SwabExpress: An end-to-end protocol for extraction-free COVID-19 testing

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Structured Abstract

Background

The urgent need for massively scaled clinical testing for SARS-CoV-2, along with global shortages of critical reagents and supplies, has necessitated development of streamlined laboratory testing protocols. Conventional nucleic acid testing for SARS-CoV-2 involves collection of a clinical specimen with a nasopharyngeal swab in transport medium, nucleic acid extraction, and quantitative reverse transcription PCR (RT-qPCR) (1). As testing has scaled across the world, the global supply chain has buckled, rendering testing reagents and materials scarce (2). To address shortages, we developed SwabExpress, an end-to-end protocol developed to employ mass produced anterior nares swabs and bypass the requirement for transport media and nucleic acid extraction.

Methods

We evaluated anterior nares swabs, transported dry and eluted in low-TE buffer as a direct-to-RT-qPCR alternative to extraction-dependent viral transport media. We validated our protocol of using heat treatment for viral inactivation and added a proteinase K digestion step to reduce amplification interference. We tested this protocol across archived and prospectively collected swab specimens to fine-tune test performance.

Results

After optimization, SwabExpress has a low limit of detection at 2-4 molecules/uL, 100% sensitivity, and 99.4% specificity when compared side-by-side with a traditional RT-qPCR protocol employing extraction. On real-world specimens, SwabExpress outperforms an automated extraction system while simultaneously reducing cost and hands-on time.

Conclusion

SwabExpress is a simplified workflow that facilitates scaled testing for COVID-19 without sacrificing test performance. It may serve as a template for the simplification of PCR-based clinical laboratory tests, particularly in times of critical shortages during pandemics.

Introduction

Since the first reported cases in the winter of 2019, the spread of the novel beta-coronavirus SARS-CoV-2 has grown into a global pandemic. The virus spreads easily from person to person and is often carried by asymptomatic individuals (3). These viral properties, in conjunction with a lack of an effective centralized response or societal adherence to public health recommendations, has led to a continued persistence of the pandemic throughout the United States (4). It is widely recognized that increased testing capacity can ameliorate the outbreak (5,6), but the prohibitive cost of testing materials and reagents as well as global supply chain problems continue to thwart efforts to reach the required scale.

Since the beginning of the pandemic, the gold standard for SARS-CoV-2 detection has been RNA extraction followed by reverse-transcription quantitative polymerase chain reaction (RT-qPCR). Specimens are traditionally collected as nasopharyngeal (NP) specimens (3) by healthcare professionals and transported in viral media (e.g. Universal Transport Media (UTM)). Worldwide reliance on this template protocol has led to global shortages in swabs, viral media, and laboratory reagents. These shortages continue to plague testing labs and impede efforts to scale. Prior literature (7,8) and the work of United Health/Quantigen (9), have established that swabs collected without transport media are acceptable for nucleic acid detection-based diagnostics, eliminating the reliance on UTM. Extraction-free protocols have also been developed to remove the need for RNA extraction reagents and streamline testing protocols. Saliva specimens have been shown to be particularly amenable to extraction-free testing protocols. For example, SalivaDirect™ – a protocol for performing SARS-CoV-2 RT-qPCR on saliva specimens without extraction (10) – had a sensitivity of 89% compared to traditionally processed anterior nares (AN) or oropharyngeal (OP) swabs, demonstrating the viability of extraction-free protocols. Unlike saliva, extraction-free methods for nasal swabs have been less sensitive than conventional protocols—likely due to PCR inhibition from transport media or saline (10–16).

Here we describe the development of an UTM and extraction-free protocol for anterior nasal dry-swabs that is compatible with RT-qPCR and does not sacrifice test performance. This protocol, which we have coined 'SwabExpress', has a low limit of detection, high sensitivity, high specificity, and superior test performance when compared to conventional extraction-based RT-qPCR protocols. We further identify and ameliorate two distinct failure modes for extraction free RT-qPCR-based testing. Widespread adoption of this approach and others like it could result in a dramatic increase in testing capacity, decrease consumables used during testing, and ultimately help curb the spread of SARS-CoV-2.

Methods

Collection of Nasal Swabs - For preliminary studies, individuals who tested positive for SARS-CoV-2 through clinical testing were identified and recruited into a study of home-based, self-collected home swabs (28). After providing consent, enrolled participants were supplied a Swab and Send kit (19) containing two swabs (Copan FloqSwab 56380CS01) delivered to their home via 2-hour delivery and were provided instructions to self collect two mid-turbinate swabs. Participants placed one swab in a tube with UTM (Becton Dickinson PN 220220) and the other in an empty, dry 15 mL conical tube for transport. For all other studies, anterior nares (US Cotton #3, distributed by Steripack) swabs were collected by the Seattle Flu Study, Husky Coronavirus Testing Program (HCT) (26) or the Seattle Coronavirus Assessment Network (SCAN) (29). Anterior nares swabs were transported in a sterile, empty conical tube directly to the lab by HCT technicians. SCAN swabs were packaged by the participant according to kit instructions and sent to the Brotman Baty Institute / Northwest Genomics Center, utilizing standard International Air Transport Association shipping procedures by courier at ambient temperature. These IRB-supervised studies were public health surveillance programs and enrolled both symptomatic and asymptomatic participants. Informed consent was obtained from adult participants and

parents/permanent legal guardians of participant children. Archived and fresh convenience specimens from these studies were chosen at random for use in the current study.

Usability Study - To recruit a sufficient number of children for the prospective usability study, participants were recruited that met broad eligibility criteria: 1. No COVID-19 symptoms, 2. no prior self-swab experience and 3. no prior medical or laboratory training. We obtained informed consent from adult participants and parents/permanent legal guardians of participant children.

