Title: Feasibility of SARS-CoV-2 virus detection from consumer-grade cotton swabs

Running Title: Alternative swabs for COVID-19 screening

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Abstract

To control the growing COVID-19 pandemic, increased testing and containment is essential, yet clinical-grade sampling supplies are expensive and rapidly being depleted. We demonstrate the feasibility of using alternative consumer-grade swabs stored in 95% ethanol rather than viral transport media to detect SARS-CoV-2 from ten hospitalized persons and hospital rooms.
Background

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) the causative agent of coronavirus disease 19 (COVID-19) has spread to 185 countries resulting in 278,892 deaths and 4,006,257 total confirmed cases as of May 11, 2020 [1-2]. Large-scale testing remains key for controlling viral spread, but sample collection supplies including swabs, viral transport media (VTM) and personal protective equipment (PPE) are being depleted in developed nations like the United States, and are in even shorter supply in low- and middle-income countries [3]. Validation of alternatives strategies such as self-administered testing using consumer-grade materials is urgently needed.

Concerns about consumer-grade materials include the presence of contaminant RNases and/or PCR inhibitors which would promote false negatives during evaluation for the presence of SARS-CoV-2 RNA by reverse transcription and quantitative polymerase chain reaction (RT-qPCR) as noted in the U.S. Centers for Disease Control (CDC) guidance for testing [4]. Similarly, the CDC-recommended VTM requires specialized ingredients and contains antimicrobials likely to interfere with downstream assessment of the microbial context of SARS-CoV-2 that may enable new insights into viral susceptibility and resistance [5]. VTM also maintains viral viability and therefore requires processing in a facility with more stringent biosafety practices compared to inactivating collection methods. Using inactivating sample collection solutions could increase the number of testing laboratories and ameliorate the risks associated with sample transport and processing. Here we demonstrate the feasibility of detecting SARS-CoV-2 RNA from patient and built-environment samples using viral-inactivating storage solutions and alternative medical-grade and consumer-grade swabs.

Methods

Swab feasibility testing

Six swab types were compared and processed following the standard SARS-CoV-2 protocol provided by the CDC [6] (Supplementary Methods). These swab types are: sterile polyester-head, plastic-shaft (‘PE’),
sterile foam-head, plastic-shaft (‘BDF’), non-sterile cotton-head, plastic-shaft (‘TMI’), non-sterile cotton-head plastic-shaft consumer-grade (‘CGp’), non-sterile cotton-head wooden-shaft consumer-grade (‘CGw’), and non-sterile cotton-head, wooden-shaft (‘Pu’). Suppliers and part numbers are provided in Supplementary Methods.

Patient and hospital environmental sampling

This study was performed with approval of the UC San Diego Institutional Review Board under protocols #150275 and #200613. All study participants were diagnosed and hospitalized with COVID-19, and both nasal samples and hospital surfaces were collected using three unmoistened swab types (PE, TMI, CGp) immediately placed in a collection tube containing 95% EtOH and stored on dry ice. Collection details and sample processing are provided in the Supplementary Methods.

Results:

We compared the efficiency of extracting and processing RNA from 200 µL of VTM eluent surrounding polyester-tipped plastic-shafted nasopharyngeal (NP) swabs (CDC protocol) to more accessible nares samples collected using PE swabs stored in viral-inactivating alcohol. RNA extraction efficiency was significantly reduced from samples stored in 95% EtOH when extracted from the eluent (n=22), but similar when extracted from the swab head itself (n=18) compared to the CDC protocol (n=39) (Figure 1a). Extracting from the swab head also provided significantly higher SARS-CoV-2 viral load compared to the EtOH eluent of the same patient samples (n=7) (Figure 1b, p=0.032). We separately compared the use of 95% EtOH vs 91% isopropanol as the storage media with human RNA and found no impact on extraction efficiency (32.1% and 35.3% recovery respectively) (ANOVA, P>0.05) (Figure 1c). However, in the presence of abundant RNase, 95% EtOH protected both human RNA and SARS-CoV-2 RNA better than 91% isopropanol (Figure 1d).
We next tested RNA recovery from a range of medical- and consumer-grade swabs (Supplementary Methods). The yield was highest from swab heads compared to eluent regardless of the swab type and whether stored in 95% EtOH (P<0.0001, U=37, Mann-Whitney) or 91% isopropanol (P<0.0001, U=28, Mann-Whitney) (Figure 1e-f). The storage solution did not impact RNA quality (Supplemental Figure 1b, Mann-Whitney, P>0.05), though swab type had a minor impact (Supplemental Figure 1c, Kruskal-Wallis P=0.0325, KW=12.17) [6]. RNA recovery ratio of swab-to-eluent and total yield varied among swab type (P<0.0001, KW=28.37, Kruskal-Wallis for eluent, and P<0.0001, KW=15.43, Kruskal-Wallis for swab-heads) (Supplemental Figure 2). CGp swabs had the highest recovery from the swab head, while TMI swabs had the highest overall recovery of RNA from both eluent and direct swab extractions (Supplemental Figure 2). Swab type did not impact the ability to detect a linear decrease of positive control human and SARS-CoV-2 RNA when extracting directly from either CDC-recommended PE swabs or CGp swabs (Supplemental Figure 3).

