One Swab Fits All: Performance of a Rapid, Antigen-Based SARS-CoV-2 Test Using a Nasal Swab, Nasopharyngeal Swab for Nasal Collection, and RT–PCR Confirmation from Residual Extraction Buffer

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Background: Point-of-care SARS-CoV-2 antigen tests have great potential to help combat the COVID-19 pandemic. In the performance of a rapid, antigen-based SARS-CoV-2 test (RAT), our study had 3 main objectives: to determine the accuracy of nasal swabs, the accuracy of using nasopharyngeal swabs for nasal collection (nasalNP), and the effectiveness of using residual extraction buffer for real-time reverse-transcriptase PCR (RT–PCR) confirmation of positive RAT (rPan).

Methods: Symptomatic adults recently diagnosed with COVID-19 in the community were recruited into the study. Nasal samples were collected using either a nasalNP or nasal swab and tested immediately with the RAT in the individual's home by a health care provider. 500 μL of universal transport media was added to the residual extraction buffer after testing and sent to the laboratory for SARS-CoV-2 testing using RT–PCR. Parallel throat swabs tested with RT–PCR were used as the reference comparators.

Results: One hundred and fifty-five individuals were included in the study (99 nasal swabs, 56 nasalNP). Sensitivities of nasal samples tested on the RAT using either nasal or nasalNP were 89.0% [95% confidence interval (CI) 80.7%–94.6%] and 90.2% (95% CI 78.6%–96.7%), respectively. rPan positivity agreement compared to throat RT–PCR was 96.2%.

Conclusions: RAT reliably detect SARS-CoV-2 from symptomatic adults in the community presenting within 7 days of symptom onset using nasal swabs or nasalNP. High agreement with rPan can avoid the need for collecting a second swab for RT–PCR confirmation or testing of variants of concern from positive RAT in this population.

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IMPACT STATEMENT

There is limited information on the performance of rapid, antigen-based SARS-CoV-2 tests (RAT) with respect to nasal swabs, and in using nasopharyngeal swabs for nasal collection. Use of NP swabs for nasal collection may be an important alternative for NP collection when nasal swabs are in limited supply or when NP swabs are in excess. At the time of writing, no literature is available on the performance of using residual extraction buffer after RAT testing for subsequent RT–PCR confirmation. Using residual extraction buffer may remove the need for the collection of a second swab for RT–PCR confirmation.

INTRODUCTION

Numerous rapid, antigen-based SARS-CoV-2 tests (RAT) have become available to help combat the COVID-19 pandemic. Most RAT are immunochromatographic assays that detect SARS-CoV-2 nucleocapsid protein and are indicated for testing of symptomatic individuals using nasopharyngeal (NP) or nasal swabs. In general, these tests should be conducted immediately after collection, although some can be stored at room temperature for longer if the swab is stored in extraction buffer (1, 2).

At time of writing, there is a paucity of data available from external third parties on RAT performance using nasal swabs. When used in community settings for individuals who are within the first 7 days of symptom(s) onset, the Panbio (Abbott, IL, USA) demonstrated a sensitivity of 86.1% [95% confidence interval (CI) 81.3%–90.0%] and specificity of 99.9% (95% CI 99.5%–100.0%) when using NP swabs (3). Compared to reverse-transcriptase real-time–polymerase chain reaction (RT–PCR) of the residual RAT extraction buffer, Abbott demonstrated a sensitivity of 98.1% (95% CI 93.2%–99.8%) and specificity 99.8% (95% CI 98.6%–100.0%) when using nasal swabs collected from individuals exposed to SARS-CoV-2 or having COVID-19 symptoms within the first 7 days (2). However, instead of collecting a second parallel NP swab for RT–PCR as the comparator, Abbott performed RT–PCR from the residual RAT extraction buffer after its use and after adding 500 µL of Universal Transport Media (UTM) (henceforth called residual Panbio or "rPan"). This testing method is not an established reference method and, therefore, the results may be bias toward enhanced performance of the RAT using nasal swabs. One study comparing nasal swabs tested on the Panbio to RT–PCR from NP swabs demonstrated poor performance of the RAT, with a positive percentage agreement of 52.6% (95% CI 42.2%–62.7%) and 22.9% (95% CI 11.0%–40.6%) on symptomatic and asymptomatic individuals, respectively (4).