Swab rehydration and elution - All work was performed within a class II biosafety cabinet with appropriate precautions. For preliminary studies each mid turbinate dry swab was placed into a 1.5 mL microfuge tube, then cut using a sterile razor blade. Next, 200 µL of low-TE [10mM Tris-HCl pH 7.5 (T2319-1L, Sigma), 0.1mM EDTA (15575020, Invitrogen)] was added to each tube and vortexed for 30 seconds. To test various buffers, 45 µL of this solution was removed and added to either 5 µL of low-TE or 5 µL of 10% Triton-X (X100-500ML, Sigma Aldrich). These two specimens constitute the undiluted eluate from the dry swabs.

For all other studies anterior nares swabs were rehydrated in 1 mL low-TE prepared in UltraPure Water (Life Technologies PN 10977023). Specimens were vortexed for 30 seconds or shaken for 1 minute and allowed to incubate at room temperature for at least 10 minutes before transfer to Matrix tubes (Thermo Fisher).

RNA extraction of specimens - 200 µL of eluate was extracted on the Magna Pure 96 using a DNA and Viral NA Small Volume Kit (Roche, 06543588001) with the universal small volume protocol and eluted into 50 µL proprietary elution buffer. Or 200 µL of eluted anterior nares specimens were extracted on the KingFisher Flex using the MagMAX Viral Pathogen II Nucleic Acid Isolation Kit with MagMAX™ Viral/Pathogen Ultra Enzyme Mix (Thermo Fisher A48383 and

A42366) and eluted in 50 µL (although circa 35 µL is eluted).

SwabExpress Specimen Preparation - 50 µL of 94 specimens were transferred to a LoBind 96 well plate (Eppendorf 30129512) using a manual 96-well pipetting system (Rainin Liquidator) with low retention tips (Rainin 17014402) with or without 5 µL of Proteinase K (Thermo Fisher A42363, proprietary concentration). The plate was sealed with foil (Eppendorf 0030127854 and 5392000013). Specimens with Proteinase K were incubated at 37˚C for 15 minutes in a convection oven (Across International 0853924003042) and then transferred to a second oven for heat inactivation at 95˚C for 15 minutes. Specimens without Proteinase K were heat inactivated at 95˚C for 30 minutes.

RT-qPCR - Each RT-qPCR reaction was performed at a final volume of 10 µL and containing 1X TaqPath RT-qPCR MasterMix (PN A15300, Life Technologies), 0.125X RNAse P TaqMan VIC assay (A30064, Life Technologies) or 1X RNAse P HEX assay (IDT), 1X SARS-Cov-2 ORF1b FAM assay (PN 4332079, Life Technologies assay# APGZJKF) or 1X Spike (S) gene (PN 4332079, Life Technologies assay# APXGVC4) and nuclease-free water (1907076, Thermo Fisher). 5 µL of specimen was added to each well. Primer sequences were designed against Wuhan-Hu-1 sequence (MN908947.3) and are proprietary to Thermo Fisher. Plates were sealed using optically clear microseal B (Biorad). Each assay was performed in technical duplicate for a total of four RT-qPCR wells per sample. RT-qPCR was then performed on the Applied Biosystems QuantStudio 6 Pro (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 using predetermined cycle thresholds. Positive controls contained purified nucleic acid with sequence that was amplified by the ORF1b and Spike gene assays.

The RT-qPCR reaction for the CDC COVID-19 diagnostic test was performed at a final volume of 20 µL. Reactions contained 1X TaqPath RT-qPCR MasterMix , nCOV-N1 FAM or nCOV-N2 FAM

primer and probe mix (10006713, IDT) and nuclease-free water (1907076, Thermo Fisher) and 5 µL of specimem was added to each well. RT-qPCR was then performed on the QuantStudio 6 Pro as above. Reported Ct values were obtained from the onboard analysis using the auto-determined cycle thresholds. Data were analyzed using Excel and R 3.5.

Preparation of inactivated viral controls - Contrived SARS-CoV-2 positive swabs were generated by collecting clinical matrix from a confirmed healthy volunteer and loaded with 2 µL of diluted heat-inactivated virion (VR-1986HK [1.6e6 virion/µL], ATCC).

Viral Inactivation Studies - Viral inactivation studies were performed at the Seattle Children's Research Institute biosafety level 3 facility. 25 µL of viral stock (isolate USA-WA1/2020 obtained from ATCC BEI Resources) with a titer of $5.8x10^6$ pfu/mL was incubated in 200 µL of TE or TE + 0.25% Triton for 10 minutes at room temperature, or in TE at 65˚C for 10 minutes. Untreated and treated SARS-CoV-2 was then added neat and at 10-fold dilutions through 10^{-7} to confluent cultures of Vero E6 cells (CRL-1586, ATCC) and 48 hours later cytopathic effects were scored after staining with crystal violet. RNA was isolated from Vero cells using a TRIzol Plus RNA Purification Kit (ThermoFisher) and the amount of SARS-CoV-2 was quantified by RT-qPCR.

Retrospective Comparison Studies - Remnant participant specimens were stored either at 4˚C or -80˚C and prepared for RT-qPCR by extraction or heat treatment or SwabExpress digestion as described above. Technicians performing testing and clinical directors interpreting results were both blinded to previous test results.

Prospective Comparison Studies - freshly acquired specimens from the SCAN and HCT studies were prepared by extraction or heat treatment or SwabExpress and tested by RT-qPCR in parallel. For prospective analyses, both technicians and clinical directors performed testing and

interpretation blinded to results from the comparator method.

