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

Alexander J. Lepak, Derrick J. Chen, Ashley Buys, Linda Stevens, and Nasia Safdar

1Division of Infectious Diseases, Department of Medicine, University of Wisconsin School of Medicine and Public Health, Madison, Wisconsin, USA; 2Department of Pathology and Laboratory Medicine, University of Wisconsin School of Medicine and Public Health, Madison, Wisconsin, USA; 3Clinical Infection Control Practitioner, UW Health University Hospital, Madison, Wisconsin, USA; 4Nursing Quality and Safety, UW Health University Hospital, Madison, Wisconsin, USA; and 5William S. Middleton Memorial Veterans Affairs Medical Center, Madison, Wisconsin, USA

Background. Multiple factors have led to an extremely high volume of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) reverse transcription polymerase chain reaction (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 1 of 4 validated assays. Key clinical and demographic data were 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 coronavirus disease 2019 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 workup 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. nasopharyngeal; RT-PCR; SARS-CoV-2; stewardship.

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 United States. 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 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
urged to 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 re-
results of repeat SARS-CoV-2 RT-PCR testing for patients who
were suspected of having COVID-19 (persons under investiga-
tion [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 in-
cludes 3 hospitals, >120 clinics, and serves >600 000 patients
in the Upper Midwest, were eligible for inclusion in this study.
Those that had >1 SARS-CoV-2 RT-PCR test 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 were not obtainable
from medical records. UW Health employees who were tested
through employee health services were also excluded. All sam-
ple were obtained via an NP sampling technique. SARS-CoV-2
RT-PCR testing was performed initially by the Wisconsin State
Laboratory of Hygiene using the Centers for Disease Control
and Prevention (CDC)–provided assay from March 12 to 21,
2020. On March 18, UW Health started in-house testing using a
laboratory-developed test based on the CDC primer-probe de-
sign targeting the N1 and N2 regions of the viral nucleocapsid
gene. Soon thereafter, additional assays were brought in-house
and validated using the Hologic Panther Fusion SARS-CoV-2
Assay (Hologic, Inc., Marlborough, MA, USA) and Cepheid
Xpert Xpress SARS-CoV-2 test (Cepheid, Sunnyvale, CA, USA);
both assays were performed according to the manufacturer’s in-
structions for use under emergency use authorization.

Advice on testing and/or retesting 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 en-
counter, an emergency room or urgent care encounter, or on
admission as part of the initial admission workup (ie, within
the first 24 hours). Inpatient testing was defined as specimen
collection that was performed after the first 24 hours during
a hospitalization. PUI vs 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 data set. 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
(eg, unexplained fever, chills, cough, shortness of breath/hy-
poxia, 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 was en-
couraged 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 preprocedure testing
for asymptomatic individuals undergoing procedures in
which exposure to oral/respiratory secretions were possible.
Preprocedure screening was required to be completed within 48
hours of a procedure, with repeat testing performed for subse-
quent procedures if screening fell outside of 48 hours from 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 cir-
culated 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 (eg, neonatal intensive care unit, pediatric and
adult intensive care units) 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 dif-
f erent clinical scenario than repeat testing of someone suspected
to have COVID-19 (ie, PUI), we separated the analysis for these
2 situations. Also, it is important to note that a small subset of
patients had >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 sta-
istics 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 the Entire Patient Population
In the analysis population, there were 660 patients with >1
SARS-CoV-2 RT-PCR test (78 children and 582 adults) who
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 the first test and repeat testing (range) for those who converted to negative was 20 (7–43) days, whereas for those who retested positive the median time between tests (range) was 15 (2–35) days. Of those who 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 and 17 days after an initial negative test.

Repeat SARS-CoV-2 RT-PCR Testing for PUI
Repeat testing as part of a PUI workup numbered 275 patients (257 adult, 18 pediatric). A repeat test (eg, a second, third, or rarely a fourth 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 workup (range of time to repeat testing, 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 after an initial positive test (range of time to repeat testing, 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 (range) was only 4 (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 from negative to positive conversion. Similar to inpatient testing, we found that 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 who had repeated negative PUI testing results as outpatients had a median time to repeat testing (range) of 14 (0–51) days.

Repeat SARS-CoV-2 RT-PCR Testing for Asymptomatic Screening
Repeat PCR testing as part of asymptomatic screening (eg, preprocedure 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

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**Table 2. Characteristics of the 6 Patients who 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, d | 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     |

Abbreviations: RT-PCR, reverse transcription polymerase chain reaction; PUI, person under investigation; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; UW/CDC, University of Wisconsin/Centers for Disease Control and Prevention; WSLH, Wisconsin State Lab of Hygiene.
inpatient testing (range) was only 4 (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 who tested repeatedly negative on asymptomatic screen testing, the median time between tests (range) was 11 (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 that 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 judgment. The most common reasons for inappropriate screening included a preprocedure screen performed for a patient who 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 before 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 who 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 (eg, 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 estimating the true sensitivity, as not all patients were serially tested and there is no agreed-upon gold standard confirmatory test.

Long and colleagues, at 2 large academic centers in the United States, 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. The number of inpatients vs outpatients for the second test is unclear, but based on the initial test location it appears that their data set included mostly outpatients. Therefore, it is possible that 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 limited our data set 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 2 studies likely explains the differences in rate of conversion. However, it is important to note that both studies suggest that 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 a limited effect as we found a number of repeat PUI tests were not indicated but still done at the discretion of the provider (ie, 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 these 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 the first and subsequent SARS-CoV-2 RT-PCR testing results noted in our study vs those noted in other studies, and the sampling site may have impacted the 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, Wisconsin (https://www.nytimes.com/interactive/2020/us/wisconsin-coronavirus-cases.html#county). The prevalence of COVID-19, testing procedures (eg, sampling technique, type of testing platform), and infection control measures (eg, PPE, hygiene measures, visitor policies, etc.) are different at each institution and could significantly affect the likelihood of discordant repeat testing results compared with initial results.

The vast majority of medical centers in the United States 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 useful data for other institutions to use when considering diagnostically important. Thus, we believe our study may provide materials, and machines are all finite and may be further constrained by commercially available platforms in which reagents, materials, and machines are all finite

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