There is benefit to using alternative swabs for nasal collection for RAT testing. In Canada, for instance, kits containing the Panbio cartridges, buffer, and NP swabs (henceforth called "RAT NP kits") were distributed to individual provinces prior to the kits containing the Panbio cartridges, buffer, and nasal swabs (henceforth called "RAT nasal kits"). This resulted in a surplus of RAT NP kits in the millions. Due to the discomfort associated with NP collection, the RAT NP kits fell out of favor for use in asymptomatic screening, especially when antigen tests that used nasal swabs became readily available, such as the BD Veritor

(BD, NJ, USA) and subsequent RAT nasal kits. To consume the surplus NP swabs, some organizations started using nasopharyngeal swabs for nasal collection (nasalNP) for asymptomatic screening with RAT, despite no evidence to document its performance when used in this context. The narrower and more flexible NP swab is not designed for testing in the large anterior nares, with lack of surface contact between the NP swab and the nasal mucosa being a major concern in specimen quality. We present an alternative method to use a NP for nasal collections, which could expand the scenarios where the RAT NP kits could be used.

We sought to address 3 important points related to RAT testing among individuals with confirmed COVID-19 who are within the first 7 days of symptom onset: (*a*) The sensitivity of RAT when using nasal swabs; (*b*) the sensitivity of RAT when using nasalNP; and (*c*) the sensitivity of SARS-CoV-2 RT–PCR testing from residual RAT extraction buffer after RAT testing (rPan).

METHODS

We recruited individuals residing within the Calgary and Edmonton Health Zones of Alberta, Canada, who recently tested positive for SARS-CoV-2 and confirmed as cases by Alberta Health Services (AHS) Public Health (AB, Canada). Diagnostic testing was performed by a Health Canada approved SARS-CoV-2 PCR assay or a laboratory-developed RT–PCR assay (see next for details). Participants were identified by an AHS Public Health confirmed case list. Oral consent by phone was obtained to collect samples in the participant's home. The symptoms of the individual were recorded at the time of consent (usually within 24 h of collecting study swabs). Individuals under the age of 18, over the age of 70, or in supportive or congregate living facilities were excluded. Eligible individuals who consented to the study had 1 nasal sample (either using nasal swab or nasalNP) and 1 throat sample collected by trained healthcare professionals. RT–PCR from throat swabs was used as the reference standard to minimize the interference NP swab collection may have on nasal collection. Our laboratory previously evaluated the sensitivity of throat swabs to NP swabs for SARS-CoV-2 detection among symptomatic individuals and discovered similar sensitivities (5). The University of Calgary Research Ethics board approved this study (REB20-444).

Healthcare workers, previously trained in nasal and throat swab collection, were given instructions on how to collect swabs from recruited COVID-19 infected individuals. For reference standard RT–PCR testing, ClassiqSwabs for throat in COPAN UTM-RT (COPAN Diagnostics, CA, USA) were used. The RAT used for this study was the Panbio, using either the nasal swab or nasopharyngeal swab provided in the Panbio kits (Abbott). Whether the individual received the nasal swab or nasalNP depended on the date of collection. Nasal samples were collected by inserting the nasal or nasalNP into one nostril, up to 2.5 cm (approximately 1 inch) from the edge of the nostril, rolling the swab 3–5 times along the mucosa, and repeating the process for the other nostril. For nasalNP, the same protocol was followed except the nostril was gently squeezed against the swab during collection to ensure adequate surface contact. Throat swabs, for reference RT–PCR testing, were collected from both sides of the oropharynx and the posterior pharyngeal wall under the uvula and subsequently added to UTM for RT–PCR testing.