Results

Usability and Reliability of Anterior Nares (AN) Swabs for At-Home Specimen Collection

We first explored the use of anterior nares (AN) swabs for specimen collection. For mass testing purposes, a swab that is widely available, inexpensive, easy to manufacture, and simple for self-collection is critical. The US Cotton #3 swabs fit these specifications; a polyester AN swab that resembles consumer-brand Q-tips (17). For the purposes of scaled observed or at-home self specimen collection or specimen collection for a child, swabbing the anterior nares anatomical site would be more comfortable, accessible and easier to describe to test users leading to fewer mistakes and better specimen collection (18,19).

Therefore, we conducted a usability study to determine both the accuracy and ease of AN swabs in a Swab-and-Send program where at-home specimen collection kits were delivered to participant residences, the participants swabbed themselves or a child while being virtually monitored by clinical study coordinators and then packaged the specimen for return to the molecular testing lab (19,20). After using the specimen collection kit, study participants completed a survey reporting their level of confidence, the kit's ease of use, and the level of discomfort experienced during swabbing. Participants were recruited from the greater Seattle area and spanned a range of ages, races, household income, and educational attainment (online **Supplemental Fig. S1; Supplemental Table S1A-D**).

The results of the usability study were very encouraging. The majority of participants reported only mild-discomfort during specimen collection with 40% of participants reporting no discomfort at all (**Fig. 1A**). A majority of study participants also found the instructions clear and felt confident that they had correctly collected their specimen (**Fig. 1B**). This was confirmed by low observed

rates of error during specimen collection using the AN swabs and during packaging for return (online **Supplemental Table S2A-B**). Molecular testing performed on these self-collected specimens confirmed this; RT-qPCR detected the human marker RNase P mRNA in 100% of swabs with an average crossing threshold value of 23.5 (SD 1.7). The amount of RNase P mRNA recovered from the AN swabs was higher than for unsupervised collection of mid-turbinate swabs, which had an average crossing threshold (Ct) value of 26.9 (SD 2.5) (**Fig. 1C**). Together these data indicate that the use of widely available polyester swabs in the anterior nares is a viable and preferable alternative for at home specimen collection.

Handling Dry Swabs in the Clinical Laboratory

Standard viral media such as Universal Transport Medium (UTM) (*e.g.* COPAN Diagnostics) have been in short supply over the course of the pandemic. These salt-rich media inhibit direct RT-qPCR, making RNA extraction a necessity and thus create an additional bottleneck in the testing process. Furthermore, automated extraction systems are expensive and their reagents and consumables are also subject to global shortages. Therefore, we focused on eliminating UTM and extraction from our testing platform. To bypass UTM, we adopted a dry swab transport and rehydration method validated by Quantigen that has been explored by other clinical testing laboratories (15,21). Next, to eliminate RNA extraction and enable direct RT-qPCR, we tested rehydration solutions for their ability to elute contrived SARS-CoV-2 specimens, compatibility with direct RT-qPCR, and simplicity. We determined that elution in low-TE (10 mM Tris pH 7.5, 0.1 mM EDTA) without other detergents was best suited for direct RT-qPCR (online **Supplemental Fig. S2**). Unlike UTM and other saline solutions, the low ionic strength of low-TE does not inhibit PCR amplification. Moreover, low-TE can be quickly prepared using reagents commonly found in laboratories.

Bypassing nucleic acids extraction poses another problem; instead of the virus being inactivated by the denaturing agents during nucleic acid extraction, the specimen eluted from the swab remains potentially infectious for SARS-CoV-2 or other pathogens and poses a risk to laboratory staff. Accordingly, specimens from both conventional UTM and rehydrated dry swabs are processed inside a class II biosafety (BSL-2) cabinet, in accordance with federal regulatory guidance. However, it is practical and beneficial for downstream steps (like preparing RT-qPCR reactions) to take place on a BSL-2 designated bench. Therefore, we compared several inactivation methods to determine which would be easiest without inhibiting PCR or causing a loss of sensitivity. Viral inactivation of coronaviruses can be achieved through the use of either detergent or heat (22). Our prior results demonstrate the negative impact of detergents on RT-qPCR (online **Supplemental Fig. S2**); therefore, we opted to deploy heat inactivation (online **Supplemental Fig. S3**). We used a protocol to heat inactivate at higher temperatures (95˚C) for 30 minutes to increase the safety margins. We also determined that this high-heat protocol had the added benefit of stabilizing the sample over time, a result concordant with another SARS-CoV-2 testing protocol in saliva (23).

Performance of Extraction-free RT-qPCR

Having developed an extraction-free RT-qPCR protocol (EF-RT-qPCR), we set out to determine its performance on both contrived and clinical specimens. To assess analytical sensitivity, we first determined this assay's limit of detection (LoD), the minimum number of SARS-CoV-2 RNA molecules that could be detected in greater than 95% of RT-qPCR reactions. To generate these contrived specimens, we inoculated AN swabs with clinical matrix collected from a healthy volunteer with dilutions of heat inactivated SARS-CoV-2. These experiments determined the EF-RT-qPCR analytical sensitivity to be 2 molecules/µl of eluate for the Orf1b assay and 4 molecules/µl of eluate for the S-gene (Spike gene) assay (online **Supplemental Table S3**). This

LoD is comparable to the LoD of many other RT-qPCR-based tests that have been issued Emergency Use Authorization from the FDA (24).

