Title: Self-Collected Anterior Nasal and Saliva Specimens versus Healthcare Worker-Collected Nasopharyngeal Swabs for the Molecular Detection of SARS-CoV-2

Running Title: Diagnostic specimen type comparison for SARS-CoV-2

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Abstract: We prospectively compared healthcare worker-collected nasopharyngeal swabs (NPS) to self-collected anterior nasal swabs (ANS) and straight saliva for the diagnosis of COVID-19 in 354 patients. The positive percent agreement between NPS and ANS or saliva was 86.3% (95% CI: 76.7-92.9) and 93.8% (95% CI: 86.0-97.9), respectively. Negative percent agreement was 99.6% (95% CI: 98-100) for NPS vs. ANS and 97.8% (95% CI: 95.3 – 99.2) for NPS vs. saliva. NPS (n=80) and saliva (n=81) detected more cases than ANS (n=70), but no single specimen type detected all SARS-CoV-2 infections.

Introduction: Rapid and accurate diagnostic tests are essential for controlling the SARS-CoV-2 pandemic. The Centers for Disease Control (CDC) currently recommends collecting and testing an upper respiratory tract specimen for initial SARS-CoV-2 diagnostic testing (1), but the most sensitive specimen type has not been defined. Nasopharyngeal swabs (NPS) have historically been considered the reference method for respiratory virus detection. In addition, anterior nasal swabs (ANS) are used routinely for influenza nucleic acid amplification testing (NAAT). Recurrent shortages of swabs and personal protective equipment (PPE), however, have prompted evaluation of alternatives to NPS including the use of patient self-collected ANS and saliva.

The advantages of ANS and saliva are the minimally invasive nature of sampling and potential for patient self-collection, which may reduce healthcare worker exposure to infectious aerosols. Saliva also has the added benefit being a “swab-free” specimen type known to contain high concentrations of SARS-CoV-2 RNA (2-4). Surprisingly few studies have assessed the performance of self-collected ANS for SARS-CoV-2 testing (5, 6). Small sample sizes and use of selected cases limits the available evidence for ANS. More performance data exists for saliva (7), but published studies vary substantially in the way the specimens were obtained. Many saliva protocols require patients to cough before pooling saliva in their mouth (2, 3, 8), entail avoidance of food, water, or tooth brushing prior to testing (9), and/or rely on

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RNA stabilization reagents as a part of the collection device. Forced cough, if performed in the presence of a healthcare worker, necessitates the need for PPE and restrictions on eating and drinking are not feasible in most healthcare settings. Furthermore, RNA stabilizers increase the cost of testing, are vulnerable to supply shortages, are not compatible with all NAAT chemistries and can be potentially toxic to use. Larger studies that compare the performance of self-collected ANS and "straight" saliva to NPS for SARS-CoV-2 detection are needed. Therefore, we performed a prospective comparative study to evaluate the performance of self-collected ANS and saliva versus healthcare provider-collected NPS for SARS-CoV-2 diagnostic testing.

Methods:

Study Subjects- Adult patients presenting to a drive-thru test center with symptoms suggestive of COVID-19 were included. Criteria for testing included the presence of at least one of the following: fever, cough, shortness of breath, sore throat, malaise, chills and/or decreased sense of smell or taste. After obtaining consent, subjects were instructed to swab both nostrils, pool saliva in their mouth without coughing and then repeatedly spit a minimum of 1 mL saliva into a sterile empty tube in the presence of a healthcare worker. Detailed instructions for the patient self-collection procedures are included in the **Supplemental material**. The NPS was collected last in the sampling sequence, with a technique matching the Infectious diseases Society of America (IDSA) and CDC guidelines for SARS-CoV-2 nucleic acid amplification testing (10). The University of Utah Institutional Review Board approved all study procedures.

Specimen collection and processing –Flocked mini-tip and foam swabs (Puritan Medical Products) were used for the nasopharyngeal and nasal collections, respectively. Swabs placed in 3 mL of sterile 1x phosphate-buffered saline (ARUP Laboratories) and straight saliva collected in a sterile empty

50 ml Falcon tube (without pre-aliquoted stabilization media) were transported to the clinical laboratory at 4°C. Study samples were stored refrigerated and tested within 5 days of receipt in the clinical laboratory, which is within our validated stability parameters for each specimen type. Saliva was then diluted 1:1 in ARUP Laboratories universal transport media™ (UTM) at the time of testing. Mixing was performed directly in the Hologic Aptima lysis tube by gently inverting the tube three times to ensure homogenization prior to testing on the instrument.

