Utility of Repeat Nasopharyngeal SARS-CoV-2 RT-PCR Testing and Refinement of Diagnostic Stewardship Strategies at a Tertiary Care Academic Center in a low Prevalence Area of the United States

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Key Points: Repeat inpatient SARS-CoV-2 testing demonstrated limited benefit at our institution with no negative-to-positive conversions. NP RT-PCR appears to have a low rate of false negative results in a low prevalence area, indicating higher than reported clinical sensitivity.

ABSTRACT

Background: Multiple factors have led to an extremely high volume of SARS-CoV-2 RT-PCR testing. Concerns exist about sensitivity and false-negative SARS-CoV-2 RT-PCR testing results. We describe a retrospective observational study examining the utility of repeat nasopharyngeal (NP) SARS-CoV-2 RT-PCR testing at an academic center in a low prevalence setting.

Methods: All patients within our health system with >1 NP SARS-CoV-2 RT-PCR test result were included. SARS-CoV-2 RT-PCR testing was performed according to one of four validated assays. Key clinical and demographic data was collected including whether the patient was inpatient or outpatient at time of the test and whether the test was performed as part of a person under investigation (PUI) for possible COVID-19 or for asymptomatic screening.

Results: A total of 660 patients had >1 NP SARS-CoV-2 PCR test performed. The initial test was negative in 638. There were only 6 negative-to-positive conversions (0.9%). All 6 were outpatients undergoing a PUI work-up 5-17 days after an initial negative result. In >260 inpatients with repeat testing, we found no instances of negative-to-positive conversion including those undergoing PUI or asymptomatic evaluation.

Conclusions: In a low prevalence area, repeat inpatient testing after an initial negative result, using a highly analytically sensitive SARS-CoV-2 RT-PCR, failed to demonstrate negative-to-positive conversion. The clinical sensitivity of NP RT-PCR testing may be higher than previously believed. These results have helped shape diagnostic stewardship guidelines, in particular guidance to decrease repeated testing in the inpatient setting to optimize test utilization and preserve resources.

Keywords: Stewardship, SARS-CoV-2, Nasopharyngeal, RT-PCR

INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic has presented numerous unprecedented challenges. One challenge was the need for novel rapid and accurate testing methods in the context of regulatory, supply chain, and resource allocation hurdles. Early reports demonstrated high accuracy and performance of real-time, reverse transcription polymerase chain reaction (RT-PCR) testing of nasopharyngeal (NP) swabs[1-3]. These were quickly adapted worldwide including numerous commercial iterations now available in the US. While this rapid development of testing methods was encouraging, it was overshadowed by the fact that massive quantities of tests would be necessary to diagnose, track, and attempt to contain the spread of the viral agent of COVID-19, SARS-CoV-2. Repeat testing of individuals has also contributed to the extremely high demand for testing. First, reports of low clinical sensitivity of the NP RT-PCR testing in certain regions and case reports of false-negative initial test results have fueled interest in repeat testing under certain clinical conditions [4]. Second, extensive and prolonged community transmission meant a high demand for testing [5]. Finally, repeated asymptomatic screening of individuals seeking care or undergoing procedures at health care facilities, as well as residents of congregate living facilities, such as skilled nursing facilities, was conducted to prevent nosocomial spread [6,7]. Left completely unchecked, the repeat testing of patients could lead to diagnostic testing congestion, undue delays in results, and exhaustion of diagnostic testing resources. Thus, diagnostic stewardship that is informed by clinical data is urgently needed. We have previously reported on the low rate of test positivity in asymptomatic outpatients getting tested before procedures or surgery [8]. In this paper we report the findings of a retrospective observational study to examine results of repeat SARS-CoV-2 RT-PCR testing for patients who were suspected of having COVID-19 (persons under investigation, or PUI) and asymptomatic patients and how the results may inform diagnostic stewardship within our academic medical center in Wisconsin.

