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Preliminary support for a "dry swab, extraction free" protocol for SARS-CoV-2 testing via RT-qPCR

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Abstract

The urgent need for massively scaled clinical or surveillance testing for SARS-CoV-2 has necessitated a reconsideration of the methods by which respiratory samples are collected, transported, processed and tested. Conventional testing for SARS-CoV-2 involves collection of a clinical specimen with a nasopharyngeal swab, storage of the swab during transport in universal transport medium (UTM), extraction of RNA, and quantitative reverse transcription PCR (RT-qPCR). As testing has scaled across the world, supply chain challenges have emerged across this entire workflow. Here we sought to evaluate how eliminating the UTM storage and RNA extraction steps would impact the results of molecular testing. Using paired mid-turbinate swabs self-collected by 11 individuals with previously established SARS-CoV-2 positivity, we performed a comparison of conventional (swab → UTM → RNA extraction → RT-qPCR) vs. simplified (direct elution from dry swab → RT-qPCR) protocols. Our results suggest that dry swabs eluted directly into a simple buffered solution (TE) can support molecular detection of SARS-CoV-2 via endpoint RT-qPCR without substantially compromising sensitivity. Although further confirmation with a larger sample size and variation of other parameters is necessary, these results are encouraging for the possibility of a simplified workflow that could support massively scaled testing for COVID-19 control.

Results

Based on prior literature (*1*, *2*) and the fact that dry swabs are employed for SARS-CoV-2 testing outside of the United States, we know that swabs collected and transported without transport media are amenable to subsequent nucleic acid detection-based diagnostics. We hypothesized that elution of dry swabs directly into a Tris-EDTA (TE) buffer would be compatible with RT-qPCR, *i.e.* skipping conventional RNA extraction altogether. TE's lower salt

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concentration than PBS or saline, and use of EDTA to chelate divalent cations required for RNA degradation, make it an attractive buffer for compatibility with downstream molecular tests.

As an initial exploration of this hypothesis, we collected two COVID-19 negative mid nasal flocked swabs and added a controlled quantity of heat-inactivated virion. The "conventional swab" was placed into the standard 3 mL of Universal Transport Media (UTM), while the "dry swab" was allowed to dry at room temperature for 6 hours prior to elution into 200 µL of TE buffer. Serial dilution was then performed on each medium prior to downstream processing. For conventional swabs, RNA was isolated using the MagnaPure 96 RNA extraction platform using 200 µL input of viral UTM solution as input and eluting into 50 µL Roche elution buffer. Dry swabs were processed by first vortexing swab in the presence of TE for 30 seconds followed by brief centrifugation. In principle, the use of detergent could enhance accessibility to the viral genome and aid in detection. Therefore, in parallel, we also tested whether the addition of 1% Triton-X to TE buffer enhanced detection of SARS-CoV-2 from dry swabs. Finally, all samples were assayed using RT-qPCR with primer-probes specific for the SARS-CoV-2 viral genome (Orf1b or Spike gene targets) or primer-probes against the human RNAse P transcript.

For both Orf1b and Spike gene targets, control results were as expected, no amplification in no template control (NTC) wells and robust detection of virion diluted in 200 µL of water or synthetic template in the positive control wells (**Figure 1A**). RT-qPCR cycle threshold (Ct) values for the viral genome with both conventional and dry swabs were consistent across replicates, and furthermore similar across the two primer probe pairs (**Figure 1A**). However, contrary to our hypothesis, the addition of detergent resulted in higher Ct values, *i.e.* less sensitive viral detection (**Figure 1B**). Furthermore, whereas dry swabs eluted into TE buffer + 1% Triton-X performed consistently worse than the conventional protocol, dry swabs eluted into TE buffer alone performed consistently better.

Finally, to determine the relationship between detergent concentration and SARS-Cov-2 sensitivity in RT-qPCR, we prepared dry swabs loaded with inactivated virion and eluted these swabs into TE buffer containing either IGEPAL, TritonX or Tween20 with final concentrations ranging from 1% to 0.05%. RT-qPCR against the viral genome showed that every 0.1% increase in detergent concentration of IGEPAL, TritonX or Tween20 resulted in an average Ct increase of 0.43, 0.40 or 0.34 cycles, respectively (**Figure 1C**). Interestingly, the relationship between detergent concentration and detection sensitivity was absent in the detection of the human RNase P transcript.

