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MAJOR ARTICLE

Evaluation of Nasopharyngeal Swab Collection Techniques for Nucleic Acid Recovery and Participant Experience: Recommendations for COVID-19 Diagnostics

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Nasopharyngeal swabs are critical to the diagnosis of respiratory infections including coronavirus disease 2019, but collection techniques vary. We compared 2 recommended nasopharyngeal swab collection techniques in adult volunteers and found that swab rotation following nasopharyngeal contact did not recover additional nucleic acid (as measured by human DNA/RNA copy number). Rotation was also less tolerable for participants. Notably, both discomfort and nucleic acid recovery were significantly higher in Asian participants, consistent with nasal anatomy differences. Our results suggest that it is unnecessary to rotate the swab in place following contact with the nasopharynx and reveal that procedural discomfort levels can differ by ethnicity.

Keywords: biological material; COVID-19; ddPCR; ethnicity; nasopharyngeal swab; participant experience; RT-ddPCR; sample quality.

Nasopharyngeal swabs are critical for accurate diagnosis of respiratory tract infections including coronavirus disease 2019 (COVID-19) [1, 2]. Specimen collection, which involves inserting a long flexible swab through the nostril along the floor of the nasal cavity to a depth of ~7 cm and into the nasopharynx, must be performed by a trained health care professional familiar with the technique and nasal anatomy [1]. There is, however, no consensus for optimal swab collection. Following contact with the nasopharynx, the World Health Organization, for example, recommends that the swab be left in place for a few seconds before withdrawal [3], while the US Centers for Disease Control and Prevention (CDC) recommends that the swab be "gently rubbed and rolled" and left in place for several seconds before withdrawal [4]. Other guidance documents recommend that the swab be rotated in place before removal [5–8]. Given that swab insertion/removal is invasive and uncomfortable [1, 9], a better understanding of the impact of postinsertion collection techniques on sample quality and patient experience may refine collection methods.

We recruited adult volunteers to undergo a nasopharyngeal swab with or without rotation and to provide saliva, an alternative COVID-19 diagnostic specimen [10–12], as a comparator. Participants rated their discomfort during the swab on an 11-point scale [13] and were asked which specimen, swab or saliva, was less unpleasant to give. We assessed nucleic acid recovery as a marker of swab collection quality [2], where human RPP30 [2] and human RNase P copy numbers were used as surrogates for DNA and RNA recovery, respectively.

METHODS

We recruited 69 participants over 3 days in July 2020. For safety reasons, participation was restricted to individuals without symptoms of COVID-19 or other respiratory illnesses, and participants were assumed to be COVID-19 negative. A single experienced health care provider collected all nasopharyngeal swabs using the Puritan UniTranz-RT transport system (Puritan Medical Products). Due to the potential for mild trauma incurred by the procedure, each participant underwent only 1 swab. To do this, participants were assigned to 1 of the 2 swab collection techniques at study entry, though they were blinded to the specific technique until immediately before the procedure. Before collection, the provider instructed participants to alternatingly apply pressure to each nasal ala to identify the less congested nostril. The provider estimated the depth to the participant's posterior nasopharynx by holding the swab

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externally from the nasal ala to tragus and viewed the nasal passage to check for mucus and obstructions (if mucus was visible, the participant was instructed to blow their nose [1]). With the participant's head tilted back slightly, the provider gently inserted the swab into the identified nostril along the lateral aspect of the nasal cavity floor and into the nasopharynx. For half the participants, the swab was removed after reaching the nasopharynx ("in-out" swab). For the remaining participants, the swab was rotated in place for 10 seconds following placement in the nasopharynx ("rotation" swab) and then removed. Swabs were immediately placed in transport medium. To help evaluate participant experience in providing samples for diagnostic purposes, participants were also asked to provide ~2 mL of saliva into a sterile container (Starplex Scientific) by focusing on pooling saliva and gently expelling it into the container, repeating until the required volume was achieved. Participants were asked to rate their discomfort during the swab on an 11-point scale [13], where "0" denoted a complete absence of discomfort and "10" denoted the most severe discomfort possible. Finally, participants were offered the hypothetical choice of providing saliva or undergoing a nasopharyngeal swab for a diagnostic purpose and asked: *Purely based on your experience today*, which sample would you prefer to give and why?