As a clinical proof-of-concept, we collected samples from ten participants admitted to the hospital for COVID-19 using TMI and/or CGp swabs alongside the recommended PE swabs, and performed RT-qPCR per CDC guidelines. All three swab types successfully detected positive control SARS-CoV-2 RNA except for one false negative CGp, and no false positives (Supplemental Figure 4). For this comparison, samples inconclusive solely for the SARS-CoV-2 N2 amplicon (I-N2 in Figure 1g) were considered positive for the presence of SARS-CoV-2 RNA based on observed and reported concerns with this primer set [7]. PE swabs were 100% concordant with NP results when extracting from the swab head compared to 67% concordance for eluent from the same samples. TMI swabs were 85% and 57% concordant while CGp were 70% and 56% concordant for swab head and eluent respectively (Figure 1g). To evaluate contamination of SARS-CoV-2 RNA on environmental surfaces, we collected and compared swabs from the inside floor and bedrail of the same participants’ rooms using the same swab types [8]. Only 3/10 bedrails had detectable SARS-CoV-2 with any swab type, while floor samples tested positive for SARS-CoV-2 RNA for at least one swab.
type in 9/10 rooms (Figure 1g). Overall, the concordance between PE swabs was ~84% both for CGp (25/30) and TMI (22/26) across participant and environmental samples (Figure 1d).

**Discussion:**

We provide evidence that nasal samples collected using more widely-available consumer-grade cotton-tipped swabs can be stored in viral-inactivating alcohol without compromising the ability to detect SARS-CoV-2. The sensitivity for detection of SARS-CoV-2 RNA was comparable between the hospital NP swabs (CDC protocol) and both TMI and CGp nasal swabs when extracting from the swab head. Negative NP results for previously positive participants may be due to viral clearance, the timing of sampling during the course of infection or inconsistencies among the standard NP swabs. Of note, wooden-shafted swabs performed poorly only when extracting from the eluent, suggesting that RNA adsorption onto the shaft rather than RT-qPCR inhibitors may be the source of interference with current eluent-based testing methods.

Cotton-tipped swabs and alcohol-based solutions are compatible with standard microbiome and metabolome analyses prohibited by VTM, and could enable more widespread assessment for SARS-CoV-2 RNA in human and environmental samples. SARS-CoV-2 was only detected on 30% of participants’ bedrails, which may have been due to routine cleaning measures and/or minimal interaction with the surfaces from heavily-sedated or intubated patients. This may be present a challenge for monitoring shared surfaces between the healthcare worker and patients. In contrast, the detection of SARS-CoV-2 RNA from the floor samples demonstrates a potentially important reservoir for viral exposure, as shoe covers are note currently recommended by the CDC. However, additional testing is needed to determine whether viable virus remains on these surfaces.

In summary, our results suggest detection of SARS-CoV-2 RNA could be performed using less expensive, consumer-grade materials. We add to the emerging body of literature supporting nasal sampling as opposed to NP sampling [10–14]. It is conceivable that patients could collect samples at home, thus reducing risk
and saving the use of PPE for healthcare workers. Further confirmatory studies using consumer-grade swabs would greatly support COVID-19 screening worldwide, particularly in resource-limited settings.

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The authors have no conflict of interest related to the work described in this manuscript.

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Figure Legends

Figure 1. Validation of alternative swabs and storage buffer (95% EtOH and 91% isopropanol) in RNA recovery and detection of COVID-19.