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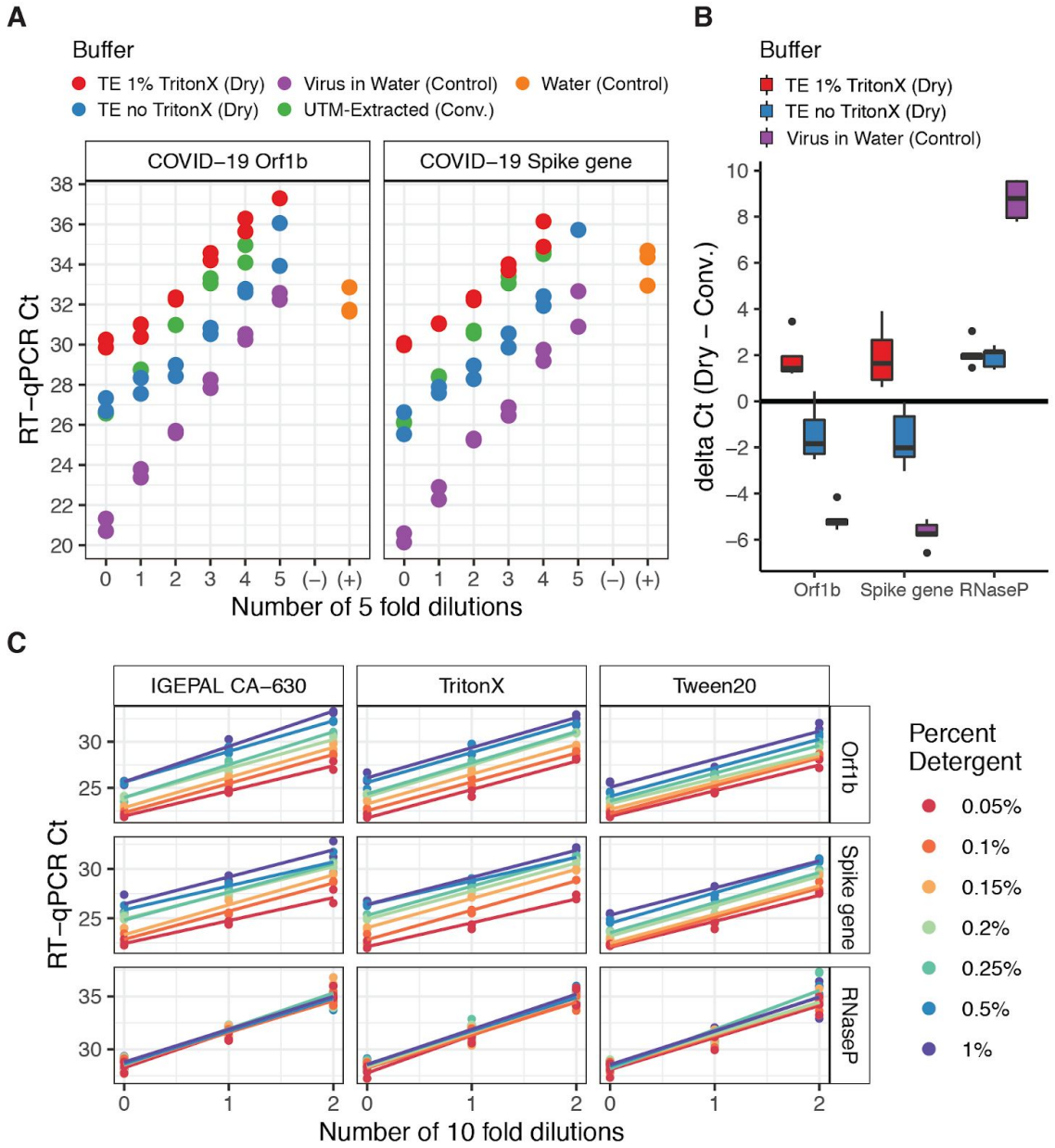

Figure 1. Comparison of RT-qPCR detection of inactivated virus from conventional and dry swabs. (A) Crossing threshold (Ct) values shown for samples comprising a self swab and inactivated SARS-CoV-2 virus for both the ORF1b primer-probe set (left) and the Spike gene primer-probe set (right). Colors correspond to unique combinations of extraction protocol or controls. All samples were measured twice in independent RT-qPCR reactions. No template control (-) wells contained either buffer or water and positive control wells (+) contained synthetic template. **(B)** Delta Ct values between conventionally processed swabs and dry processed swabs at matched dilutions for this contrived experiment. **(C)** Ct values for three probes (rows: Orf1b, Spike, Rnase P) assayed in buffers containing one of three detergents (columns: IGEPAL CA-360, TritonX, Tween20) across ten-fold dilutions. Linear model (colored line) was fit for observations (colored points) at each detergent percent.