Next we tested the performance of EF-RT-qPCR compared to our clinically validated RT-qPCR laboratory-developed test on archived AN specimens. In this assay each sample is tested in 4 independent RT-qPCR reactions, comprising two SARS-CoV-2 assays (Orf1b and Spike) in duplicate, and is multiplexed with a RNase P assay in every well (**Fig. 2A** and online **Supplemental Fig S4**). Following RT-qPCR, a clinical result is determined by the number of replicates displaying SARS-CoV-2 amplification: positive (3 or 4 of 4 wells), low-positive/inconclusive (2 of 4 wells) and negative (0 or 1 of 4 wells). Head-to-head comparison between EF-RT-qPCR and a reference standard extraction-based RT-qPCR assay on matched specimens established that EF-RT-qPCR was 100% specific (56/56 negative specimens) and 91.0% sensitive (61/67 - 56 positive and 5 low-positive) (**Fig. 2B**). Comparison of the mean delta Ct (∆Ct) values between the two assays showed that eliminating extraction did decrease analytical sensitivity. We observed an average increase of 1.96, 2.45, and 4.00 cycles for Orf1b, Spike and RNase P assays, respectively. Indeed, the 6 specimens not detected by EF-RT-qPCR had an average Ct with the extraction-based RT-qPCR assay of 34.13 for Orf1b and 35.29 for Spike.

Owing to an unstable supply chain, while validating the EF-RT-qPCR protocol, our clinical laboratory was forced to switch from the Roche Magna Pure 96 to the Thermo Fisher KingFisher Flex automated nucleic acids extraction platform. The relative sensitivity, specificity and ∆Ct

values between 619 prospective specimens run in parallel on both the KingFisher Flex (extraction) and EF-RT-qPCR were comparable to results of the retrospective study on stored specimens. EF-RT-qPCR detected SARS-CoV-2 in 100% of specimens that were positive by the extraction method with a 99.4% specificity (**Tables S5-S7**).

Addition of Proteinase K reduces amplification interference

After deploying EF-RT-qPCR as our clinical testing platform, we repeatedly observed two undesirable outcomes that were not observed in our validation studies. First, for 0.9% of specimens (n = 383/43,539), amplification of the human RNase P internal control was undetected in 2 or more of the 4 reactions (**Fig. 2B**; online **Supplemental Table S7**). These specimens were classified as "failures" and each test was repeated before releasing the result. Second, for 0.5% of specimens (229/43,539), we sporadically observed the presence of strong amplification (Ct < 30) in a single well for one of the SARS-CoV-2 targets in specimens where the three other wells were undetected (online **Supplemental Table S8)**. However, upon repeat RT-qPCR, both with and without extraction, all 4 wells of the SARS-CoV-2 reactions for these specimens were undetected.

We noted that some of the specimens that produced these problematic outcomes had excess mucous or other nasal secretions. Therefore, we hypothesized that the addition of proteinase K (ProK) digestion could ameliorate both RNase P failures and the spurious SARS-CoV-2 amplification by digesting mucins and other potentially interfering proteins in the nasal specimens (25). We compared RT-qPCR results for 1,222 clinical specimens prepared by the 30 minute 95˚C heat treatment with those digested with ProK for 15 minutes prior to heat treatment at 95˚C for 15 minutes. We observed approximately 10-fold fewer RT-qPCR reactions with failed RNase P

amplification -- 27 of 4,888 without ProK vs 2 of 4,888 with ProK -- reducing the failure rate to 0.04% (**Fig. 3A,** online **Supplemental Table S9**), and improved RNase P detection (∆Ct -0.88) (online **Supplemental Fig. S5**). Furthermore, the addition of a ProK digestion step eliminated spurious amplification of SARS-CoV-2 targets.

In the 4,888 specimens processed both with and without ProK, ProK-treated specimens had decreased Ct values (mean decrease of 1.22 for Orf1B, and 0.97 for Spike). This increased sensitivity was also reflected in the ability to accurately classify archived SARS-CoV-2 positive specimens with Ct values > 28 (**Fig. 3B**). Repeatability and reproducibility were also improved with the addition of ProK (**Fig. 3C**). Upon addition of ProK, on SARS-CoV-2 positive samples, our protocol had a higher concordance (93.3%) versus without ProK (90%) or specimens extracted on the KingFisher Flex (86.6%) (online **Supplemental Table S10**). After this optimization we named our final protocol "SwabExpress" -- consisting of a dry AN swab, followed by ProK digestion and direct RT-PCR. Finally, we prospectively compared performance on 1169 specimens run in parallel on the SwabExpress and KingFisher Flex (extraction) platforms. Positive and negative clinical concordance was excellent; there was 100% concordance for positives results, 99.91% concordance across negatives with a small ∆Ct value of 0.37 for the Orf1b target and 1.46 for the S target between the two assays (**Fig. 3D**, online **Supplemental Table S11**).

<u>SwabExpress is compatible with other SARS-CoV-2 RT-qPCR assays</u>

Our laboratory-developed test uses custom Orf1b and Spike-gene assays for detecting SARS-CoV-2. To establish that the SwabExpress protocol was compatible with the widely used CDC N1 and N2 assays, we performed RT-qPCR on 75 positive specimens and 92 negative specimens

with the N1 and N2 assays performed in parallel on the SwabExpress platform and extraction-based RT-qPCR platform. The results were 100% concordant between our custom assays and the CDC assays. Ct values for positive samples were delayed when prepared by SwabExpress protocol compared to the Roche Magna Pure 96. However, this difference did not change the clinical interpretation of these samples (**fig. S6**). For the N1 assay, extracted specimens had an average Ct of 19.22 ± 3.67 versus 21.79 ± 4.33 with SwabExpress (∆Ct of 2.57). For the N2 assay, extracted specimens had an average Ct of 18.31 ± 3.73 versus Cts of 19.80 ± 3.72 for SwabExpress (∆Ct of 1.49) (**Table S12).**

SwabExpress is time and cost effective

A dry-swab, extraction-free RT-qPCR protocol comprises the minimal components of a diagnostic test. Although the addition of a proteinase K digestion adds $0.14 USD to the reagent cost for each sample, this cost is warranted. The addition of proteinase K reduces the repeat rate, reduces the chances of a false positive result from interfering substances during PCR amplification, improves the performance of the test, and in our hands, outperformed a suboptimal yet widely used automated extraction system (Thermo KingFisher Flex™).