SARS-CoV-2 detection **-** All specimens were analyzed using the Hologic Aptima SARS-CoV-2 transcription mediated amplification (TMA) assay (Hologic Inc.) which is FDA EUA approved for NPS and ANS. Samples producing an invalid TMA result were repeated using the original specimen and a 1:1 dilution in UTM. Discrepant NAAT results across specimens collected from the same patient triggered repeat testing using the Hologic Panther Fusion (Hologic Inc.), a real-time RT-PCR platform, to assess crossing thresholds (Cts) as a surrogate measure of RNA concentration. As per the manufacturer's package insert, Cts of ≤ 42 by PCR are considered positive.

Statistical methods **–** The standard of care NPS results by TMA were used as the benchmark for assessments of test agreement. GraphPad Quick Calcs software was used to calculate kappa coefficients (k) and proportions (*p* value) by the Chi Square test. Percent positive or negative agreement for categorical variables were calculated in Microsoft Excel using the Analyse-it software package.

Results: A total of 1104 specimens were collected from 368 unique patients between May 29th and June 25th, 2020. The average age of study participants was 35 (range 18-75 years), 47% female and 53% male. Saliva samples from 12 patients (3.3%) generated invalid TMA results due to automated sample processing errors or internal control failure and an additional 2 patients did not provide adequate saliva

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volume for testing. Repeat testing in response to invalid results did not resolve sample failures. Patients with missing saliva data (n=14) were excluded from the primary analysis.

Tables 1 and 2 contain the summary of all TMA results. There was near perfect qualitative agreement across sample types (NPS vs. saliva κ=0.912 [95%CI: 0.86-0.96]; NPS vs. ANS κ=0.889 [95%CI: 0.84-0.95]). In all, 66 (18.6%) patients had SARS-CoV-2 detected in all 3 specimen types, 13 (3.7%) in 2 specimens, 7 (2.0%) in 1 specimen, and 268 (75.7%) had completely negative testing. Of the 13 patients that were positive by two of the three specimen collections, 9 (69.2%) had SARS-CoV-2 detected by NPS and saliva, 3 (23%) were positive by NPS and ANS and a single patient (7.7%) was positive by saliva and ANS. The 7 single specimen positives included 2 (28.6%) infections detected by NPS only and 5 (71.4%) by saliva only. Positivity rates were higher for NPS (22.5%; 80/354) and saliva (22.9%; 81/354) compared to ANS (19.7%; 70/354) alone, but this did not reach statistical significance (p = 0.408 for the NPS vs. ANS comparison). The greatest case detection rate combines NPS sampling with saliva (23.6%; 86/354).

Adequate residual sample volume was available for 15 of 20 discordant specimen sets to perform repeat PCR testing. **Figure 1** and **Table S1** display the Ct values across discordant specimen sets. The average Ct values for NPS positive only or saliva positive only specimens were 27.0 (range 19.7 – 32.7) and 28.2 (range 18.3 – 37.5), respectively. Similar Ct ranges (22.0-35.7) were seen in the NPS positive/ANS negative specimens, with an average Ct value of 28.3. Interestingly, 3 specimens (1 saliva and 2 ANS) initially reported as negative by TMA had low levels of viral RNA detected by RT-PCR upon repeat testing (average Ct 35.7; range 33.4-37.3).

Discussion: Sensitive detection of SARS-CoV-2 RNA is critical for patient management decisions, hospital infection prevention, and curbing the ongoing Public Health emergency. The selection and adequate collection of clinical specimens plays an essential role in diagnostic test performance, and this holds true

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for sensitive NAAT methods. Both the CDC (1) and IDSA (10) endorse use of NPS or ANS (either healthcare worker or patient collected) for the diagnosis of COVID-19. However, little data exists comparing the performance of different sample types collected from the same patient, at the same time, and using U.S. Food and Drug Administration (FDA) authorized NAAT platforms.

This study represents one of the largest prospective specimen type comparisons to date and demonstrates excellent agreement between provider-collected NPS and patient-self collected saliva and ANS. The majority (91.9%) of patients with positive results had SARS-CoV-2 nucleic acid detected in at least two specimen types concurrently. NPS and saliva samples had the greatest positivity rates overall. Given that all participants had a strong clinical suspicion for COVID-19, and molecular testing in general has very high specificity, it is likely that the NPS or saliva positive only specimens are true positives; but the lack of an accepted external reference standard precludes calculations of clinical sensitivity and specificity. Even though there was excellent qualitative agreement across specimen types, relying on ANS alone could have missed infection in 10 to 11 patients compared with NPS or saliva, respectively. Missed COVID-19 cases have major clinical implications affecting isolation decisions for symptomatic patients and are a lost opportunity for contact tracing.