METHODS

All patients, including adult and pediatric patients, in the University of Wisconsin health system (UW Health), which includes three hospitals, over 120 clinics, and serves >600,000 patients in the Upper Midwest, were eligible for inclusion in this study. Those that had more than one SARS-CoV-2 RT-PCR testing from March 12, 2020, to May 5, 2020 were included in the analysis. Patients who were not cared for at our hospitals or clinics were excluded as reasons for testing and clinical information was not obtainable from medical records. UW Health employees that were tested through employee health services were also excluded. All samples were obtained via NP sampling technique. SARS-CoV-2 RT-PCR testing was performed initially by the Wisconsin State Laboratory of Hygiene using the CDC-provided assay from March 12-21, 2020. On March 18, UW Health started in-house testing using a laboratory-developed test based on the CDC primer-probe design targeting the N1 and N2 regions of the viral nucleocapsid gene. Soon thereafter, additional assays were brought in-house and validated including Hologic Panther Fusion SARS-CoV-2 Assay (Hologic, Inc., Marlborough, MA) and Cepheid Xpert Xpress SARS-CoV-2 test (Cepheid, Sunnyvale, CA); both assays were performed according to the manufacturer's instructions for use under emergency use authorization.

Advice on testing and/or re-testing was disseminated to all providers via daily emails and updated continuously on the institutional COVID-19 resource website. Outpatient testing was defined as specimen collection in an outpatient clinic encounter, an emergency room or urgent care encounter, or on admission as part of the initial admission work-up (i.e. within the first 24 hours). Inpatient testing was defined as specimen collection that was performed after the first 24 hours during a hospitalization. PUI versus asymptomatic screening designation was based on CDC PUI criteria at the time of testing and was manually determined based on chart review for each patient in the dataset. In the initial stages of testing at our facilities, only PUIs were tested for SARS-CoV-2. PUI testing in the outpatient setting was performed according to early CDC criteria, which limited testing to moderately or

severely ill patients; over time testing was liberalized to those with high risk conditions and symptoms compatible with COVID-19. Repeat testing in the outpatient setting was performed if patients presented a second time and met the aforementioned criteria. No specific time to presentation was considered an exclusion to repeat testing in the outpatient setting. Inpatient PUI testing was mandated for those with symptoms consistent with possible COVID-19 (e.g. unexplained fever, chills, cough, shortness of breath/hypoxia, loss of smell or taste, fatigue, vomiting or diarrhea, and/or sore throat). Daily screening for respiratory symptoms was performed in the inpatient setting and repeat testing encouraged for those with changes in symptoms consistent with possible COVID-19 disease. Providers were advised, though, to only repeat PUI testing on inpatients after discussion with, and verbal approval by, the on call COVID-19 infectious disease physician, but this was not actively enforced. This meant most repeat PUI tests for inpatients met clinical suspicion for high-risk or high likelihood of the patient actually having COVID-19 despite a first negative test.

On March 28, 2020, we initiated pre-procedure testing for asymptomatic individuals undergoing procedures in which exposure to oral/respiratory secretions were possible. Pre-procedure screening was required to be completed within 48 hours of a procedure, with repeat testing performed for subsequent procedures if they fell outside of 48 hours of the first test or if the original procedure was rescheduled to more than 48 hours after the test result. For example, a patient having surgery under general anesthesia on hospital days 1, 4, and 7 would have 3 separate tests, each occurring within 48 hours of each surgery. Advice on which procedures met criteria was circulated to all providers, but, as with repeat PUI testing, proper test utilization was not actively enforced.

Finally, admission screen testing for all individuals admitted to certain units (e.g. NICU, pediatric and adult ICU's) was performed routinely throughout the period and eventually expanded on April 21, 2020, to every admission irrespective of reason or unit location.

Given that repeat screening of asymptomatic individuals was a much different clinical scenario than repeat testing of someone suspected to have COVID-19 (i.e. PUI), we separated the analysis for these two situations. Also, it is important to note that a small subset of patients had more than 2 tests performed and may have had both repeat PUI testing and repeat asymptomatic screening over the study period. Each subsequent test was considered a separate repeat test in the analysis and therefore the total number of tests is greater than the study population. We present descriptive statistics to summarize the data. The University of Wisconsin Institutional Review Board determined this study to be exempt.