Upon adoption, SwabExpress approximately doubled laboratory capacity. First, hands-on technician time previously spent preparing and running extraction systems, went towards accessioning and processing additional samples. Second, the SwabExpress protocol increases scale by using a convection oven that can process up to 6 96-well plates simultaneously. This throughput greatly exceeds the single 96-well plate processed by commercial automated extraction systems. Further scaling of the SwabExpress protocol can be accomplished through

the purchase of additional or larger ovens, although RT-qPCR instruments used during amplification and readout still pose a substantial bottleneck in the testing protocol.

Along with the substantial cost of purchasing automated extractors, the consumables required for their operation cost between $4 and $5 per sample. By eliminating extraction, and transport medium, SwabExpress reduces the associated costs by more than 90% (~$0.20 per sample). In all, SwabExpress offers a time and cost-saving alternative to nucleic acids extraction using readily available reagents, which reduces dependence on a heavily burdened supply chain (**Fig. 4, Table S13**).

Discussion

Here we present SwabExpress, an end-to-end diagnostic platform optimized for faster and simpler low-cost detection of SARS-CoV-2 from nasal swabs without the use of nucleic acid extraction (**Figure 4)**. This protocol was so named for its ease, rapid turn around and simplicity -- dry swabs, without extraction, enhanced with proK digestion. By eliminating transport media and extraction from the workflow, we have decreased cost per sample and reduced supply chain pressure for the lab. Because of the reduced cost and the ability to process many more specimens in parallel, our lab's capacity markedly increased with its adoption. Importantly, we gained efficiency without sacrificing accuracy; our results suggest that the simplified SwabExpress protocol (direct elution from dry swab into low-TE + proteinase K → RT-qPCR) is as sensitive as the conventional PCR protocol (swab → UTM → RNA extraction → RT-qPCR). SwabExpress has supported scaled testing in our lab with over 91,000tests performed to date and allowed us to

support large testing endeavors such as the Husky Coronavirus Testing Program for the University of Washington (26).

There are some caveats to consider. Even with the addition of proteinase K, specimens with excess mucous fail to amplify RNase P. Since adding this proteinase digestion step, 18/12,991 specimens have had two or more RNase P reactions fail (0.1%) and our laboratory reflexes these few specimens to an extraction protocol. However, 0.1% compares favorably when compared to a protocol with extraction where the failure rate due to failed RNase P is 1% (215/22,546). In addition, the unknown presence of inhibitors precludes comparison of Ct values between specimens; therefore, studies directly comparing Ct values from different specimens may not yield accurate results.

We have observed a marked loss of viral RNA after freeze-thaw cycles for specimens stored in low-TE compared to specimens stored in commercial UTM. We detect a ΔCt of about 2.5 for specimens after -80℃ storage in low-TE, whereas the ΔCt for specimens retested after storage at -80℃ in UTM has historically been negligible. This impacts the ability to use these specimens for downstream applications such as genomic sequencing.

Several improvements can be incorporated into the SwabExpress platform. First, the ability to detect multiple pathogens from one assay can be explored. It is likely that SwabExpress will be compatible with other enveloped viruses such as influenza and respiratory syncytial virus. Multiple targets can be detected in many qPCR systems and the reemergence of these viruses as COVID-19 prevention control measures are relaxed will be of interest to monitor. Second, the most labor intensive part of the SwabExpress protocol is sample accessioning. Receiving individual 10 ml

tubes and transferring the eluate to 96-well format takes approximately 2.5 minutes per sample. Receiving nasal swabs in 96-well compatible, lab-ready transport tubes would streamline the process considerably (27). Third, incubation times for proteinase K digestion, heat inactivation, and RT-qPCR could be further optimized to save additional time during the testing process.

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 protocol evaluated here, including thousands of real-world, self-collected nasal swabs, 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 for public health surveillance applications. This includes gold-standard tests like RT-qPCR, but also potentially new modalities like Swab-Seq (*11*). The operationalization of the mass distribution and return of such lab-ready collection devices is a significant effort that should begin now.

Ethics Approval: Sequencing and analysis of specimens from the Seattle Flu Study, the Hospitalized and Ambulatory Adults with Respiratory Viral Infections (HAARVI) study and the SCAN study were approved by the Institutional Review Board at the University of Washington (protocols STUDY00006181, STUDY00000959, STUDY00007628, STUDY00010432, STUDY00011148). Informed consent was obtained for all participant specimens.

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Patents: S. Kosuri, Ginkgo Bioworks - Licensing for SwabSeq multiplexed assay development, Harvard U - Royalties from patents relating to genome editing, UCLA - Royalties from patents relating to multiplexed assays.

