-CoV-2 antigen assays offer the promise of rapid and portable testing but so far lack the analytic sensitivity of nucleic acid tests, with positive percent agreement ranging from 80% to 84%.[7] Novel technology to detect SARS-CoV-2 nucleic acid, such as reverse tran-

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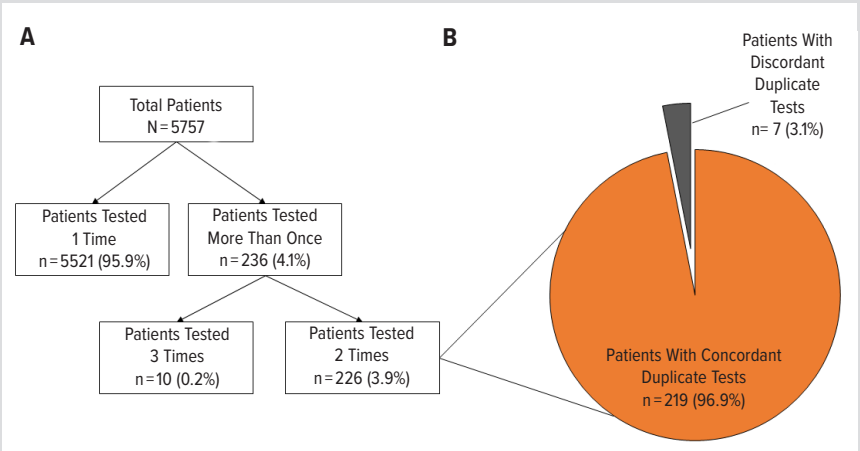
Figure 1. Breakdown of 5757 Consecutive Patients Who Underwent Nasopharyngeal SARS-CoV-2 RT-PCR Testing by (A) All Patients and (B) Patients Who Were Tested Twice



Initial Test Result	Number of Patients (%)
Positive	272 (4.7%)
Negative	5485 (95.2%)

Initial Test Result	Number of Patients (%)
Positive	1 (0.4%)
Negative	225 (99.6%)

scription-loop mediated isothermal amplification (RT-LAMP) or clustered regularly interspaced short palindromic repeats (CRISPR)-based systems, may bridge the gap in the need for rapid, portable, and accurate assays; however, they are not yet readily available.[7]

At present, RT-PCR SARS-CoV-2 assays are the cornerstone of diagnostic testing for SARS-CoV-2 infection, but while their analytical performance is well documented, their clinical performance in terms of test results correlating with a patient's disease state remain uncertain. Early reports have questioned the clinical sensitivity of RT-PCR SARS-CoV-2 assays performed on a single nasopharyngeal specimen.[8-13] Repeat testing may mitigate the impact of false negative results to public safety and health care workers but comes at the expense of possible overutilization of a presently scarce resource.

Herein, we review the patterns and utility of repeat SARS-CoV-2 RT-PCR testing in 5757 consecutive patients who were tested through UW Health Clinical Laboratories from March 11 through April 13, 2020.

METHODS

The UW Health laboratory information system was queried for all SARS-CoV-2 tests performed through UW Health Clinical Laboratories from March 11, 2020 through April 13, 2020. Retrospective analysis was performed on the data set that was assembled and then segregated into patients who were tested 1, 2, or 3 times within the sampled period. No patient in the data set received more than 3 tests.

During the sampled period, SARS-CoV-2 testing was solely performed by nucleic acid amplification technique on nasopharyngeal swab specimens collected on flocked swabs and transported in either commercial universal transport media or M4RT media. Specimens were tested on 1 of 3 assays: (1) CDC 2019-Novel Coronavirus Real-Time RT-PCR Diagnostic Panel performed at the Wisconsin State Laboratory of Hygiene (WSLH CDC method), (2) modified CDC 2019-Novel Coronavirus Real-Time RT-PCR Diagnostic Panel with alternative Roche LightCycler 480 amplification validated and performed at UW Health Clinical Laboratories (UW CDC method), or (3) Panther Fusion SARS-CoV-2 (Hologic, Inc) performed at UW Health Clinical Laboratories (UW Panther method).