RESULTS

Repeat SARS-CoV-2 RT-PCR Testing for Entire Patient Population

In the analysis population there were 660 patients with >1 SARS-CoV-2 RT-PCR test (78 children and 582 adults) that were cared for in our health system. The results of repeat testing for the total population are shown in **Table 1**. Initial tests were positive in 22 (3%) patients and negative in 638 (97%) patients. In those initially positive, there were 12 patients who converted from positive to negative on a repeat test. The median time between first test and repeat test for those that converted to negative was 20 days (range 7-43 days), whereas for those that retested positive the median time between tests was 15 days (range 2-35 days). Of those that tested negative initially (n=638), there were only 6 conversions to positive (0.9% negative-to-positive conversion rate), which was noted on repeat tests done between 5 to 17 days after an initial negative test.

Repeat SARS-CoV-2 RT-PCR Testing for PUI

Repeat testing as part of a PUI work-up numbered 275 patients (257 adult, 18 pediatric). A repeat test (e.g. second, third, or rarely 4th test) for PUI occurred in 63 inpatients and 212 outpatients. For inpatients, 6 patients tested positive for SARS-CoV-2 on a repeat PUI test and all 6 were known to be positive from their first test that was done as part of an initial PUI work-up (range of time to repeat testing was 2-19 days). The remainder of inpatients tested negative on repeat PUI testing. Within this cohort, we found 5 instances in which an inpatient

had a negative PUI test following an initial positive test (range of time to repeat testing was 8-29 days). Thus, 52 of 52 inpatients who tested initially negative for PUI evaluation were negative on repeat testing. Said another way, we found no cases of inpatients demonstrating conversion from a negative test to a positive test on repeat PUI testing. The median time to repeat PUI testing for inpatients was only 4 days (range 0-26 days). It is also noteworthy that 45% of patients with repeat PUI testing in the inpatient setting did not have a clear alternative diagnosis.

Out of 212 outpatients with a repeat PUI test, 13 tested positive but only 7 were known to be positive from prior testing. Thus, 6 outpatients converted from an initial negative test result to a positive test result (**Table 2**). It is notable that 4 of the 6 had a testing interval >10 days between negative to positive conversion. Similar to inpatient testing, we found 5 patients had tested positive on an initial test and converted to negative on repeat testing in the outpatient setting, and the time between first test (positive) and second test (negative) was 10-43 days. Those that had repeated negative PUI testing results as outpatients had a median time to repeat testing of 14 days (range 0-51 days).

Repeat SARS-CoV-2 RT-PCR Testing for Asymptomatic Screening

Repeat PCR testing as part of asymptomatic screening (e.g. pre-procedure or admission screening) for SARS-CoV-2 was performed in 431 patients. Inpatient repeat screening was performed on 215 patients with 248 repeat screening tests (29 had >2 screening tests for repeated procedures over a prolonged hospitalization), and all were negative on repeat screening. Therefore, similar to PUI testing we failed to demonstrate any inpatient negative-to-positive conversions for asymptomatic screening. The median time to repeat screen for asymptomatic inpatient testing was only 4 days (range 0-35 days). Outpatient asymptomatic screening, largely performed for planned procedures, occurred in 216 patients. There were 2 positives, both of which were known to be positive from an initial test. One patient was positive on asymptomatic screening 35 days after previously testing positive and the second

was positive on asymptomatic screening 16 days after previously testing positive. For the remainder of outpatients that tested repeatedly negative on asymptomatic screen testing, the median time between tests was 11 days (range 0-52 days).

Diagnostic Testing Stewardship

As part of the data analysis we examined the appropriateness of PUI testing and asymptomatic screening in the context of institutional guidance that was conveyed at the time of testing. We found 29.6% of repeat PUI testing and 31.4% of repeat asymptomatic screens likely should not have been performed based on institutional guidance. The most common reason for inappropriate PUI repeat testing was provider judgement. The most common reasons for inappropriate screening included a pre-procedure screen performed for a patient that never had a procedure (n=61), a screen performed with inappropriate timing in relation to the procedure leading to additional screening (n=33), and a screen performed for a procedure that did not meet institutional guidelines to perform screening prior to said procedure (n=66).