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Next, to test whether this method was applicable to real-world samples, we collected pairs of conventional and dry swabs from 11 adult study participants. Participants who had a positive nasopharyngeal (NP) swab as part of routine clinical care were recruited for enrollment into the Hospitalized and Ambulatory Adults with Respiratory Viral Infections (HAARVI) study at the University of Washington. These clinical samples were established to be COVID-19-positive via testing at the University of Washington's Virology lab (*3*). Paired conventional and dry mid-nasal swabs were then self-collected via Seattle Flu Study (SFS) home self-swab kits delivered to participants' homes within 1-2 days after they had tested positive, and returned via delivery service at ambient temperature (*4*, *5*).

Upon receipt, conventional swabs (received in UTM) underwent RNA extraction and were then assayed by RT-qPCR. The paired dry swab was stored at 4˚C prior to processing 1-5 days later. Dry swab samples were eluted and assayed on two separate days by two different technicians. All assayed samples passed quality control with robust RNAse P detection in every well, consistent Ct values between technical replicates, and expected results for the NTC and positive control samples.

A head-to-head comparison of RT-qPCR Ct values showed that dry swabs collected and eluted directly into TE with no RNA extraction steps performed comparably to conventionally collected and processed swabs (**Figure 2A**). Similar to the contrived experiment described earlier, real-world dry swabs eluted into TE yielded lower Ct values than paired, conventionally processed swabs. Once again, the addition of 1% TritonX to the dry swab eluate diminished SARS-CoV-2 detection, but not for RNAse P.

Applying the algorithm used for our lab-developed clinical NWGC SARS-CoV-2 RT-PCR test to results from the 11 individuals (all of whom had tested positive in routine clinical care 1-2 days earlier), dry swabs eluted in TE identified 9 positive samples (one inconclusive), while the conventionally processed swabs identified 8 positive samples (two inconclusive) (**Table 1**). These preliminary results suggest that dry swabs eluted directly into a simple buffered solution (TE) can potentially support molecular detection of SARS-CoV-2 via endpoint RT-qPCR while maintaining sensitivity.

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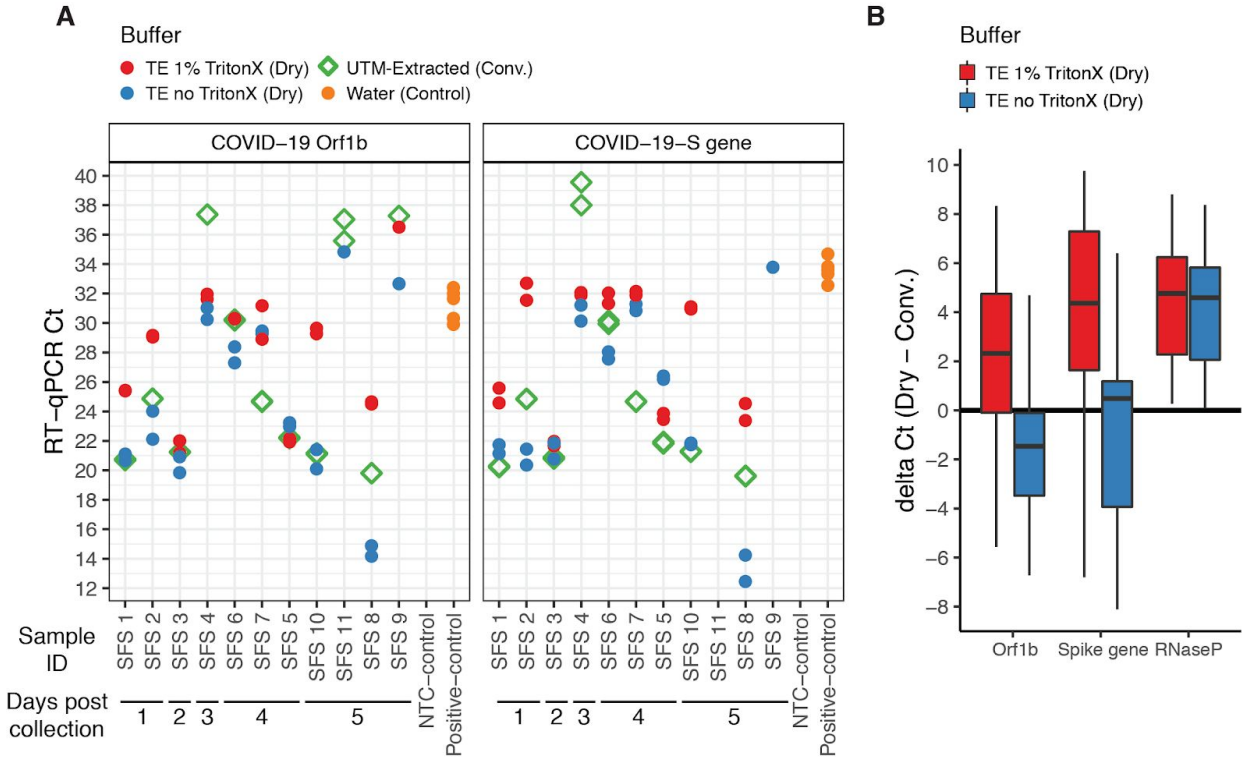