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References

1. Cheng MP, Papenburg J, Desjardins M, Kanjilal S, Quach C, Libman M, et al. Diagnostic Testing for Severe Acute Respiratory Syndrome–Related Coronavirus 2 [Internet]. Annals of Internal Medicine. 2020. page 726–34. Available from: http://dx.doi.org/10.7326/m20-1301

2. Botti-Lodovico Y, Rosenberg E, Sabeti PC. Testing in a Pandemic — Improving Access, Coordination, and Prioritization [Internet]. New England Journal of Medicine. 2021. page 197–9. Available from: http://dx.doi.org/10.1056/nejmp2025173

3. Cevik M, Tate M, Lloyd O, Maraolo AE, Schafers J, Ho A. SARS-CoV-2, SARS-CoV, and MERS-CoV viral load dynamics, duration of viral shedding, and infectiousness: a systematic review and meta-analysis. Lancet Microbe. Elsevier BV; 2021;2:e13–22.

4. Brauner JM, Mindermann S, Sharma M, Johnston D, Salvatier J, Gavenčiak T, et al. Inferring the effectiveness of government interventions against COVID-19. Science [Internet]. American Association for the Advancement of Science; 2021 [cited 2021 Apr 23];371. Available from: https://science.sciencemag.org/content/371/6531/eabd9338.abstract

5. Larremore DB, Wilder B, Lester E, Shehata S, Burke JM, Hay JA, et al. Test sensitivity is secondary to frequency and turnaround time for COVID-19 surveillance. medRxiv [Internet]. 2020; Available from: http://dx.doi.org/10.1101/2020.06.22.20136309

6. Hellewell J, Russell TW, Beale R, Kelly G, Houlihan C, Nastouli E, et al. Estimating the effectiveness of routine asymptomatic PCR testing at different frequencies for the detection of SARS-CoV-2 infections [Internet]. bioRxiv. medRxiv; 2020. Available from: http://medrxiv.org/lookup/doi/10.1101/2020.11.24.20229948

7. Moore C, Corden S, Sinha J, Jones R. Dry cotton or flocked respiratory swabs as a simple collection technique for the molecular detection of respiratory viruses using real-time NASBA [Internet]. Journal of Virological Methods. 2008. page 84–9. Available from: http://dx.doi.org/10.1016/j.jviromet.2008.08.001

8. Emerson J, Cochrane E, McNamara S, Kuypers J, Gibson RL, Campbell AP. Home Self-Collection of Nasal Swabs for Diagnosis of Acute Respiratory Virus Infections in Children With Cystic Fibrosis. J Pediatric Infect Dis Soc. 2013;2:345–51.

9. U.S. Department of Health and Human Services Food and Drug Administration. Policy for Coronavirus Disease-2019 Tests During the Public Health Emergency [Internet]. US Food and Drug Administration. 2020 [cited 2021 Mar 20]. Available from: https://www.fda.gov/media/135659/download

10. Vogels CBF, Brackney D, Wang J, Kalinich CC, Ott I, Kudo E, et al. SalivaDirect: Simple and sensitive molecular diagnostic test for SARS-CoV-2 surveillance [Internet]. Infectious Diseases (except HIV/AIDS). medRxiv; 2020. Available from: https://www.medrxiv.org/content/10.1101/2020.08.03.20167791v1.abstract

11. Smyrlaki I, Ekman M, Lentini A, Rufino de Sousa N, Papanicoloau N, Vondracek M, et al. Massive and rapid COVID-19 testing is feasible by extraction-free SARS-CoV-2 RT-PCR [Internet]. Infectious Diseases (except HIV/AIDS). medRxiv; 2020. Available from:

https://www.medrxiv.org/content/10.1101/2020.04.17.20067348v4.abstract

12. Lübke N, Senff T, Scherger S, Hauka S, Andrée M, Adams O, et al. Extraction-free SARS-CoV-2 detection by rapid RT-qPCR universal for all primary respiratory materials. J Clin Virol. 2020;130:104579.

13. Bruce EA, Huang M-L, Perchetti GA, Tighe S, Laaguiby P, Hoffman JJ, et al. Direct RT-qPCR detection of SARS-CoV-2 RNA from patient nasopharyngeal swabs without an RNA extraction step. PLoS Biol. 2020;18:e3000896.

14. Merindol N, Pépin G, Marchand C, Rheault M, Peterson C, Poirier A, et al. SARS-CoV-2 detection by direct rRT-PCR without RNA extraction. J Clin Virol. 2020;128:104423.

15. Padgett LR, Kennington LA, Ahls CL, Samarasinghe DK, Tu Y-P, Wallander ML, et al. Polyester Nasal Swabs Collected in a Dry Tube are a Robust and Inexpensive, Minimal Self-Collection Kit for SARS-CoV-2 Testing [Internet]. Available from: http://dx.doi.org/10.1101/2020.10.09.20210302

16. Ñique AM, Coronado-Marquina F, Mendez Rico JA, García Mendoza MP, Rojas-Serrano N, Simas PVM, et al. A faster and less costly alternative for RNA extraction of SARS-CoV-2 using proteinase k treatment followed by thermal shock. PLoS One. 2021;16:e0248885.

17. U.S. Department of Health and Human Services Food and Drug Administration. FDA, Gates Foundation, UnitedHealth Group, Quantigen, and U.S. Cotton Collaborate to Address Testing Supply Needs [Internet]. US Food and Drug Administration. 2020 [cited 2021 Apr 23]. Available from: https://www.fda.gov/news-events/press-announcements/coronavirus-covid-19-update-fda-gates-foundation-unitedhealth-group-quantigen-and-us-cotton

18. Truong M, Pfau B, McDermot E, Han PD, Brandstetter E, Richardson M, et al. Comparable specimen collection from both ends of at-home mid-turbinate swabs. J Clin Microbiol [Internet]. 2021; Available from: http://dx.doi.org/10.1128/JCM.03073-20

19. Kim AE, Brandstetter E, Wilcox N, Heimonen J, Graham C, Han PD, et al. Evaluating Specimen Quality and Results from a Community-Wide, Home-Based Respiratory Surveillance Study. J Clin Microbiol [Internet]. 2021; Available from: http://dx.doi.org/10.1128/JCM.02934-20

20. Chu HY, Englund JA, Starita LM, Famulare M, Brandstetter E, Nickerson DA, et al. Early Detection of Covid-19 through a Citywide Pandemic Surveillance Platform. N Engl J Med [Internet]. 2020; Available from: http://dx.doi.org/10.1056/NEJMc2008646

21. Parikh BA, Wallace MA, McCune BT, Burnham C-AD, Anderson NW. The Effects of “Dry Swab” Incubation on SARS-CoV-2 Molecular Testing. [Epub] J Appl Lab Med March 29, 2021, as doi: 10.1093/jalm/jfab010.