DISCUSSION

In this retrospective observational study, we demonstrated a number of important findings to inform ongoing utilization of SARS-CoV-2 RT-PCR testing resources at our institution. We believe the biggest lesson learned for our institution is that we found no cases of conversion from a negative to a positive result for inpatients undergoing a repeat PUI test or a repeat asymptomatic screen test. Other reports of the clinical sensitivity of RT-PCR testing for COVID-19 have demonstrated a sensitivity range of 80-95% for PUI [9-13]. These studies therefore suggest that a subset of patients may test negative when in fact they are positive, and consequently repeat testing may be warranted to identify these patients. However, in our study, we failed to find a single occurrence of negative-to-positive conversion in 308 repeat tests in the inpatient setting. It is noteworthy that the median time to repeat testing was only 4 days and repeat PUI testing at our center was, in general, directed at those that

were negative but had high risk or high likelihood based on clinical factors of having COVID-19 disease. Thus, we conclude that the PCR testing methods (e.g. specimen collection and testing platforms), combined with infection control measures, likely lead to higher sensitivity and lower likelihood of false-negative testing results in a low prevalence area than previously suggested. We acknowledge that the study design is not amenable to estimate the true sensitivity as not all patients were serially tested and there is no agreed upon gold-standard confirmatory test.

A recent study by Long and colleagues at two large academic centers in the US have recently published a similar study examining the rates of conversion from negative to positive NP SARS-CoV-2 RT-PCR test results when performed within 7 days of each other [9]. Out of 626 patients, 22 (3.5%) converted from negative to positive. It is unclear the number of inpatients versus outpatients for the second test, but based on the initial test location it appears their dataset included mostly outpatients. Therefore, it is possible ongoing community exposure could occur within the repeat testing window in the study. In our population, we had only 6 patients convert from negative to positive out of 638 patients. Within those 6 conversions, 2 of those converted within a 7 day window from the first test. When we limit our dataset to repeats within 7 days, we had 330 patients who had a repeat test within 7 days of an initial test leading to a conversion rate of 0.6%. Differences in prevalence, infection control measures (inpatient and ambulatory) and/or compliance with those measures, and the number of asymptomatic screens done between the two studies likely explains the differences in rate of conversion. However, it is important to note that both studies suggest false negative NP SARS-CoV-2 RT-PCR results may be much lower than previously believed.

Our results have important implications for improving diagnostic stewardship of SARS-CoV-2 RT-PCR testing at our facility. Indeed, we continue to discourage repeat testing for PUI in the inpatient setting unless it is discussed and approved by an Infectious Disease physician.

This restriction, though, has limited effect as we found a number of repeat PUI tests were not indicated but still done at the discretion of the provider (i.e. ordered without approval from Infectious Diseases). Certainly, one way to improve diagnostic stewardship would be to institute prospective monitoring of PUI orders, especially in the inpatient setting. As we demonstrated negative-to-positive conversions for PUI testing in the outpatient setting, consistent with ongoing community spread of COVID-19, we believe it is advisable to continue to recommend and perform aggressive patient testing for PUI in this setting.

For asymptomatic screening we have also modified our procedures based on this data. We have recently extended the repeat asymptomatic screening to testing only once every 7 days for asymptomatic inpatients undergoing certain procedures, which have also been revised to include mainly aerosol generating procedures rather than all procedures. As above with PUI testing, though, we found numerous examples of providers ordering screening tests when not indicated. Some of this is not unexpected because plans for a procedure are sometimes quite fluid, and thus there were instances where patients were screened (1) in anticipation of a procedure that never occurred, (2) for a procedure that was delayed, necessitating another screening test as it fell outside the testing window, or (3) for a procedure that was later deemed not necessary. Active prospective monitoring of the ordering could improve diagnostic stewardship practices in these situations as well.