Statistical analyses were performed using Microsoft Excel software (Version 2016). Comparisons between groups were performed by Student *t* test, Fisher exact test, or chi-square test, as appropriate. Results were considered statistically significant if a 2-tailed *P* value was less than 0.05.

Data collection and analysis were determined by the University of Wisconsin Health Sciences Institutional Review Board to qualify as quality improvement and, thus, considered exempt from institutional review board approval.

RESULTS

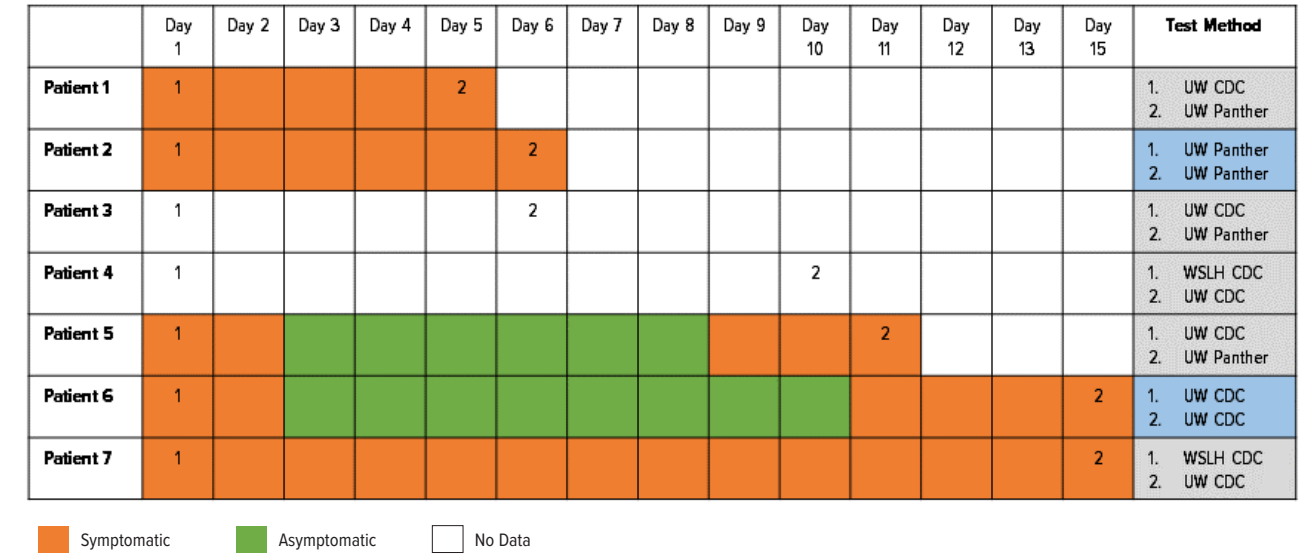
Patient Characteristics

Between March 11 and April 13, 2020, 5757 patients were tested for SARS-CoV-2 through UW Health Clinical Laboratories. The vast majority of patients was tested just once (n = 5521, 95.9%), with a small proportion tested twice (n = 226, 3.9%) or 3 times (n = 10, 0.2%) (Figure 1A). Based on the results of each patient's initial test, the overall prevalence of disease in the tested population was 4.7% (Figure 1A). The mean age of those tested once was 45 years and was not significantly different than those who were tested more than once (Table 1). Interestingly, the proportion of patients tested was skewed toward females, with approximately 65% females and 35% males, but was not significantly different between those who were tested once versus multiple times (Table 1). Of note, however, the proportion of patients with initial negative test results was significantly higher in the group tested multiple times (99.2%) compared to the group tested once (95.2%) (Table 1).