22. Darnell MER, Taylor DR. Evaluation of inactivation methods for severe acute respiratory syndrome coronavirus in noncellular blood products. Transfusion 2006;46:1770–7.

23. Ranoa DRE, Holland RL, Alnaji FG, Green KJ, Wang L, Brooke CB, et al. Saliva-Based Molecular Testing for SARS-CoV-2 that Bypasses RNA Extraction [Internet]. Cold Spring Harbor Laboratory. 2020 [cited 2020 Dec 31]. page 2020.06.18.159434. Available from:

https://www.biorxiv.org/content/10.1101/2020.06.18.159434v1

24. Resilience Health [Internet]. [cited 2021 Mar 19]. Available from: https://www.resiliencehealth.com/tests.html

25. Mallmann L, Schallenberger K, Demolliner M, Eisen AKA, Hermann BS, Heldt FH, et al. Pre-treatment of the clinical sample with Proteinase K allows detection of SARS-CoV-2 in the absence of RNA extraction [Internet]. Available from: http://dx.doi.org/10.1101/2020.05.07.083139

26. Weil AA, Sohlberg SL, O'Hanlon JA, Casto AM, Emanuels AW, Lo NK, et al. SARS-CoV-2 epidemiology on a public university campus in Washington state [Internet]. bioRxiv. medRxiv; 2021. Available from: http://medrxiv.org/lookup/doi/10.1101/2021.03.15.21253227

27. Rhinostics product information [Internet]. Rhinostics. [cited 2021 Jun 21]. Available from: https://rhinostics.com

28. McCulloch DJ, Kim AE, Wilcox NC, Logue JK, Greninger AL, Englund JA, et al. Comparison of Unsupervised Home Self-collected Midnasal Swabs With Clinician-Collected Nasopharyngeal Swabs for Detection of SARS-CoV-2 Infection. JAMA Netw Open. 2020;3:e2016382.

29. Greater Seattle Coronavirus Assessment Network (SCAN) [Internet]. [cited 2021 Feb 19]. Available from: https://scanpublichealth.org/

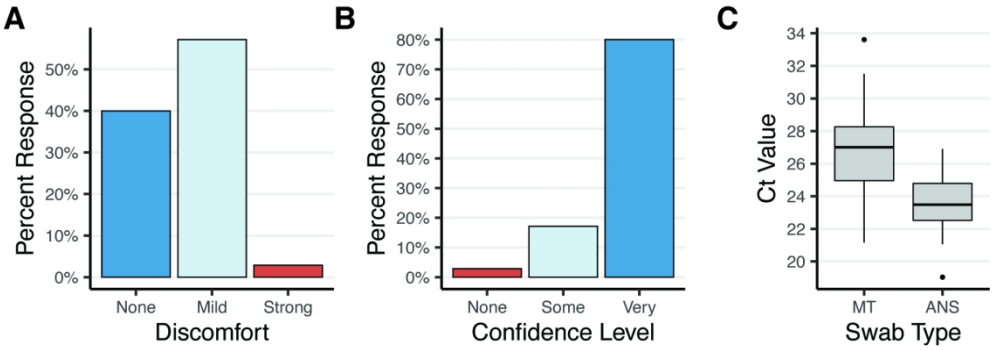
Figure 1. Polyester anterior nares swabs are both comfortable and easy to use. (A,B) Study participants' (n=35) self reported **(A)** discomfort and **(B)** confidence during self administration of an anterior nares swab at home. **(C)** Boxplot depicting the RT-qPCR crossing threshold values for RNaseP from self-administered anterior nares swabs (ANS) and mid-turbinate (MT) swabs.

Figure 2. Extraction free RT-qPCR set-up and test performance. (**A**) Assay layout of the EF-RT-qPCR test. One sample is assayed in four wells on a 384 well-plate. Each sample is tested for two probes, in duplicate. RNase P is assayed in each well. (B) Mean Ct values for 67 specimens processed by EF-RT-qPCR and extraction-based RT-qPCR. Reactions with no amplification by one preparation protocol are demarcated with red or blue points as indicated.

Figure 3. Addition of Proteinase K improves test performance. (A) Observed percentage of test failures with and without the addition of Proteinase K. **(B)** Archived samples with Ct > 28 reprocessed with either KingFisher Flex Extraction (left), Extraction-Free RT-PCR (middle), or SwabExpress (Extraction-Free RT-PCR + ProK) (right). Colors signify the number of samples and their classifications. **(C)** Box and whisker plots depicting the average delta Ct between replicate wells for SARS-Cov2 positive specimens. Red points indicate outliers. ΔCt values were more consistent upon addition of Proteinase K. **(D)** Mean Ct values of matched specimens run through the automated KingFisher extraction system (left) or using SwabExpress (SE). Specimens which were detected in only one of the two protocols are displayed as green points.