Swabs were processed within 5 hours of collection. Total nucleic acids were extracted from 1 mL of medium on a NucliSens easyMAG (BioMérieux) and eluted in 60 µL. Eluates were split into 3 aliquots and frozen at –80°C until use. Droplet digital polymerase chain reaction (ddPCR) and reverse transcriptase (RT) ddPCR were used to quantify human RPP30 copy numbers and RNase P transcript numbers, respectively. In this technology, each sample is fractionated into 20 000-nL-sized water-in-oil droplets before amplification with sequence-specific primers and fluorescent probes, and input template concentrations are calculated at the end point using Poisson statistics. The RPP30 assay, described previously, yields a final measurement of cells/µL extract [2]. In the RNase P assay, nucleic acid extracts were combined with the CDC-developed RNAseP-specific primer/probe set [14], XhoI restriction enzyme (New England Biolabs), and the One-Step RT-ddPCR Advanced Kit for Probes (BioRad). Primer and probe sequences are as follows: Forward Primer-AGATTTGGACCTGCGAGCG, Reverse Primer-GAGCGGCTGTCTCCACAAGT, Probe-FAM-TTCTGACCT-ZEN-GAAGGCTCTGCGCG-3IABkFQ (Integrated DNA Technologies; ZEN = internal ZEN quencher; 3IABkFQ = 3' Iowa Black Quencher). Droplets were generated using an Automated Droplet Generator (BioRad) and cycled at 50°C for 60 minutes, 40 cycles of (94°C for 30 seconds and 55°C for 1 minute), and 98°C for 10 minutes and analyzed on a QX200 Droplet Reader using QuantaSoft software, version 1.7.4 (BioRad). Measured RNase P copies were normalized to input volume to determine RNase P copies/µL extract. RNase P levels were also assessed using real-time RT-PCR with the same primer/probe set on a Roche Lightcycler 480 according to the CDC protocol [15]. All 3 assays were performed on independent extract aliquots to avoid freeze-thaw. All ddPCR and RT-ddPCR assays were performed in duplicate, and results were averaged between replicates. Nonparametric statistics were used for all correlations and between-group comparisons. Contingency tables were analyzed using Fisher's exact test. Lin's concordance coefficient was used to calculate concordance between replicates. As the purpose of this study was to evaluate nasopharyngeal swab collection techniques, human DNA/RNA was not quantified in saliva samples.

Patient Consent Statement

This study was approved by the Providence Health Care/University of British Columbia and Simon Fraser University Research Ethics Boards. All participants provided written informed consent.

RESULTS

The median age of the 69 participants (interquartile range [IQR]) was 42 (36–54) years; 43 (62%) were female. Self-reported ethnicities were 44 (64%) White, 21 (30%) Asian (including 14 East, 1 Central, 3 South, and 3 Southeast), and 4 (6%) other (including 2 Latino and 2 mixed ethnicity). For 5 (7.2%) participants, the nasopharyngeal swab was unsuccessful due to obstruction despite attempts through both nares, leaving 64 (34 "in-out" and 30 "rotation") swabs for analysis. Discomfort scores ranged from 1 (minimal discomfort) to 10 (maximum discomfort) in both swab groups, with no significant difference between them (median [IQR], 5 [3.75–5] for "in-out" vs 4.5 [4–6] for "rotation"; $P = .51$) (Figure 1A). This suggests that most of the discomfort occurs during swab insertion/withdrawal, a notion that is supported by the significantly higher discomfort reported by participants with occlusions ($P < .001$) (Figure 1B). However, responses to additional study questions suggested that swab rotation was less tolerable. First, though most participants preferred giving saliva, 10 of 34 (29.4%) participants in the "in-out" group preferred the swab, vs only 3 of 30 (10%) participants in the "rotation" group (Fisher exact test $P = .068$) (Figure 1C), citing that the swab was easier, faster, and/or generally less unpleasant than giving saliva. Moreover, 2 participants in the "rotation" group mentioned that they had previously undergone an "in-out" swab and that the additional rotation made the procedure more uncomfortable. One added that, given the choice between "in-out" swab and saliva, they preferred the swab, but given the choice between "rotation" swab and saliva, they preferred saliva. Discomfort scores did not differ by sex ($P = .85$) or age (Spearman's $\rho = 0.05$; $P = .7$). Of note, however, Asian participants reported significantly higher discomfort scores compared with White participants (median [IQR], 5 [4–7] vs 4 [3.5–5], respectively; $P = .047$) (Figure 1D).

