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Accessioning and automation compatible anterior nares swab design

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ABSTRACT

The COVID-19 pandemic has resulted in an unparalleled need for viral testing capacity across the world and is a critical requirement for successful re-opening of economies. The logistical barriers to near-universal testing are considerable. We have designed an injection molded polypropylene anterior nares swab, the Rhinostic, with a screw cap integrated into the swab handle that is compatible with fully automated sample accessioning and processing. The ability to collect and release both human and viral material is comparable to that of several commonly used swabs on the market. SARS-CoV-2 is stable on dry Rhinostic swabs for at least 3 days, even at 42 °C, and elution can be achieved with small volumes. To test the performance of the Rhinostic in patients, 119 samples were collected with Rhinostic and the positive and negative determinations were 100 % concordant with samples collected using Clinical Laboratory Improvement Amendments (CLIA) use approved nasal swabs at a clinical lab. The Rhinostic swab and barcoded tube set can be produced, sterilized, and packaged cost effectively and is designed to be adopted by clinical laboratories using automation to increase throughput and dramatically reduce the cost of a standard SARS-CoV-2 detection pipeline.

1. Introduction

As of November 1st 2020, at least 47 million cases of COVID-19 and over 1 million deaths have been reported world-wide ("COVID-19 Map - Johns Hopkins Coronavirus Resource Center, 2021."). To determine if a patient has COVID-19, in most cases, a nasopharyngeal (NP) swab is collected by a trained professional. The swab is then deposited in 1−3 mL of transport media followed by RNA purification and RT-qPCR. NP swabs are around 15 cm in length with a collection head coated with short synthetic filaments, flock, or spun fibers (Callahan et al., 2020); collection is often an uncomfortable process. The high demand for testing during this pandemic has outstripped the supply of NP swabs (and many other critical reagents for testing) resulting in a testing bottleneck (Garnett et al., 2020; Pfeiffer, 2020). These supply limitations together with a drive towards patient self-collection has spurred the development of alternatives to the standard NP swab. A promising alternative is anterior nares (AN) swabs, commonly referred to as nasal swabs. AN swabs offer a testing sensitivity similar to NP swabs (Irving et al., 2012; Péré et al., 2020) but are easier to use and more comfortable for the patient.

The choice of swab and collection device can have a major impact on the testing speed in clinical labs. Upon receiving samples, a typical procedure in a testing facility is to first accession the delivered patient samples by scanning the barcoded label to upload relevant patient data into the system, then swabs are manually removed from each collection tube and disposed of. The sample transport media is then processed to purify RNA, which is used as input for RT-qPCR. The initial steps in this procedure are hard to automate, slow, and expose staff to infection. Standard 1D barcoding systems and the manual removal of swabs is time consuming and thus costly. There are machines that can perform the entire procedure from accessioning to results, one tube at a time, e.g. a cobas® 8800, but this process is slow, 1056 tubes per 8-h shift ("Run new COVID-19 Coronavirus test on cobas® 6800/8800 Systems, " n.d.), and the machines are expensive.

In an effort to meet the dramatic increase in demand for nasal swabs, several groups have designed and 3D printed new swabs (Alghounaim

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et al., 2020; Callahan et al., 2020). The performance of these swabs is comparable to that of standard swabs; however, they aim to reproduce the existing status quo, rather than to address some of the limitations caused by the standard swab design. An ideal swab would be one that is comfortable for patients to self-administer without sacrificing performance, while also allowing for automated specimen accessioning and processing. Additionally, the swab would be made from non-absorbent material, allowing samples to be eluted into smaller volumes of transport media than those used in the current procedure, rendering the sample more concentrated and allowing for more sensitive detection of viral RNA. Due to the COVID-19 pandemic, there is a need for very high throughput clinical laboratories in non-traditional as well as traditional settings. For this reason, many clinical laboratories have been established at universities to allow for schools to safely re-open. Here we present the Rhinostic, a swab that 1) performs as well as existing AN swabs; 2) is compatible with direct input to RT-qPCR for extraction free SARS-CoV-2 detection (Smyrlaki et al., 2020); and 3) enables automated sample accessioning and processing; the collection system is compatible with 96-well format automated sample accessioning and processing.