There are limitations to our study results and generalizability to other institutions as this was a single center, retrospective, observational study where the only type of specimen collected was an NP swab. Therefore, we were unable to examine the impact of sampling site on differences in congruency between first and subsequent SARS-CoV-2 RT-PCR testing results noted in our study versus those noted in other studies and sampling site may impact testing results [14]. Second, our results were noted in an area of the country with a prevalence of 4-6 per 100,000 population in Dane County, WI (https://www.nytimes.com/interactive/2020/us/wisconsin-coronavirus-cases.html#county).

Prevalence of COVID-19, testing procedures (e.g. sampling technique, type of testing platform), and infection control measures (e.g. PPE, hygiene measures, visitor policies, etc.) are different at each institution and could significantly affect the likelihood of discordant repeat testing results compared to initial results.

The vast majority of medical centers in the US are utilizing commercially available platforms in which reagents, materials, and machines are all finite and may be further constrained by voluminous testing, making diagnostic stewardship critically important. Thus, we believe our study may provide useful data for other institutions to use when considering diagnostic stewardship. In summary, we did not observe any inpatient negative-to-positive conversions for PUI or asymptomatic screening purposes. This has led us to further refine our inpatient and ambulatory testing procedures to optimize SARS-CoV-2 RT-PCR testing resources. Further diagnostic stewardship may be enhanced through prospective monitoring of tests ordered, particularly for inpatients.

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Conflicts of Interest

All authors report no conflicts of interest in relation to this study.

Patient Consent Statement

The design of the work has been approved by local ethical committees or that it conforms to standards currently applied in the country of origin, and includes the name of the authorizing body which should be stated in the paper. As stated in manuscript above “The University of Wisconsin Institutional Review Board determined this study to be exempt.” Specifically, the IRB determined the study met criteria for exempt human subjects research as it was secondary research for which patient consent is not required. The exemption is on file locally.

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Table 1. Overall results of repeat testing based on patient status at time of testing. Note that total numbers add up to more than the total number of patients (n=660) as some patients had >2 tests with various combinations of inpatient and outpatient status as well as PUI versus asymptomatic screens.

Initial and repeat COVID-19 PCR testing result	Outpatient Status			Inpatient Status		
	Number of Patients	Number of Repeat Tests	Median (range) Time to repeat testing (days)	Number of Patients	Number of Repeat Tests	Median (range) Time to repeat testing (days)
Negative -> Negative	420	469 (PUI = 219, AS = 250)	13 (0-52)	257	308 (PUI = 60, AS = 248)	4 (0-35)
Negative -> Positive	6	7 (PUI = 7, AS = 0)	13 (5-17)	0	0 (PUI = 0, AS = 0)	
Positive -> Negative	7	7 (PUI = 7, AS = 0)	31 (10-43)	5	8 (PUI = 8, AS = 0)	17 (8-29)
Positive -> Positive	7	12 (PUI = 9, AS = 3)	15 (7-35)	6	8 (PUI = 8, AS = 0)	13 (2-19)

PUI, person under investigation; AS, asymptomatic screen

Table 2. Characteristics of the 6 patients that converted from a negative to a positive SARS-CoV-2 RT-PCR test result on repeat testing.

Gender	Age (y)	Test 1 Result	Test 1 Type	Test 2 Result	Test 2 type	Time between tests 1 and 2 (days)	Reason for repeat test	Patient Status
Male	85	negative	Panther Fusion	positive	Panther Fusion	17.4	PUI	outpatient
Male	58	negative	UW/CDC RT-PCR	positive	Panther Fusion	5.4	PUI	outpatient
Female	25	negative	Panther Fusion	positive	Panther Fusion	6.1	PUI	outpatient
Female	28	negative	UW/CDC RT-PCR	positive	UW/CDC RT-PCR	13.9	PUI	outpatient
Female	52	negative	WSLH RT-PCR	positive	UW/CDC RT-PCR	13.8	PUI	outpatient
Male	70	negative	UW/CDC RT-PCR	positive	Panther Fusion	11.5	PUI	outpatient

WSLH, Wisconsin State Lab of Hygiene