Nasal samples, using either a nasal swab or nasalNP, were tested immediately on the Panbio cartridge as per the manufacturer's instructions (2). After RAT testing, 500 μL of UTM was added to the residual tube containing the used swab. Throat swabs and residual RAT tubes containing 500 μL of UTM were transported to the laboratory at room temperature, stored at 4 °C on arrival and

tested within 72 hours of collection. Reference standard testing was performed by RT–PCR using either a laboratory-developed E gene assay or the Cobas SARS-CoV-2 assay. Our laboratory developed E gene RT-PCR assay was previously validated and demonstrated similar performance to the US CDC and WHO RT–PCR assays (6). The other RT–PCR assay was the Cobas® SARS-CoV-2 test on the Cobas 6800 instrument, which was run according to the manufacturer's instructions and also performs similarly to the US CDC and WHO RT–PCR assays (7, 8). For our laboratory-developed E gene RT–PCR assay, 200 µL of UTM were extracted on the MagMAX Express-96 or Kingfisher Flex (ABI) using the MagMAX-96 Viral RNA Isolation Kit (Thermo Fisher Scientific, MA, USA) or the PurePrep Pathogen Kit (MolGen, CA, USA) according to the manufacturer's instructions, and eluted into a volume of 110 µL.

The laboratory developed E gene RT–PCR assay, the samples were considered positive for SARS-CoV-2 when the E gene cycle threshold (Ct) value was <35. If the Ct was ≥35, amplification from the same eluate was repeated in duplicate and was considered positive if at least 2/3 results had a Ct <41. For the Cobas SARS-CoV-2 test, as per the manufacturer, a positive result was defined as 2/2 targets positive, or 1 or more targets were positive in duplicate. If 1 or 2 targets were positive and duplicate testing was negative, the result was considered indeterminate.

Sensitivity was calculated with Clopper–Pearson 95% confidence intervals (CI). Statistical analysis was performed using Pearson Chi-squared test for categorical variables and *t*-test for continuous variables using STATA (v.14.1).

RESULTS

One hundred and sixty-seven individuals were recruited (106 nasal swabs, 61 nasalNP). Twelve individuals were excluded: 4 RAT results were not recorded, 2 RT–PCR results were not completed due to leaking containers, 3 individuals had symptom onset >7 days, 2 individuals were under 18 years of age, and 1 individual did not have a throat sample collected for reference RT–PCR testing. Individual characteristics of the remaining 155 individuals (99 nasal swabs, 56 nasalNP) are provided in Table 1. E gene Ct values between RAT nasal swabs and nasalNP are provided in Fig. 1.

For the individuals from which the 99 nasal samples were collected, cough was the most frequent symptom at enrollment (43.4%), followed by headache (43.4%), fevers/chills (34.3%), pharyngitis (30.3%), sinus congestion (26.3%), myalgias (25.3%), malaise (23.2%), rhinorrhea (17.2%), ageusia (10.1%), nausea/vomiting (9.1%), anosmia

Table 1. Characteristics of symptomatic individuals with COVID-19 tested within 7 days of symptom onset with RAT, using either nasal swabs (*n* = 99) or nasalNP (*n* = 56).

Characteristic	Nasal swab (*n* = 99)	nasalNP (*n* = 56)	*P* value
Male gender	43.4%	42.9%	NS
Mean age in years (median, range)	39.4 (38.6, 18.5–73.4)	37.8 (36.3, 20.4–60.3)	NS
Mean throat swab RT–PCR Ct value (median, range)	29.7 (29.8, 18.5–38.2) (*n* = 79*)	29.7 (29.5, 21.3–37.5) (*n* = 46**)	NS
Mean rPan Ct value (median, range)	23.2 (22.4, 15.4–35.8) (*n* = 86)	22.2 (20.8, 15.2–33.9) (*n* = 53)	NS
Mean duration of symptoms from collection date in days (median, range)	3.9 (4, 1–7)	3.9 (4, 2–7)	NS

NS: not significant.
* 5 were positive on Cobas with no Ct value available.
** 3 were positive on Cobas with no Ct value available.