No single sample type detected all potential COVID-19 cases and discrepant results were not always explained by high Ct values (i.e. low RNA concentrations near the limit of detection of the test). There are several potential explanations for “false negative” results. First, inadequate swab collection technique is possible. We did not include a host genomic marker to assure presence of respiratory epithelial cells on the swab, nor did we compare self-collection to healthcare provider-collected ANS. Previous respiratory virus studies, however, suggest that self-collected is equivalent to provider-collected ANS (11). We also did not evaluate the impact of swab type on SARS-CoV-2 detection. This study relied on foam nasal swabs and flocked NP swabs, so the results may not be generalizable to other swab types. Additionally, the level of viral replication in the nasopharynx or posterior

oropharynx/salivary glands may vary over the course of infection. We did not collect information on the duration or type of symptoms at the time of specimen collection, which is an additional limitation of the study. Lastly, in an attempt to exclude RNA degradation in straight saliva as a potential explanation for "false negatives", we performed stability studies at ambient and refrigerated temperatures for up to 5 days and saw no reduced TMA or PCR signal (data not shown).

In conclusion, NPS and saliva were clinically superior to ANS alone for the detection of SARS-CoV-2 in symptomatic patients. These observations, along with other recent reports (9, 12), suggest that straight saliva is an acceptable specimen type for symptomatic patients, especially if swab or PPE supplies are limited. However, not all patients could provide adequate volume and saliva is a complex matrix that requires clinical laboratories to validate this specimen type on their respective NAAT platforms. Saliva processing also required an additional pipetting step to dilute the specimen in UTM prior to testing. Additional processing has workflow and ergonomic implications for the clinical laboratory. Despite sample dilution, an increased indeterminate or invalid rate was observed for saliva (3.3% for saliva vs. 0% for swabs in saline). This could be related to issues of sample viscosity affecting the automated pipetting and/or internal control inhibition. Repeat testing of the original specimen (diluted 1:1 in UTM) did not resolve invalid results, and therefore, is not recommended. We did not test whether a higher dilution factor (e.g., 1:2 or 1:3) with proportionally more UTM would reduce the invalid rate without losing sensitivity. Regardless of the approach, repeat testing and recreation of dilution series increases time to results, labor and the overall cost of testing.

Combination testing with simultaneous sample collection from multiple anatomic sites may increase SARS-CoV-2 detection rates slightly, but multisite testing could be impractical given current swab and reagent shortages. Requiring two separate NAAT reactions would also increase costs. Given ongoing supply limitations, validating multiple specimen types provides redundancy and allows clinical

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laboratories options for testing. Ultimately, the availability of materials, staffing and cost considerations will influence what testing can be offered by individual laboratories.

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164 **Table 1. Qualitative Result Comparisons across All Specimen Types**

A.

Nasopharyngeal \ Saliva	+	-	Total
+	75	5	80
-	6	268	274
Total	81	273	354

B.

Nasopharyngeal \ Anterior Nasal	+	-	Total
+	69	11	80
-	1	273	274
Total	70	284	354

c.

Saliva \ Anterior Nasal	+	-	Total
+	67	14	81
-	3	270	274
Total	70	284	354

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166 Abbreviations: Positive (+), Negative (-)

167 **Table 2. Percent Agreement between Nasopharyngeal Swabs and Alternative Specimen Types**

	Saliva vs. Nasopharyngeal	Nasal vs. Nasopharyngeal
Positive Agreement (%)	93.8 (95% CI: 86.0-97.9)	86.3 (95% CI: 76.7-92.9)
Negative Agreement (%)	97.8 (95% CI: 95.3-99.2)	99.6% (95% CI: 98.0 – 100.0)

Figure 1. RT-PCR cycle thresholds (Ct) values for discordant NPS, saliva, and ANS specimen sets

Footnote: Three way comparison of Ct values with solid lines linking RT-PCR results across specimen types