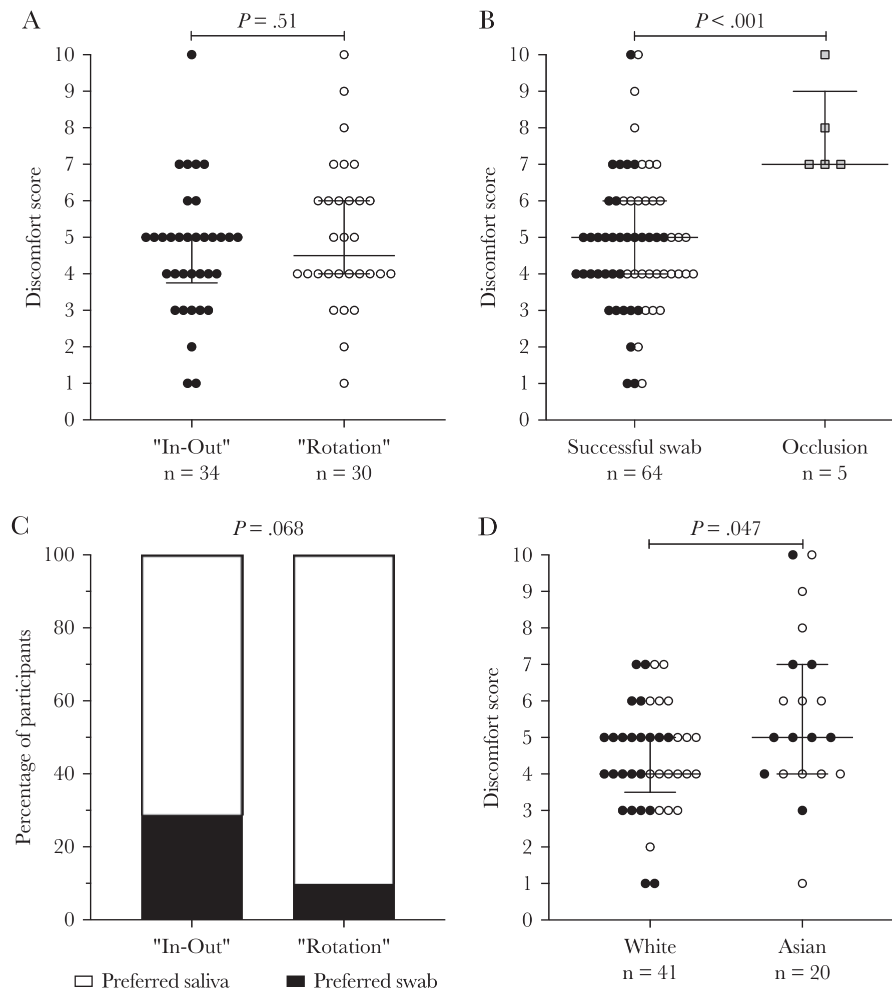

Figure 1. Differences in participant experience by nasopharyngeal swab technique and ethnicity. A, No difference in discomfort score between "in-out" (black circles) and "rotation" (white circles) swab groups was observed. B, Significantly higher discomfort was reported in participants with occlusion (gray squares) compared with those with a successful swab (black and white circles, denoting the groups described in (A). C, A greater proportion of "rotation" swab participants preferred to give saliva compared with "in-out" swab participants. D, Significantly higher discomfort scores were reported in Asian compared with White participants. Individuals of other ethnicities were excluded due to low numbers (n = 4).