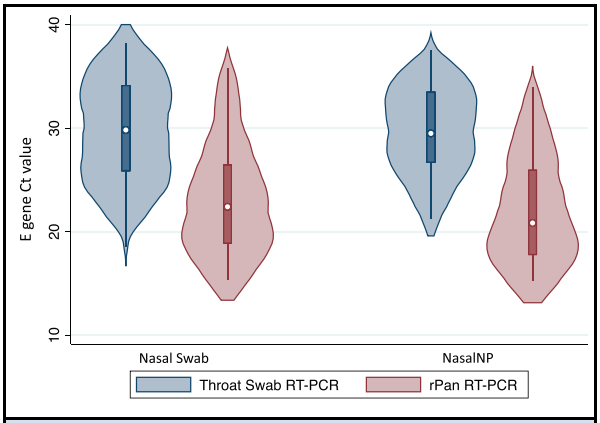

Fig. 1. Violin plot of Ct values from throat swab RT–PCR and rPan RT–PCR between RAT nasal swabs and nasalNP.

Table 2. Results of RAT nasal ($n = 99$) and RAT nasalNP ($n = 56$) compared to RT–PCR in symptomatic individuals with COVID-19 tested within 7 days of symptom onset.

		Throat RT–PCR	
		Positive	**Negative**
RAT nasal	Positive	74	7
	Negative	10	8
RAT nasalNP	Positive	44	2
	Negative	5	5

(8.1%), shortness of breath (2.0%), and other (10.1%; loss of appetite, metallic taste, diarrhea, chest pain, arthralgia, and/or abdominal pain).

For the individuals from which the 56 nasalNP samples were collected, cough was the most frequent symptom at enrollment (44.6%), followed by headache (44.6%), fevers/chills (35.7%), pharyngitis (26.8%), sinus congestion (26.8%), myalgias (33.9%), malaise (32.1%), rhinorrhea (21.4%), ageusia (8.9%), nausea/vomiting (8.9%), anosmia (10.7%), shortness of breath (8.9%), and other (25.0%; loss of appetite, gastrointestinal upset, chest pain, back pain, dizziness, arthralgia, chest tightness, diarrhea, paresthesia, metallic taste, and/or conjunctivitis).

When compared to parallel throat swabs tested with RT–PCR, and assuming any positive is a true positive result, the sensitivity of the RAT nasal swabs and nasalNP was 89.0% (95% CI 80.7%–94.6%) and 90.2% (95% CI 78.6%–96.7%), respectively (Table 2). Only 8 oropharyngeal samples were tested using the Cobas® SARS-CoV-2 test (5 from RAT nasal, 3 from RAT nasalNP). Excluding these 8 samples, sensitivity of the RAT nasal swabs and nasalNP was 88.4% (95% CI 79.7%–94.3%) and 91.7% (95% CI 80.0%–97.7%), respectively. There were 10 and 5 false negatives from the RAT nasal swabs and RAT nasalNP, respectively (Table 2). Throat RT–PCR results had higher E gene Ct values, on average, in RAT negative vs RAT positive samples but was not statistically significant (see Supplemental Table 2).

The total number of positive throat RT–PCR samples was 133. Excluding one sample that had insufficient quantity for rPan, the positivity agreement of rPan compared to throat RT–PCR was 127/132 (96.2%). Of the 5 rPan negative, throat RT–PCR positive samples, 4 had an E gene Ct value >30 (E gene Ct 25.5, 31.3, 34.6, 36.6 and 38.1). Differences between RAT, rPan, and throat RT–PCR positivity agreement is provided in Fig. 2. There were 8 and 7 RAT negative, rPan positive samples taken from nasal and nasalNP, respectively. For nasal swabs, the mean Ct values of rPan between RAT negative and RAT positive samples were 32.0 and 22.3, respectively ($P < 0.001$). For nasalNP, mean Ct values of rPan between RAT negative and RAT positive samples were 31.0 and 20.9, respectively ($P < 0.001$).