2. Materials and methods

2.1. Swab design

The swabs were designed in SolidWorks (Dassault Systèmes) and manufactured using single-shot rapid injection molding (Protolabs) from medical grade FHR P5M4R polypropylene (Flint Hills), a material compatible with autoclaving (121 °C, 20 min), ethylene oxide, gamma radiation, and e-beam sterilization. The stacked rings of the swab head enable collection of nasal matrix without the need for an absorbent coating, using a design previously developed at the Wyss Institute. The cap cavity is compatible with automated decapping robot systems using a square profile adapter head, while the 2 mm pitch external threading mates with the interior threading of sample collection tubes from several major manufacturers (e.g. Matrix, Micronics, and LVL). As the new swab we developed in this study is useful for the collection of nasal samples for diagnostic tests we call it the Rhinostic swab, and the swab is being manufactured by a company called Rhinostics Inc. (See Declaration of Competing Interest).

2.2. Absorption of liquid by swab

The swabs used in this study were weighed on an analytical balance before and after a 15 s incubation in 1 mL of nuclease free water. Six replicates were measured and results are reported in Table 1.

2.3. Anterior nares self-swabbing to compare swab performance

We compared several swab types for performance in anterior nares (AN) specimen collection: Rhinostic, Procter & Gamble (P&G) Blue prototype, Wyss Institute flocked prototype, Puritan hydraflock, Puritan foam, Puritan polyester, US Cotton, and Microbrush®. Per CDC guidelines, volunteers were instructed to insert the AN swab 0.5 inch into a nostril, rotate three times along the membrane of the nose firmly and leave in place for 10–15 seconds, remove, and then repeat this procedure on the other nostril with the same swab to collect nasal matrix (CDC, 2020). The volunteer was then instructed to place the used swab in a dry 1.5 mL microcentrifuge tube and break the handle if necessary so the tube could close for transport. Prior to RT-qPCR reactions all swabs were suspended in nuclease free 1x PBS. All experiments in this study were approved by the Institutional Review Board of the Harvard Faculty of Medicine, IRB20−0581, and informed written consent was obtained from volunteers.

2.4. RT-qPCR

RT-qPCR reactions were prepared to reach a final volume of 10 μL using 8 μL of master mix and 2 μL of sample. For all experiments swab eluant was input to the RT-qPCR reaction prior to sample inactivation. The Luna Universal One Step RT-qPCR kit (NEB) was used for all RT-qPCR reactions. The master mix protocol was adjusted to include 0.25 U/μL of RNaseIn Plus (Promega) for every 10 μL reaction. RT-qPCR reactions were run on the QuantStudio 6 or QuantStudio 7 Real Time PCR system (Thermo Fisher Scientific) following the manufacturer recommended Luna RT-qPCR protocol. For all reactions, melt curves were used to determine if products were specific or non-specific. All non-specific T_ms, > 1 °C from the expected melting temperature, are presented as having a Ct of 40. All experiments included at least one negative control which was either 1x PBS or water. The sequences of all primers used are listed in Table S1.

2.5. Recovery of human mRNA from AN swabs

SARS-CoV-2 negative volunteers performed AN swabbing as directed with each type of swab tested (Fig. 2C, Table 1) to collect nasal matrix. There were three biological replicates for each AN swab measurement, taken on at least two different days. For every condition in which a swab was tested, an unused swab, without nasal matrix, was processed in parallel as a negative control. To recover the sample from the swabs, all swabs were suspended in 200 μL of 1x nuclease free PBS, vortexed for 10 s, spun down in a microcentrifuge, and input directly to the RT-qPCR for GAPDH mRNA detection (Fig. 2C).