a) Human RNase P gene (Rp) RNA extraction control comparison across sample types. Clinical gold-standard polyester-tipped plastic-shafted NP swabs stored in VTM and extracted from 200 µL of eluent (left, n=39) have significantly lower higher copy numbers compared to 200 µL EtOH eluent from PE nares swabs (middle, n=22), but not when extracted from the EtOH-preserved swab head (right, n=18). One-way ANOVA with Tukey’s multiple comparison VTM eluent vs EtOH eluent p=<0.001, EtOH eluent vs EtOH swab p=0.001, VTM vs EtOH swab p = 0.266. b) Extrapolated viral RNA copy number from nares samples collected with BD polyester swabs in the hospital stored in 95% EtOH and extracted from either the eluent or swab from the same sample(one-tailed paired Student’s T-test p=0.032). c) Proportion of recovered RNA across three storage buffers: None, 95% EtOH, and 91% isopropanol (ns, ANOVA p>0.05). d) Evaluation of RNaseA inhibition by 95% EtOH (grey) and 91% isopropanol (blue) (multiple t-test) using either the human Rp or SARS-CoV-2 N1 primer set. e) Comparison human RNA recovery across six swab types (PE=polyester ‘commercial’, BDF=BD foam ‘commercial’, TMI=BD TMI ‘commercial’, CGp=plastic ‘consumer grade’, Pu=Puritan ‘commercial’, CGw=wood ‘consumer grade’), extracted from 200µL eluent (blank bar) or the swab head. Recovery for each swab type is normalized to the CDC recommended method (eluent from PE swab). A ‘2’ would indicate there was 2x more RNA recovered whereas a 0.5 would indicate a 50% reduction in RNA recovery. f) Total RNA copies per extraction for all samples which are grouped by sample-type (eluent or swab head) and storage buffer (95% EtOH or 91% isopropanol). Pairwise comparisons performed within sample-type (not significant) and across sample-type controlling for storage buffer (Mann-Whitney, U=test statistic). g) Demonstration of consumer-grade CGp and bulk TMI swab congruence compared to clinical-grade hospital tests using polyester-tipped plastic shafted NP swabs across ten patient rooms. Samples positive (+, dark blue background) or negative (-, orange background) for N1, N2, and Rp by CDC guidelines. Samples positive for N1 and Rp, but negative for N2 are labeled inconclusive for N2 (I-N2, blue) but
considered positive for comparisons. Samples positive for N2 and Rp, but negative for N1 (I-N1, light blue) are inconclusive. Samples not measured for a given patient are labeled as NaN (light grey). (P<0.05 = *, P<0.01 = **, P<0.001 = ***, P<0.0001 = ****)

Figures

Figure 1. Validation of alternative swabs and storage buffer (95% EtOH and 91% isopropanol) in RNA recovery and detection of COVID-19.
Supplementary Methods

VTM versus EtOH sample comparison

Nasopharyngeal (NP) swabs were collected according to CDC guidelines and stored in viral transport media (VTM). For comparison, sterile polyester-head, plastic-shaft (‘PE’, BBL Culture swab REF-220135, Becton, Dickinson and Company) were used to collect nares samples by rotating the dry swab head in the nares for approximately 30 seconds from lab members, patients, or healthcare workers, and then immediately placed in 95% EtOH. Eluent nucleic acid extractions were performed on 200 µL of the swab eluent (either VTM or EtOH) using the Omega Mag-Bind® Viral DNA/RNA 96 Kit (catalog# M6246-03), which only uses chemical lysis and does not include a bead beating step. For nucleic acid extraction from the swab head, the MagMAX Microbiome Ultra kit (Cat#A42357, Thermo Fisher Scientific) was used. For the direct comparison of SARS-CoV-2 extraction efficiency, we extracted EtOH eluent and swab separately from the same samples of COVID-19 patients with approval of the UC San Diego Institutional Review Board under protocols #150275 and #200613.

Patient and hospital environmental sampling

All study patients were diagnosed and hospitalized with COVID-19 with approval of the UC San Diego Institutional Review Board under protocols #150275 and #200613. Both nasal samples and hospital surfaces were collected using three unmoistened swab types (PE, TMI, CGp; see Table 1). Nasal samples were collected by inserting the swab into one nostril to the depth of approximately 2-3 cm and rotated for 5-10 seconds. Hospital surfaces sampled included the floor inside the patient’s room (approximately 1x1 square foot area) and the patient’s bedrail. All swabs were immediately placed in a collection tube containing 0.5-1 mL 95% ethanol and stored on dry ice and processed for RNA or total nucleic acid extraction (Supplementary Methods).

RT-qPCR for VTM and 95% EtOH comparison using polyester-tipped plastic swabs
SARS-CoV-2 detection was performed following a slightly miniaturized version of the CDC protocol. Each RT-qPCR reaction contained 4µl RNA template, 100nm forward and reverse primers, 200nm probe, 3µl TaqPath (catalog# A15299, Thermo), and RNAse free water to a total reaction volume of 10µl. All primers and probes were ordered from IDT (catalog# 10006606). RT-qPCR was performed on the Bio-rad CFX384 Touch Real-Time PCR Detection System following the CDC thermocycling guidelines. Serial dilutions of the Hs_RPP30 Positive Control plasmid (catalog# 10006626, IDT) or 2019-nCoV_N_Positive Control plasmid (catalog# 10006625, IDT) were included to extrapolate human RNase P and SARS-CoV-2 copy numbers, respectively.