DISCUSSION

Our study has 3 important findings. First, similar sensitivity of RAT can be achieved when using nasal swabs (sensitivity 89.0%) compared to nasopharyngeal swabs (sensitivity 86.1%) among individuals who are within the first 7 days of

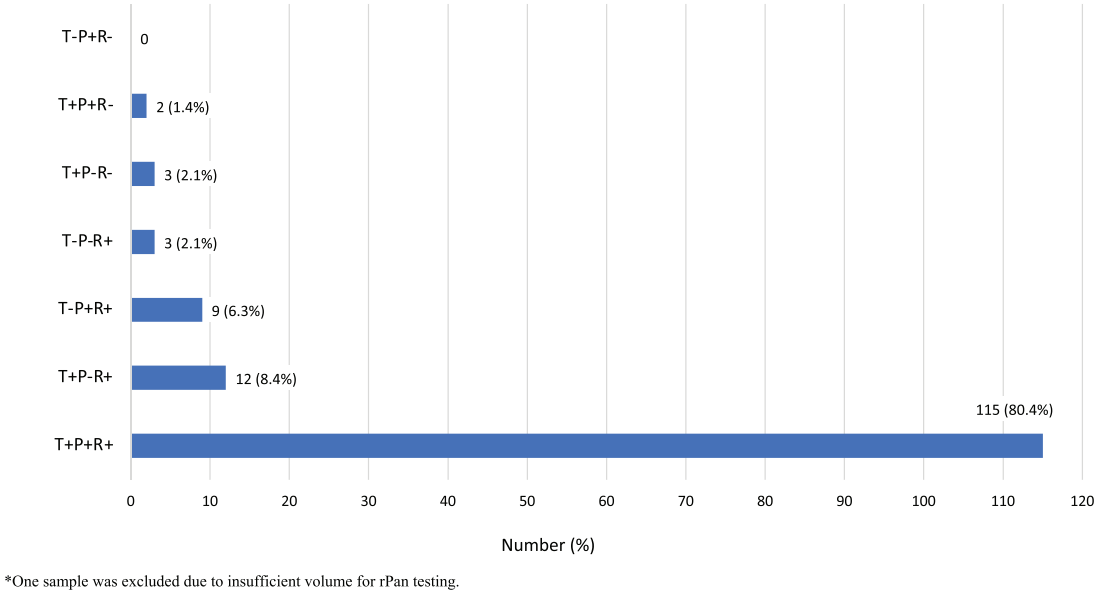

Fig. 2. Results between throat RT–PCR (T), RAT nasalNP/nasal (P), and rPan (R). *N* = 144*. *One sample was excluded due to insufficient volume for rPan testing.

symptom onset (3). Second, nasalNP can achieve similar sensitivity (90.2%) to nasal swabs, suggesting that nasalNP can be used as an alternative if used correctly. Third, confirming positive RAT results by RT–PCR can be achieved by adding 500 μL of UTM to the residual RAT extraction buffer and testing using RT–PCR (rPan).

We have previously demonstrated that the sensitivity and specificity of a RAT, the Panbio, when using NP swabs among individuals with symptoms ≤7 days are 86.1% and 99.9%, respectively (3). The study presented here demonstrated that nasal and nasalNP tested on the same RAT to have similar sensitivity to NP swabs. This is contrary to the findings of Agullo et al. who found that the Panbio had poor sensitivity/PPA for nasal (44.7%) and NP swab (57.3%). They also reported poor sensitivity in symptomatic patients tested with a nasal swab (52.6%), which was worse if individuals had symptoms >7 days at the time of testing (4). However, many studies have demonstrated higher performance of the Panbio with nasopharyngeal swabs, with sensitivity ranging from 72.6% to 86.1% among individuals with symptoms ≤7 days (3, 9–12).