2.6. Contrived samples using packaged synthetic SARS-CoV-2 spiked onto unused swabs

AccuPlex SARS-CoV-2 verification panel v2 (Seracare), a packaged synthetic virus, containing the N gene, E gene, ORF1a, S gene, and RdRp was used to simulate the expected viral recovery from AN swabs near the limit of detection (Fig. 2D). 10 μL of 100 copies/μL packaged synthetic virus was directly applied to the collection head of each swab. Swabs were left in a fume hood for about 20 min until the swabs appeared dry to the eye indicating absorption of the packaged synthetic virus into the collection material. At least three biological replicates were used for every swab tested and replicate data was collected on at least two different days. Swabs were then inserted into a 1.5 mL microcentrifuge

Table 1
Absorption properties of anterior nares swab types tested in this study.

Swab	Collection Material	Average volume absorbed (μL)	Standard deviation (μL)	Purchasable as of 03/24/21
Rhinostic	Polypropylene	14.4	2.2	Y
P&G Blue[1]	Polypropylene	0.7	1.0	Y
Wyss flock	Polypropylene and polyester flock	65.8	3.9	N
Puritan hydraflock	Polyester flock	154.1	8.9	Y
Puritan foam	Polyurethane foam	41.3	14.4	Y
Puritan polyester	Polyester	155.9	9.6	Y
US Cotton	Cotton	168.8	25.4	Y
Microbrush	Nylon Flock	64.9	10.1	Y

[1] Since submission, the rights to the P&G Blue prototype was purchased by Rhinostics Inc. and are available for purchase.

tube containing 200 μL of 1x nuclease free PBS, vortexed for 10 s, spun down in a microcentrifuge, and 2 μL was input directly to RT-qPCR for N gene detection. The positive control was 10 μL of 100 copies/μL packaged synthetic virus directly input to 190 μL of nuclease free 1x PBS and the negative control was 1x nuclease free PBS.

2.7. Remnant clinical samples

NP swabs from SARS-CoV-2 patient samples were purchased from BocaBiolistics, FL. The NP swabs are remnant samples obtained through BocaBiolistics and partner labs that were de-identified by BocaBiolistics with their IRB reviewed and approved SOP for de-linking specimens. These NP swabs arrived in 1−3 mL of viral, multitrans, or universal transport media (VTM, MTM, or UTM). 40 μL of each remnant sample media was aliquoted and frozen at −80 °C to limit freeze-thawing of samples. Samples were handled in a biosafety cabinet. Samples were only removed from the biosafety cabinet after being added to the RT-qPCR plate and sealed. Heat inactivation then occurred during the RT-qPCR process.

2.8. Contrived samples from a clinical source spiked onto swabs with nasal matrix

Nasal matrix was collected from volunteers as described above using Rhinostic and Puritan foam swabs. 5 μL of remnant clinical sample media, with either a higher (~1600 copies/μL), or lower titer (~140 copies/μL), was applied to the collection head of used swabs with nasal matrix, and swabs were air dried in the BSL2+ biosafety cabinet for 20 min. Each swab was then placed in a 1.5 mL microcentrifuge tube containing 200 μL of 1x nuclease free PBS, manually spun for 10 s in the media, and 2 μL was directly input to the RT-qPCR for both N gene (Fig. 2E) and GAPDH mRNA detection (Fig. S2C). To assess maximum possible viral recovery from the swab, the positive control was 5 μL of either the higher or lower titer remnant clinical swab sample in 195 μL of 1x nuclease free PBS. Negative controls were unused Rhinostic and Puritan foam swabs suspended in 200 μL of 1x nuclease free PBS. Four biological replicates were performed for each titer and type of swab tested.