Concordant vs Discordant Results in Patients Tested Twice

Of the 226 patients who were tested twice, 160 (71%) had persis-

Figure 2. Details of Patients Who Had an Initial Negative Test and Subsequent Positive Test



Boxes shaded grey denote patients who were tested by different methods for the first and second test. Boxed shaded blue denote patients who were tested by the same method for the first and second test. For test methods: UW CDC = modified CDC 2019-Novel Coronavirus Real-Time RT-PCR Diagnostic Panel (CDC) with alternative Roche LightCycler 480 amplification validated and performed at UW Health Clinical Laboratories; UW Panther = Panther Fusion SARS-CoV-2 (Hologic, Inc.) performed at UW Health Clinical Laboratories; and WSLH CDC = CDC 2019-Novel Coronavirus Real-Time RT-PCR Diagnostic Panel (CDC) performed at the Wisconsin State Laboratory of Hygiene.

tent symptoms and 51 (23%) had a second test performed before undergoing an aerosolizing procedure (Table 2). Thirteen patients (6%) had duplicate testing performed in error, where initial testing was ordered in the emergency department or outpatient clinic and then ordered again hours later when the patient was admitted to the hospital while initial test results were pending. Two patients (1%) had a second test performed prior to transfer to a nursing facility that required documentation.

Of all patients who were tested twice, results were concordant for 219 (97%) and discordant for 7 (3%) (Figure 1 B). All 7 patients with discordant results had persistent symptoms and an initial negative result, followed by a subsequent positive result. Of the 219 patients with concordant results, 218 had 2 negative results. The single patient who had concordant positive tests had repeat testing performed in accordance to transfer requirements for a nursing facility. And of the 160 patients tested twice for persistent symptoms, 153 (96%) had concordant results and 7 (4%) had discordant results.

The sampling period encompassed a time span when SARS-CoV-2 testing for UW Health Clinical Laboratories expanded to include 3 RT-PCR assays performed at 2 laboratories (see Methods). To evaluate if mismatched testing methodology contributed to discordant results, we compared the proportion of patients tested with the same assay to those tested with different assays for concordant (54.8% and 45.2%, respectively) and discordant results (28.6% and 71.4%, respectively) and found no signif-

Table 1. Comparison of Characteristics of Patients Who Received a Single vs Multiple Nasopharyngeal SARS-CoV-2 RT-PCR Tests

	Single Test (n=5521)	Multiple Tests (n=236)	*P* value
Age, years			
Mean (SD)	45 (18)	46 (20)	*P*=0.61
Median (Range)	43 (<1-98)	45 (1-94)	
Sex			
Male	1899 (34.4%)	82 (34.9%)	*P*=0.89
Female	3609 (65.5%)	153 (65.1%)	
Initial test result			
Negative	5258 (95.2%)	234 (99.2%)	*P*=0.002
Positive	263 (4.8%)	2 (0.8%)	

Table 2. Comparison of Patients Who Had Concordant vs Discordant Results on 2 Nasopharyngeal SARS-CoV-2 RT-PCR Tests

	Patients With 2 Concordant Results (n=219)	Patients With 2 Discordant Results (n=7)	*P* value
Reason for second test			
Persistent symptoms	153 (70.8%)	7 (100%)	
Preprocedure	51 (22.6%)	0	
Facility requirement	2 (0.9%)	0	
Ordering error	13 (5.8%)	0	
Interval between tests, days			
Mean (SD)	9.9 (6.4)	9.4 (3.8)	*P*=0.86
Median (Range)	9 (0- 28)	10 (5- 14)	
Testing method			
Same assay	120 (54.8%)	2 (28.6%)	*P* = 0.25
Different assays	99 (45.2%)	5(71.4%)	

icant difference ($P = 0.25$, Fisher exact test) (Table 2). Likewise, we evaluated whether the interval between testing may have contributed to discordant results and found the mean interval between tests for patients who had concordant results—9.9 days—was not significantly different from patients who had discordant results (mean = 9.4 days; $P = 0.86$, Student t test) (Table 2).