Use of rapid antigen-based tests for the detection of SARS-CoV-2 among symptomatic individuals has many potential benefits to the COVID-19 pandemic response. Although confirmatory testing of negatives is recommended, identifying positives at the point of care has several advantages. It can speed important public health measures (such as contact tracing and isolation) and facilitate testing outside of a laboratory. Moreover, it has significant benefits for the laboratory in terms of decreasing error and improving laboratory processes. For instance, decreasing the number of positive samples entering the laboratory can decrease the risk of false positive results by reducing the probability of SARS-CoV-2 contamination during RT–PCR testing. In addition, the decrease in positive samples can improve efficiencies in other laboratory processes, such as pooling.

Our study provides key insights into the use of alternative sample methods for RAT testing, and

unique ways to pursue RT–PCR confirmation and/or variant of concern testing on RAT positive samples. With the large rollout of asymptomatic screening for COVID-19 occurring across the world, these results have important implications. Instead of spending significant time, effort, and costs into replacing nasopharyngeal swabs with nasal swabs for RAT testing, our study has shown that adequate performance can be achieved when using nasalNP.

Furthermore, PCR testing may be necessary to either test for variants and/or to confirm positive results from RAT (13). Depending on where the specimen was collected, obtaining a second sample for RT–PCR confirmation or variant testing may prove inconvenient, or time-consuming. False negatives are also possible due to a potential delay in obtaining a second sample for confirmatory testing. Differences in sample collection may also lead to discrepant results, as was observed in our study. To mitigate this, we found that adding UTM to the residual RAT extraction buffer (rPan) can be used as an alternative for RT–PCR confirmation from a second collected sample. Based on our study, rPan was more likely to yield a positive result than RT–PCR from a throat sample (Fig. 2). Of course, we cannot guarantee that similar performance will be observed when used for SARS-CoV-2 detection among asymptomatic individuals, as viral loads will likely be lower in this context. However, as observed in this study, RT–PCR testing of residual RAT extraction buffer was often positive when RAT is negative, suggesting that almost all RAT positive samples should be detected with RT–PCR testing of the residual extraction buffer, regardless of the population being tested.

Our study was predominately restricted to individuals within the community who had symptoms $\leq$7 days. As such, our study is unable to provide any conclusions about RAT performance among individuals admitted to hospital, in congregate living facilities, who are asymptomatic, and individuals with symptoms >7 days. An additional limitation of our study was the testing of known COVID-19 patients rather than prospective testing of suspect cases. While there were several instances in our study where the RAT was positive and the throat RT–PCR was negative, subsequent rPan was positive in each case. This implies that accuracy can fluctuate from factors other than test performance, mainly from differences in sample collection. It is also worth noting that NP swabs may have slightly improved sensitivity compared to oropharyngeal swabs for SARS-CoV-2 RT–PCR testing (14) and, therefore, our sensitivity of the nasal swabs and NasalNP for RAT testing may be higher than if NP swabs were used as the reference method. However, our own local data have demonstrated similar performances between oropharyngeal and NP swabs .

The RAT nasal kit was able to detect SARS-CoV-2 in most individuals with COVID-19 who are symptomatic within the first 7 days. If necessary, NP swabs can be used as an alternative for nasal sample collection (nasalNP) and confirmation and/or variant testing of positive RAT results can be done by RT–PCR testing of residual RAT extraction buffer (rPan) as opposed to collection of a second sample. Given the speed, low-complexity, acceptable performance, and more tolerated sample type, the RAT nasal test is a suitable COVID-19 test when used in the right setting, especially when rapid identification of positive patients is critical.

SUPPLEMENTAL MATERIAL

Supplemental material is available at *The Journal of Applied Laboratory Medicine* online.

Nonstandard Abbreviations: RAT, Rapid antigen-based SARS-CoV-2 tests; NP, nasopharyngeal; RT–PCR, reverse-transcriptase real-time–polymerase chain reaction; UTM, universal transport media; rPan, RT–PCR testing from residual RAT extraction buffer after RAT testing; nasalNP, nasopharyngeal swabs for nasal collection; AHS, Alberta Health Services Public Health; Ct, cycle threshold; CI, confidence intervals.

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