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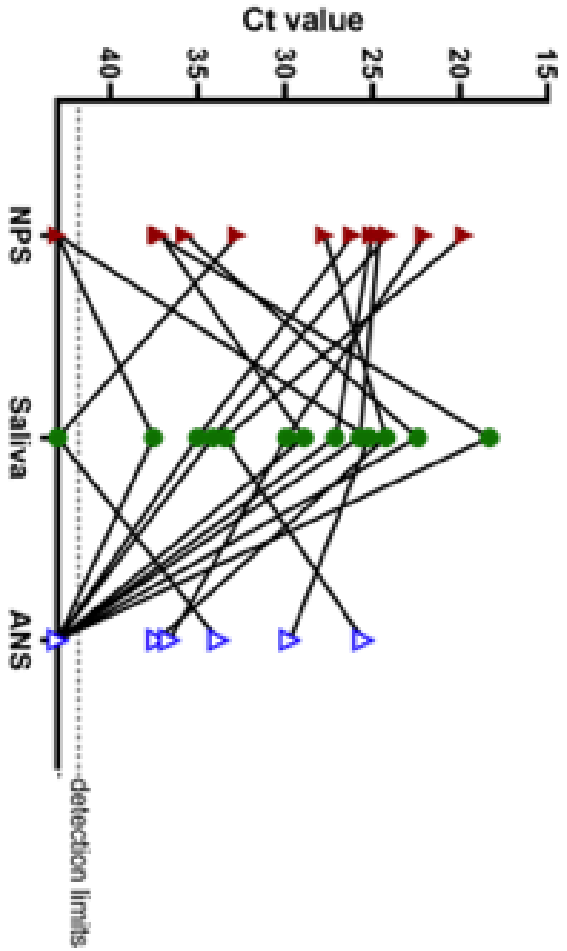

Supplemental Material

Table 1. Discrepant analysis of paired NPS, saliva and ANS samples

Study #	NPS		Saliva		ANS	
	Initial TMA	**Repeat PCR (Ct)**	**Initial TMA**	**Repeat PCR Ct)**	**Initial TMA**	**Repeat PCR (Ct)**
1	Detected	24.1	Detected	34.1	Not Detected	Not Detected
2	Detected	32.7	Not Detected	Not Detected	Detected	33.8
3	Detected	25	Detected	27.1	Not Detected	Not Detected
4	Detected	24.6	Detected	25.7	Not Detected	Not Detected
5	Detected	27.7	Detected	24.2	Not Detected	37.3
7	Detected	22	Detected	29.9	Not Detected	36.6
9	Detected	19.7	Not Detected	33.4	Detected	25.5
10	Detected	35.7	Detected	22.4	Not Detected	Not Detected
12	Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected
17	Detected	26.1	Detected	35	Not Detected	Not Detected
11	Not Detected	37.1	Detected	28.9	Not Detected	Not Detected
13	Not Detected	37.3	Detected	18.3	Not Detected	Not Detected
14	Not Detected	Not Detected	Detected	37.5	Not Detected	Not Detected
16	Not Detected	Not Detected	Detected	Not Detected	Not Detected	Not Detected
18	Not Detected	Not Detected	Detected	25.2	Detected	29.7

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Healthcare worker instructions to collect nasopharyngeal swab

1. Use a nasopharyngeal flocked, synthetic fiber mini-tip swabs with plastic or wire shafts
2. Tilt patient's head back 70°
3. Insert flexible shaft mini-tip swab through nares parallel to palate (not upwards) until
    a. Resistance is met, OR
    b. Distance is equivalent to the distance from the patient's ear to their nostril
4. Gently rub and roll swab
5. Leave swab in place for several seconds to absorb secretions
6. Slowly remove swab while rotating it
7. Immediately place swab in sterile tubes containing transport media

Patient Instructions for self-collected anterior nasal swab

1. Open the swab package without touching the swab tip.
2. Take the swab out of the package and hold it by the handle.
3. Hand the swab to the patient.
4. Tilt head back slightly (approximately 20 degrees).
5. Gently insert the swab approximately 1 inch into the nostril (or until resistance is met) following a horizontal pathway, parallel to roof of mouth. **DO NOT** point the swab tip upwards toward the eyes.
6. The entire swab tip should be in the nostril. The patient may feel some pressure with insertion, but this should not hurt.
7. Rotate the swab three times leaving the swab in place for several seconds to absorb sections.
8. Remove the swab from the nostril without touching the tip of the swab.
9. Using the same swab, **repeat the same process for the other nostril.**
10. After sampling both nostrils, have the patient place the swab in the pre-opened transport tube so that the tip comes in to contact with the liquid at the bottom of the tube.
11. The provider will snap the swab handle at the break point, **securely** screw on the tube cap and make sure the tube is labeled with the patient's information.