Figure 2. Detection of SARS-CoV-2 viral RNA from matched conventional and dry swabs from study participant samples. (A) Cycle threshold (Ct) values are shown for 11 participant samples and controls (colors) for both the ORF1b primer-probe set (left) and the Spike gene primer-probe set (right). Study participant samples are ordered and labeled by the number of days between collection of the dry swab and measurement of the eluate (bottom). All measurements were made in technical duplicate. **(B)** Distribution of delta Ct values between paired conventionally processed swab and dry swab for the three targets are shown.

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Test Result	UTM	Dry Swab into TE	Dry swab into TE + 1% Triton
Positive	8	9	9
Inconclusive	2	1	0
Negative	1*	1*	2

*SFS-11 is negative in TE and SFS-9 is negative in UTM

Table 1. Number of positive, inconclusive and negative samples from each swab type and resuspension buffer. Samples were positive, negative or inconclusive according to the NWGC CLIA SARS-CoV-2 RT-PCR laboratory developed test. Positive samples have SARS-CoV-2 targets detected at Ct values < 40 for at least 3 of 4 replicates, inconclusive have 2 replicates with Ct < 40 and negative samples have 1 or 0 replicates with Ct < 40.

Discussion

Here we present preliminary results demonstrating the potential for a "dry" collection and processing method for faster and simpler detection of SARS-CoV-2 from nasal swabs. Our results further suggest that the simplified protocol (direct elution from dry swab into TE → RT-qPCR) may be as sensitive as the conventional protocol (swab → UTM → RNA extraction → RT-qPCR). SARS-CoV-2 Ct values for the simplified protocol were consistently lower, including when using both contrived and real-world positive samples, although the differences were not statistically significant and are likely in part due to the elution of dry swabs into a smaller volume. Additional testing using samples collected from a larger cohort of study participants and under a broader range of environmental stressors (e.g. temperature stability studies) is warranted.

Massive scaling and deployment of SARS-CoV-2 testing is essential to curtailing the COVID-19 pandemic, and will likely be necessary well into the future. The dry swab protocol evaluated here, including in the context of real-world self-collection, would markedly simplify the workflow for RT-qPCR, the most widely deployed testing paradigm, by eliminating the need for viral transport media and RNA extraction, both of which are currently experiencing significant supply chain challenges. Looking forward, we envision that nasal swabs -- self-collected into laboratory ready, barcoded tubes and transported dry -- could potentially serve as a common input to a range of SARS-CoV-2 nucleic acid tests. This includes gold-standard tests like RT-qPCR, but also potentially new, more scalable modalities including RT-LAMP (*6–8*) and Swab-Seq (*11*).

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The operationalization of the mass distribution and return of such lab-ready collection devices is a significant effort that should begin now.

Methods

Collection of Nasal Swabs

Individuals who tested positive for SARS-CoV-2 through routine clinical testing were recruited into this study. Based on identification of a laboratory-confirmed positive through the automated laboratory alert system, individuals were contacted and enrolled into a study to compare performance of various swab types. After providing consent, enrolled participants had a swab and send kit (*4*) containing two swabs (Copan FloqSwab 56380CS01) delivered to their home via 2-hour delivery and were provided instructions to self collect mid nasal swabs. Participants placed one swab in UTM (Becton Dickinson PN 220220) and the other in an empty 15 mL conical tube for transport. Both swab types were packaged by the participant according to kit instructions and sent to the Brotman Baty Institute / Northwest Genomics Center, utilizing standard IATA shipping procedures at ambient temperature. All samples were tested utilizing the Northwest Genomics Center's SARS-CoV-2 RT-PCR Laboratory Developed Test (extraction and RT-qPCR described below).