2.9. Assessment of stability of SARS-CoV-2 on swabs with nasal matrix over time

To assess the stability of the SARS-CoV-2 virus on swabs with nasal matrix over time, two volunteers self-swabbed three independent times with both Rhinostic and Puritan foam swabs for a total of six swabs at each time point. The handles of the Puritan foam swabs were broken in order to safely close the collection vial, a 1.5 mL microcentrifuge tube. Several remnant clinical samples were mixed together to generate a pooled sample with a viral titer of ~10,200 copies/μL. The pooled sample was then aliquoted into 50 μL volumes and refrozen at −80 °C. At each time point (72, 48, 24, 2, and 0 h) an aliquot was thawed and 3 μL of pooled sample was applied to each swab. One Rhinostic and one Puritan foam swab with nasal matrix from each volunteer was incubated dry at room temperature (25 °C) or 42 °C in a 1.5 mL microcentrifuge tube to asses stability at room temperature or elevated temperatures that may occur during transport. A matched Rhinostic or Puritan foam swab with nasal matrix from each volunteer was immediately put into a 1.5 mL microcentrifuge tube containing 0.4 mL of 1x nuclease free PBS to assess the relative stability of a wet swab vs dry swab. Additionally, 3 μL of the pooled remnant clinical sample was applied to an unused Rhinostic and unused Puritan foam swab at each time point and kept dry over the time course at 25 °C, to assess the effect of nasal matrix on viral recovery. At the end of the time course, dry swabs were suspended in 0.4 mL of 1x nuclease free PBS. The samples from both wet and dry tubes were mixed by vortexing for 10 s, then spun down in a microcentrifuge. 2 μL of each sample was directly input to RT-qPCR for N gene (Fig. 3B and D) and GAPDH detection (Fig. 3C and E). The positive control was 3 μL of the pooled sample in 197 μL of 1x nuclease free PBS at time 0, and the negative control was unused Rhinostic and Puritan foam swabs in 200 μL of 1x nuclease free PBS at time 0.

2.10. Clinical patient validation

A total of 119 patient samples were collected with a Rhinostic within two days of a validated positive or negative result from a sample collected with a CLIA use approved Class I exempt nasal swab and tested in an approved RT-qPCR assay, such as Xpert® Xpress from Cepheid, by a clinical lab (Fig. 4, Table S2, supplemental data file). Samples were obtained from volunteers as approved by the Harvard Medical School Institutional Review Board (IRB 20-0581). Informed written consents were obtained by all volunteers. Nine individuals were also asked to self-swab with the US Cotton swab, and five individuals swabbed with a FLOQ swab side-by-side with the Rhinostic for a direct comparison of clinical performance. Rhinostic swabs were transported attached to 1.0 mL Matrix storage tubes. US Cotton swabs were collected in BD Vacu-container tubes, and FLOQ swabs were collected in their respective collection tubes due to their larger size. Rhinostic swabs were suspended in 200 μL of 1x nuclease free PBS whereas US Cotton and FLOQ swabs were suspended in 400 μL of 1x nuclease free PBS due to the absorptive property of the swab. All swabs were agitated and 2 μL of unpurified sample was used as input to the RT-qPCR for N gene and GAPDH detection. Swabs were kept dry at room temperature for 1–4 days before analysis. Each sample was run in two or three technical replicates and samples were called positive for SARS-CoV-2 if at least two replicates had specific signal. Technical replicates for paired samples presented in Fig. 4A and B were averaged before plotting; replicates with a Tm corresponding to an aberrant product was excluded. Samples with nonspecific products, based on Tm, in at least two technical replicates were declared as not determined (ND) $\geq$ 40 (Fig. 4B). Data on patient comfort and satisfaction with the Rhinostic was not collected as a part of this study.