Patient instructions for saliva collection

1. Open the saliva tube package and remove the contents.
2. Hand the open collection device to the patient
3. Ask the patient to pool saliva in their mouth
4. Repeatedly spit into the saliva tube
5. Fill the tube with saliva at least to the fill line (3mL), going over is o.k., a minimum of half the way to the fill line (1.5mL) is required.

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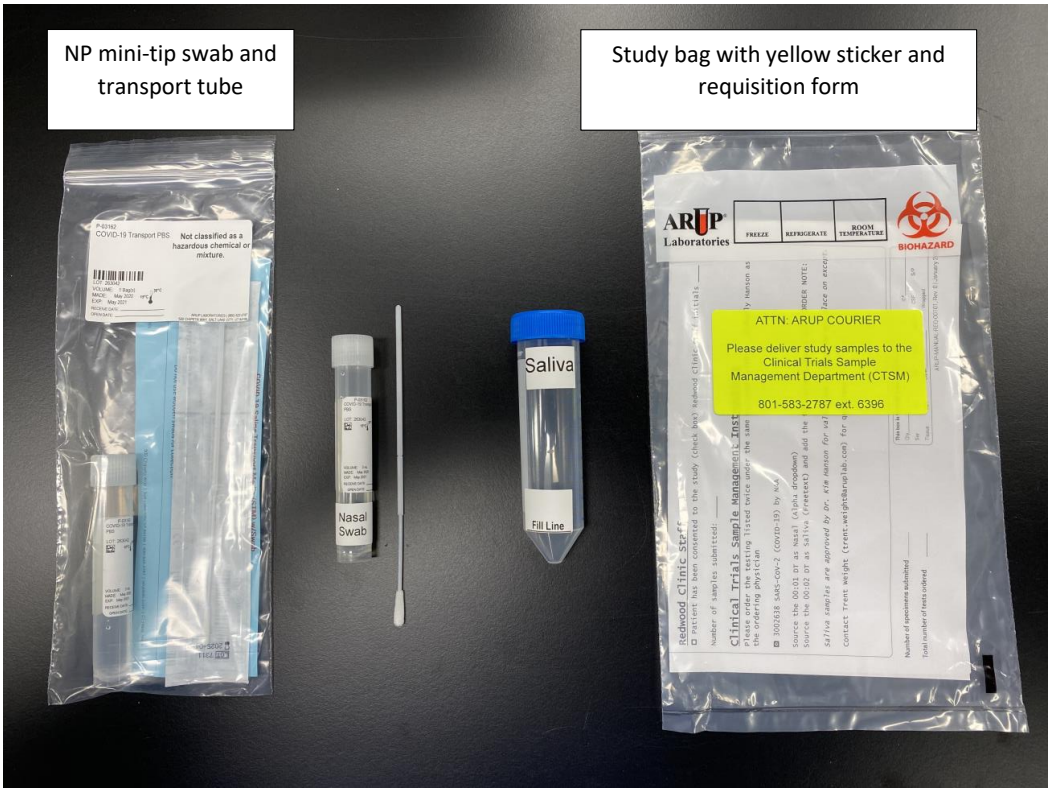
6. Massaging the cheek may help stimulate saliva production
7. Hand the tube back to the healthcare provider
8. **Securely** screw the cap on to the saliva transport tube
9. Make sure the tube is labeled with the patient's information.

Study Kit



Study kit contents

1) NP swab and transport tube (packaged separately)
2) Nasal swab and transport tube
3) Saliva transport tube
4) Requisition form
5) Collection Instructions
6) Consent summary document

ARUP Universal Transport Media

Material Description	Amount (units)
Bovine Serum Albumin	175 g
L-Cysteine Hydrochloride	8.4 g
Gelatin	175 g
L-Glutamic Acid	25.2 g
HEPES	210 g
Vancomycin	175 mL
Amphotericin B	280 mL
Phenol Red	385 mg
Sodium Bicarbonate	12.25 g
Colistin	606 mg
HBSS (10X)	3.5 L
Clinical Laboratory Reagent Water	31.5 L
Sucrose	2.397 kg

ARUP 1X Phosphate buffered saline

Material Description	Amount (units)
Sodium Chloride	876.6 g
Sodium Phosphate, Dibasic	113.6 g
Potassium phosphate (KH2PO4)	40.8 g
Water, Molecular Grade	100 L

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