RPP30 (DNA) and RNase P (RNA) copy numbers were measured as surrogates of nucleic acid recovery. Concordance between replicates was high for both targets (Lin's concordance coefficient: RPP30 = 0.98; $P < .0001$; RNase P = 0.91; $P < .0001$), and measurements of both targets correlated strongly with one another (Spearman's $\rho = 0.84$; $P < .0001$). Both RPP30 and RNase P levels varied markedly regardless of swab technique: RPP30 levels extended over a 42-fold range (from 42 to 1751 cells/µL extract) (Figure 2A), while RNase P levels extended over a 20-fold range (from 183 to 3570 copies/µL extract) (Figure 2B). Moreover, we found no significant differences in RPP30 levels by swab technique (median [IQR], 500 [235–738] cells/µL extract for "in-out" vs 503 [398–685] for "rotation"; $P = .83$) (Figure 2A) or RNase P values by swab technique (median [IQR], 1338 [610–2039] RNase P copies/µL extract for "in-out" vs 1309 [973–1789] for "rotation"; $P = .84$) (Figure 2B). Together, this indicates that swab rotation does not recover more nucleic acid and suggests that the amount of cellular material recovered is participant-specific. Indeed, when stratified by ethnicity, nucleic acid recovery was significantly higher in Asians, who reported on average higher discomfort levels (see Figure 1D). Specifically, the median RPP30 levels (IQR) were 610 (430–780) vs 431 (223–621) cells/µL extract from Asian vs White participants ($P = .026$) (Figure 2C), while median RNase P levels were 1629 (1167–2095) vs 1193 (531–1758) RNase P copies/µL extract from these same groups ($P = .038$) (Figure 2D). No significant differences in RPP30 or RNase P levels were observed by sex, age, or recruitment date (a surrogate of nucleic acid extraction run). RNAse P levels were also measured using the CDC 2019-nCoV real-time RT-PCR diagnostic assay [15]. The resulting cycle threshold (C_t) values correlated strongly with those measured using RT-ddPCR (Spearman's $\rho = -0.9$; $P < .0001$) and yielded results entirely consistent with those described above (data not shown).