3. Results

3.1. Swab design for automated accessioning and analysis

NP swabs are long, making it challenging to use these swabs with automation-compatible tubes. AN swabs in contrast do not need to be as long as NP swabs and can be designed with a shorter handle, opening up the possibility of making AN swabs that can be directly paired with automation-compatible tubes for an effective collection system. As part of the design, Rhinostic swabs have a cap that can be directly screwed onto a 96-well format automation-compatible tube, such as a 1.0 mL Matrix tube (Thermo Fisher Scientific) (Fig. 1A). The swabs were made by single shot injection molding with medical grade polypropylene (Fig. S1 and Methods). Injection molding of swabs allows for high volume production at low prices. While the swabs can fit onto many tubes, we believe the optimal design is in collection tubes pre-labeled (by the manufacturer) with a serialized Type 128 1D barcode plus human readable code on the side with a matching 2D data matrix barcode on the bottom (Fig. 1). This design allows for the collection tube and swab to be accessioned and used by the patient in an unobserved manner without having to pre-register each barcode manually, reducing costs and labor. In addition, the matching 2D barcode on the bottom allows a whole rack of tubes to be accessioned in seconds by a barcode reader.

3.2. Swab performance

We compared the Rhinostic to several other swabs on the market or under development at the time this study was conducted (Fig. 2A, Table 1). First, we tested for absorption of water. Water absorption is sometimes used as a proxy for the amount of material that a swab will

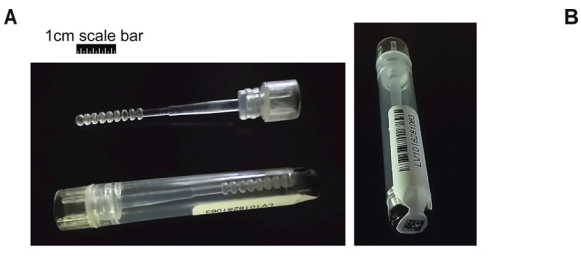

Fig. 1. 96-well format automation and accession compatible AN swab design. (A) Custom injection molded AN swab that can be produced at large scale and is compatible with 96-well format automation. A sample tube compatible with the Rhinostic swab is shown with barcodes on the side and bottom. The Rhinostic swab is 4.9 cm long with a collection head length of 1.6 cm. 1 cm scale bar shown for reference. (B) 96-well rack of swabs and tubes with 2D matrix codes printed on the bottom of the tubes, allows for rapid accessioning.