Clinical information was available for 5 of the 7 patients with discordant results (Figure 2). All five were symptomatic at the time of their first and second test. Three patients had persistent symptoms for 5, 6, and 14 days before their second test and 2 patients reported an asymptomatic interval of approximately 6 and 8 days prior to becoming symptomatic again (Figure 2).

Characteristics of Patients Who Were Tested 3 Times

Ten patients were tested 3 times, with a mean interval of 8.4 days between the first and second test and 6 days between the second and third test. Nine patients had concordant negative-negative-negative results, and 1 patient had discordant positive-negative-negative results. The patient with the discordant results was tested 8 and 9 days after her initial positive test to fulfill requirements for 2 negative SARS-CoV-2 tests prior to transfer to a nursing facility.

DISCUSSION

As SARS-CoV-2 began to spread across the United States in early 2020, local experience in Wisconsin with SARS-CoV-2 virus testing was limited. In March 2020, our institution began operationalization of coordinated plans to test patients for SARS-CoV-2 by RT-PCR from nasopharyngeal swabs. From March 11 to April 13, 2020, 5757 patients were tested, with 236 (4%) patients receiving multiple tests. To date, our institution has not implemented any "hard stops" to prevent repeat testing. However, guidance was issued early when testing began to discourage repeat testing within 7 days, and an accompanying "best practice alert" appeared when providers placed electronic orders for testing within 7 days of the last order.

The majority (71%) of repeat tests in our sample were performed on patients with persistent upper respiratory infection symptoms who initially tested negative. Repeat testing in this population was likely driven, in part, by early reports raising concerns regarding false negative results with nucleic acid amplification assays performed on nasopharyngeal swab specimens.[8-11] However, our findings show that 153 of 160 of the patients who were tested twice for persistent symptoms had concordant negative results on their second test, giving a negative predictive value (true negative / true negative + false negative) of 96% for this population. We found no significant difference in the interval between testing for patients with concordant results versus discordant results. Likewise, there was no significant difference in the proportion of patients who were tested with matched or mismatched methodologies between the concordant and discordant groups. This is important to note, as scarcity of testing—especially at the beginning of the pandemic in the US—necessitated that health systems adopt multiple testing methodologies in order to maintain and expand testing capacity.

The cause of the false negative results is uncertain, though pre-analytical error stemming from the clinical sensitivity of nasopharyngeal swab samples during the course of disease appears to be the biggest concern, given the reliance on sampling technique and our evolving understanding of the viral dynamics of SARS-CoV-2 infection. Virus shedding in the nasopharynx appears to peak within 5 days after symptom onset, after which the sensitivity of nasopharyngeal swabs begins to decline, with an overall detection rate of 40% in swabs taken after 5 days and no viral isolates obtained beyond 8 days after symptom onset.[14] Accordingly, poor nasopharyngeal sampling technique and suboptimal timing of sampling may have contributed to the false negative results. Alternatively, given that we found 2 patients with an asymptomatic interval within a relatively prolonged interval between testing of 11 and 14 days (Figure 2), there is a possibility that some patients with discordant results acquired SARS-CoV-2 in the interval between testing.

This study is limited by the low detected disease prevalence in the sampled population and the lack of a "gold standard" COVID-19 test. In settings of high disease prevalence, clinical suspicion combined with supportive imaging findings can serve as a gold standard and supersede negative laboratory testing results.[8,10,15] However, when the detected disease prevalence is low, we need to turn to an alternate source of truth, such as evidence of seroconversion, to accurately determine the clinical sensitivity of nasopharyngeal SARS-CoV-2 RT-PCR testing; these studies are currently underway.

CONCLUSION

The clinical sensitivity of nasopharyngeal SARS-CoV-2 RT-PCR testing is yet to be determined. However, this study demonstrates that repeat nasopharyngeal SARS-CoV-2 RT-PCR testing of patients with initially negative results, but who have persistent upper respiratory infection symptoms, in a presumptive low disease prevalence setting yields concordant negative results 96% of the time. This information may be of utility in the current circumstance where scarcity of SARS-CoV-2 testing is a factor in both institutional and public health decisions.

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