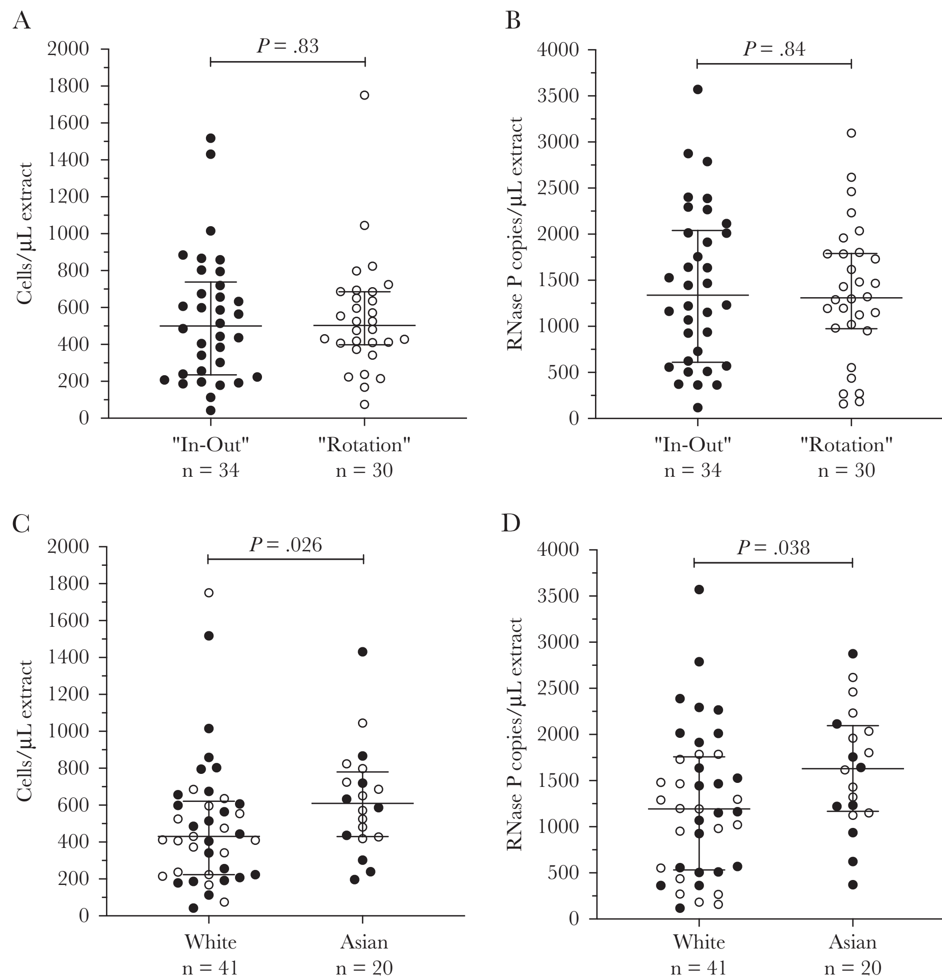

Figure 2. Differences in nucleic acid recovery by nasopharyngeal swab technique and ethnicity. A and B, No difference in DNA (RPP30, cells/μL extract (A)) or RNA recovery (RNase P, RNase P copies/μL extract (B)) between the "in-out" (black circles) and "rotation" (white circles) swab technique groups was observed. C and D, Significantly higher levels of DNA (RPP30, cells/μL extract (C)) and RNA (RNase P, RNase P copies/μL extract (D)) were recovered on swabs from Asian compared with White participants. Individuals of other ethnicities were excluded due to low numbers (n = 4).

DISCUSSION

Our observations have the potential to improve nasopharyngeal sample collection. For 7.2% of individuals, nasopharyngeal sampling was not possible due to obstructions (eg, nasal polyps or deviated septum), and the procedure caused significantly more discomfort in these individuals. Providers should be aware of the frequency and discomfort implications of such occlusions and should be issued appropriate guidance (eg, do not force swab; sample "midturbinate" area of the nasal cavity if the nasopharynx cannot be reached and note the swab location). Our observations further indicated that, despite being widely recommended, swab rotation upon contact with the nasopharynx does not enhance nucleic acid recovery and is less tolerable. We speculate that swab saturation is essentially achieved during entry and very brief resting in the nasopharynx, such that rotation does not recover additional material. As we followed guidance to remove excess mucus before swabbing (as it can reduce the collection of desired cellular material [16]), our results are unlikely attributable to mucus collection. By extension, our results further suggest that, while sometimes recommended [8], swabbing both nares is an unnecessary practice.

The marked spread in discomfort scores was also notable. Though the average score in our study (5 on an 11-point scale) is similar to previous reports for nasopharyngeal swabs (an average of 3 on a 6-point scale [9]), the variation in discomfort levels from minimal to extreme underscores the need for providers to be mindful of interindividual differences in experience. Intriguingly, Asian participants reported on average 1-point greater discomfort than White participants. This may be related to differences in the shape, contour, and/or size of the nasal cavities and nasopharynx. Indeed, after adjustment for weight, age, and sex, a study of facial anthropometric differences by ethnicity reported significantly lower nasal volumes (measured at 0–4 cm from the nostril), lower mean cross-sectional nasal area (at 0–6 cm), and longer distances to the minimal cross-sectional area in

Asian compared with White individuals [17], differences that could affect individual experience during the procedure. Our recovery of higher levels of nucleic acid on swabs from Asian participants is also consistent with narrower nasal passages in this group compared with White individuals [17]. Specifically, narrower nasal passages could increase both swab discomfort and mucosal contact, though marked variation in nucleic acid recovery across all ethnicities was noted. Nevertheless, health care providers should be sensitive to differences in discomfort levels across diverse populations.

Some limitations of our study merit mention. We assume that total human DNA/RNA targets are appropriate markers of respiratory pathogen collection quality [2], which is consistent with the inclusion of RNase P in the CDC 2019-nCoV real-time RT-PCR diagnostic panel as part of quality control [15]. While respiratory epithelial cells, which are the targets of SARS-CoV-2 and other respiratory pathogens, exhibit a unique transcriptional profile that could facilitate their selective quantification [18], these cells are estimated to represent 85% of all human cells in this anatomical region [18], suggesting that the majority of human DNA/RNA quantified here is derived from this most relevant cell type. As swabs vary in design, the absolute discomfort scores and nucleic acid quantities recovered may not be applicable to all swabs, though our general observations should be. Protocol differences also prevent direct comparison of recovered nucleic acid across studies (eg, the swab, silica input during nucleic acid extraction, and elution volumes differed between the present and a previous study by our group [2]).

CONCLUSIONS

When performing nasopharyngeal sampling, rotation of the swab upon contact with the nasopharynx does not enhance sample quality. Swab rotation also increases the procedure duration, which represents an additional disadvantage in the context of mass screening. The observation that procedural discomfort levels differ significantly by ethnicity underscores the need for care providers to be sensitive to such differences and more broadly underscores the importance of diverse participant representation in health research. Review and standardization of nasopharyngeal swab collection guidance notes should be a priority in the current COVID-19 pandemic.

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Prior presentation. This work has not been previously presented at any conference or meeting.

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