**Alternative swab list**

Six swab types were used: sterile polyester-head, plastic-shaft (‘PE’, BBL Culture swab REF-220135, Becton, Dickinson and Company); sterile foam-head, plastic-shaft (‘BDF’, Flock PurFlock REF-25-3606-U-BT, Becton, Dickinson and Company); non-sterile cotton-head, plastic-shaft in use by The Microsetta Initiative (‘TMI’, SKU#839-PPCS, Puritan Medical Products); non-sterile cotton-head plastic-shaft consumer-grade (‘CGp’ Part #165902, CVS Caremark Corp.); non-sterile cotton-head wooden-shaft consumer-grade (‘CGw’, Part#858948, CVS Caremark Corp.); and non-sterile cotton-head, wooden-shaft (‘Pu’, REF-806-WC, Puritan Medical Products).

**Controls for RNA extraction efficiency testing**

Approximately 600 ng of purified, DNA-free human lung RNA (Cat#AM7968, Thermo Fisher Scientific) was pipetted onto each of the six swab types in triplicate and stored in two storage solutions (500 μL 95% ethanol (EtOH) and 500 μL 91% isopropanol). Two sets of six, 10-fold serial dilutions of human RNA were included as controls. The same quantity of RNA (600 ng) was added to either 95% EtOH (n=6) or 91% isopropanol (n=6) in the presence of 25 µg RNaseA to determine the resistance offered against RNase contaminants. Four negative (swab only) and four positive (swab + 500 ng spiked human RNA + 5
μL spiked SARS-CoV-2 RNA (~20,000 copies per ul) controls were included, in addition to three, 10-fold serial dilutions of this mixture in 95% EtOH.

**Extraction and RT-qPCR of hospital swabs and controls**

All samples were processed according to the manufacturer’s protocol using the MagMAX Microbiome Ultra kit (Cat#A42357, Thermo Fisher Scientific) and eluted into 70 μL buffer. For RT-qPCR, 5 μL sample was processed in duplicate with averaged Ct values, using the standard SARS-CoV-2 protocol provided by the CDC (Cat# 2019-nCoV-EUA-01[15]). Both the swab head and 200 μL of eluent were processed for nasal samples, but only the swab head was processed for floor, bedrail and spiked control samples using the same methods.

**Statistics and visualizations**

Visualizations and statistical comparisons performed using PRISM 8.0 and the limit of detection determination were consistent with CDC recommendations whereby samples with a Ct value greater than 40 are omitted [16].
Supplementary Figures Legends

Supplemental S1. Impacts of storage solution or swab type on RNA quality as measured by RNA Tapestation High Sensitivity kit. a) All direct-swab extracted RNA grouped by storage buffer and swab type. b) Samples grouped by storage buffer, no significant difference in RNA integrity number (RIN) values between storage buffers (95% EtOH vs. 91% isopropanol) (Mann-Whitney). c) Swab extracts grouped by swab type only and compared to determine if swab type has an impact on RNA quality (Kruskal-Wallis test).

Supplemental Figure S2. Impacts of sample-type (eluent vs. swab head) on RNA recovery by swab used. Comparison human RNA recovery across six swab types (PE=polyester ‘commercial’, BDF=BD foam ‘commercial’, TMI=BD TMI ‘commercial’, CGp=plastic ‘consumer grade’, Pu=Puritan ‘commercial’, CGw=wood ‘consumer grade’), a) extracted from 200 μL eluent or b) swab head (Group comparison using Kruskal-Wallis)

Supplemental Figure 3. Demonstration of impact of swab or storage buffer on human RNA and SARS-CoV-2 detection. a) Positive (swab + SARS-CoV-2 RNA + human RNA, n=4) and negative (swab only, n=4) controls from hospital processing experiment. b) Serial dilution of positive controls in 95% EtOH to demonstrate conserved extraction efficiency across biomass. Three swab types:circle-PE, square=CGp, triangle=TMI.

Supplemental Figure 4. Hospital built environment and patient screen for SARS-CoV-2. Demonstration of consumer-grade CGp and bulk TMI swab congruence compared to CDC polyester in a hospital setting across ten patient rooms and three sampling environments (bedrail, floor, and nasal swab) including negative and positive controls. Samples positive (+, dark blue background) or negative (-, orange background) for N1, N2, and Rp by CDC guidelines. Samples positive for N1 and Rp, but negative for N2 are labeled inconclusive for N2 (I-N2, blue) but considered positive for comparisons.
Samples positive for N2 and Rp, but negative for N1 (I-N1, light blue) are inconclusive. Samples not measured for a given patient are labeled as NaN (light grey). All data except controls are identical to those in Figure 1g.

Supplementary Figures

Supplemental S1. Impacts of storage solution or swab type on RNA quality as measured by RNA Tapestation High Sensitivity kit.

Supplemental Figure S2. Impacts of sample-type (eluent vs. swab head) on RNA recovery.
Supplemental Figure 3. Demonstration of impact of swab or storage buffer on human RNA and SARS-CoV-2 detection.

Supplemental Figure 4. Hospital built environment and patient screen for SARS-CoV-2.