Figure 4. SwabExpress workflow. (1) Anterior nares swabs are collected and (2) transported dry to the lab. Upon receipt, (3) each swab is then hydrated with low-TE buffer, aliquoted into a 96 well plate, and (4) proteinase K is added to every well. (5) The eluted specimens are digested

and heat-inactivated in a laboratory oven before (6) they are loaded as the template in a RT-qPCR reaction. The cost listed includes reagents and consumables.



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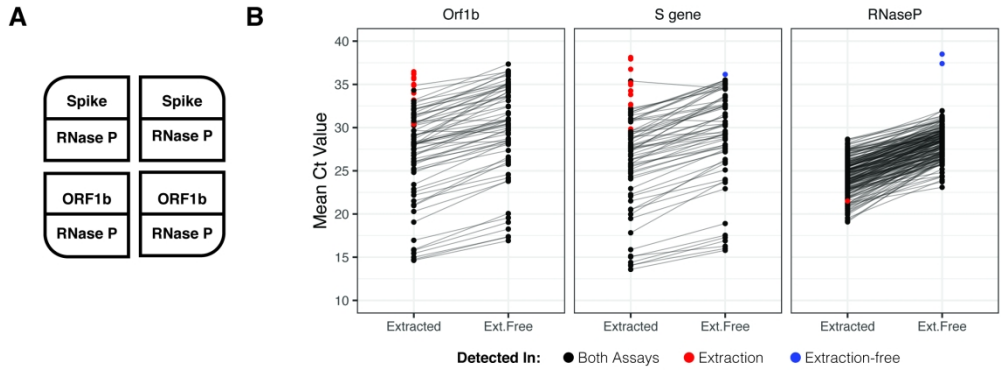

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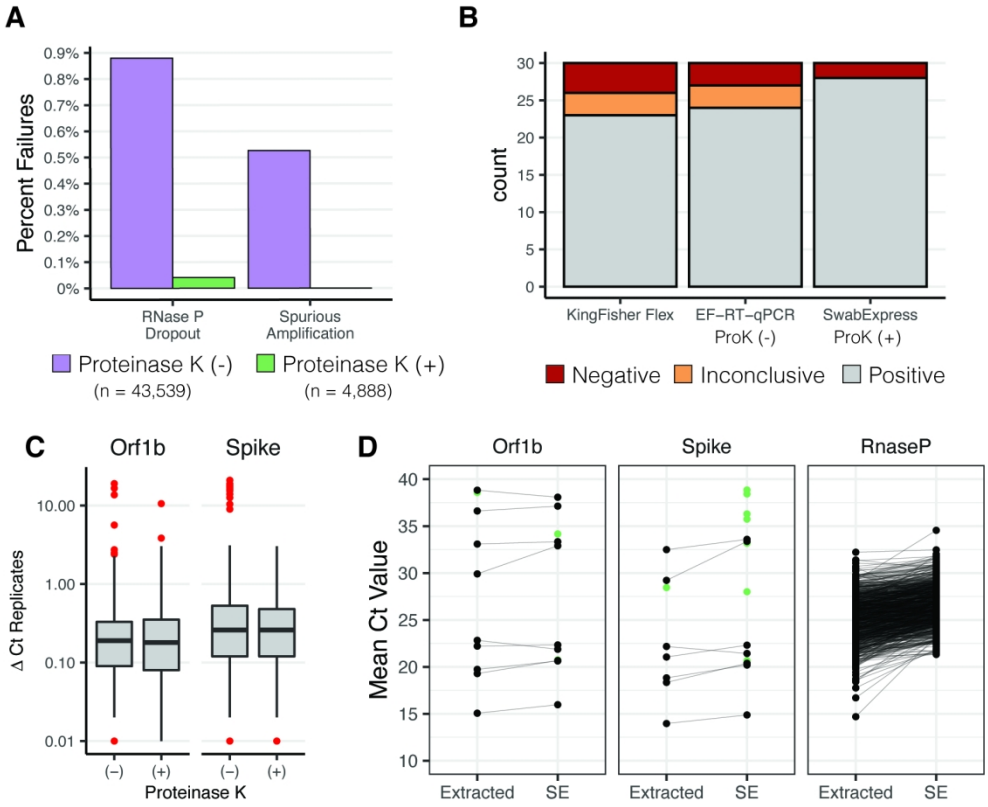

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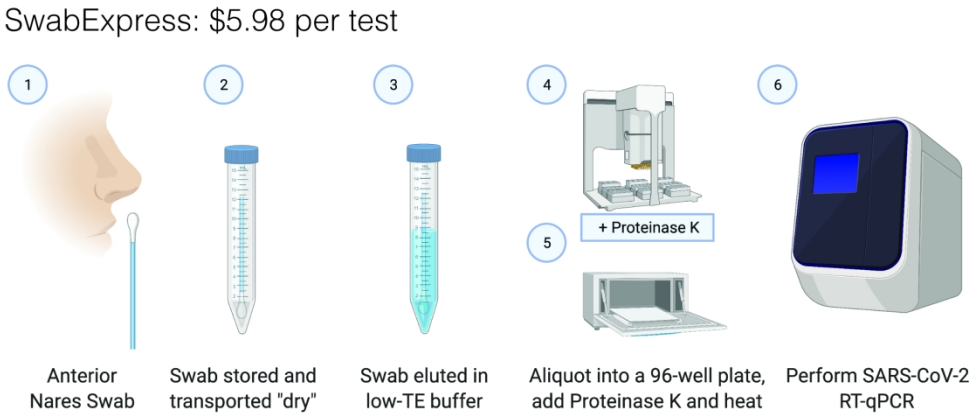

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