Preparation of inactivated viral controls

For mock SARS-CoV2 positive swab samples, two healthy volunteer self-swabs were administered and collected. Each swab was then loaded with 2 µL of heat-inactivated virion (VR-1986HK [1.6e6 virion/µL], ATCC). The conventionally processed swab was prepared by placing the virion loaded swab into 3 mL of UTM (Becton Dickinson PN 220220) and then allowing it to rest at room temperature for 6 hours. Five fold dilutions were then made using UTM as the diluent. After loading with 2 µL of heat-inactivated virion, each dry swab was allowed to dry to completion for 6 hours at room temperature in an uncapped 15 mL conical tube.

RNA extraction of samples in UTM

For swabs stored in UTM, 200 µL of UTM was extracted on the Magna Pure 96 using a DNA and Viral NA Small Volume Kit (06543588001, Roche) with the universal small volume protocol and eluted into 50 µL proprietary elution buffer.

Dry Swab elution

All work was performed within a biosafety cabinet with appropriate precautions. Each collected dry swab was first removed from its 15 mL conical transport container and placed into a 1.5 mL eppendorf tube (15575020, Eppendorf). Swabs were then cut using a sterile razor blade such that each eppendorf tube could be closed. Next, 200 µL of Tris-EDTA [10mM Tris-HCl pH 7.5 (T2319-1L, Sigma), 0.1mM EDTA (15575020, Invitrogen)] was added to each eppendorf tube and vortexed for 30 seconds. Eppendorf tubes containing swabs were then placed in a microfuge to collect eluate. To test various buffers, 45 µL of this solution was removed and added to either 5 µL of TE or 5 µL of 10% Triton-X (X100-500ML, Sigma Aldrich). These two

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samples constitute the undiluted eluate from the dry swabs. The real-world dry swabs presented in this study were processed on 2 separate days, and by 2 different operators.

RT-qPCR

For each primer-probe pair a RT-qPCR working solution was prepared. Each RT-qPCR reaction was run at a final volume of 20 µL and contained 5 µL 4x TaqPath RT-qPCR MasterMix (PN A15300, Life Technologies), 0.5 µL 20x RNAse P TaqMan VIC Probe (A30064, Life Technologies), 0.33 µL of SARS-Cov2 ORF1b FAM probe (PN 4332079, Life Technologies assay# APGZJKF) or spike (S) gene (PN 4332079, Life Technologies assay# APXGVC4) and 9.17 µL nuclease-free water (1907076, Thermo Fisher). After dispensing 15 µL of this master mix to each well of a 384 well plate (Applied Biosystems PN 4309849), 5 µL of sample was added to each well. Each assay is run in technical duplicate for a total of four RT-qPCR per sample. RT-qPCR was then performed on the Quantstudio 6 Pro. The thermo-cycler was programmed to 25˚C for 2 minutes, 50˚C for 15 minutes, 98˚C for 3 minutes, followed by 40 cycles of 98˚C for 3 seconds and 60˚C for 30 seconds. Reported Ct values were obtained from the onboard analysis performed by the Quantstudio 6 using the default settings. No template controls (NTC) included either TE, TE + 1.0% Triton-X, or water. Positive controls contained purified nucleic acid with sequence that was amplified by the ORF1b and Spike gene primer-probe sets.

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Ethics Approval: Sequencing and analysis of samples from the Seattle Flu Study and the Hospitalized and Ambulatory Adults with Respiratory Viral Infections (HAARVI) study were approved by the institutional review board at the University of Washington (protocols STUDY00006181 and STUDY00000959). Informed consent was obtained for all participant samples.

Competing interests: Sriram Kosuri owns stock and is an employee of Octant Inc., who have developed SwabSeq, a method that will likely benefit from dry swab and extraction free protocols becoming standard. Helen Chu is a consultant for Merck and GlaxoSmithKline. Jay Shendure is a consultant with Guardant Health, Maze Therapeutics, Camp4 Therapeutics, Nanostring, Phase Genomics, Adaptive Biotechnologies, and Stratos Genomics, and has a

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research collaboration with Illumina.

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