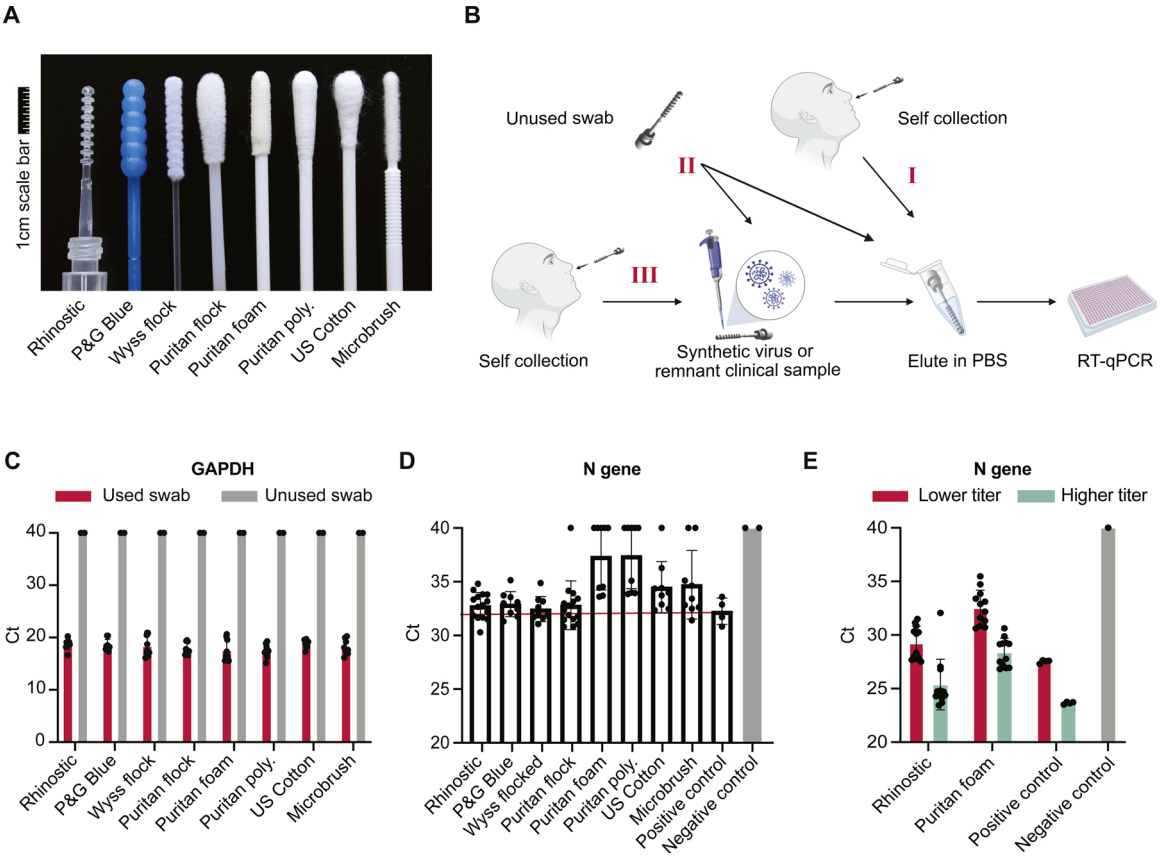

Fig. 2. Comparison of swab performance. (A) AN swabs tested in this study, from left to right: Rhinostic, Procter & Gamble (P&G) Blue, Wyss Institute flocked prototype, Puritan hydraflock, Puritan foam, Puritan polyester, US Cotton, and Microbrush®. 1 cm scale bar shown for reference. (B) Schematic of swab experiments performed in C-D. **Scheme I**; SARS-CoV-2 negative volunteer self-collected nasal matrix on a swab. **Scheme II**; unused swab, without nasal matrix, was either treated with packaged synthetic SARS-CoV-2 virus or left untreated (clean, unused swab). **Scheme III**; SARS-CoV-2 negative volunteer self-collected nasal matrix on a swab which was then treated with packaged synthetic SARS-CoV-2 or SARS-CoV-2 remnant clinical sample (Methods). All samples were eluted in PBS and used as direct input to RT-qPCR assays. Images were created with BioRender.com. (C) RT-qPCR quantitation of human GAPDH mRNA from used swabs containing nasal matrix (pink bars) or matched unused swabs (grey bars). (D) RT-qPCR quantitation of the SARS-CoV-2 N gene from packaged synthetic virus applied to clean, unused swabs. The grey bar is the negative control, PBS input into RT-qPCR. The pink line is a guideline for complete recovery based on the positive control. (E) RT-qPCR quantitation of SARS-CoV-2 N gene from swabs in the presence of nasal matrix spiked with a lower (~140 copies/μL, pink bars) or higher (~1600 copies/μL, green bars) titer remnant clinical sample. The grey bar is the negative control, PBS, and the positive controls are the lower or higher titer remnant clinical samples directly input to RT-qPCR. RT-qPCR data in C-E all show technical replicates of at least 3 biological experiments (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

collect (Harry et al., 2013; Zasada et al., 2020), although it does not necessarily correlate with effective collection of cells and viral particles. The Rhinostic, as well as the Procter and Gamble (P&G) Blue swab absorbed very little water compared to the majority of available swabs on the market or prototypes (Table 1). This lack of absorption is likely because polypropylene is more hydrophobic than collection materials such as cotton and spun polyester.

To test swab performance more directly, we measured the performance of 8 different AN swabs using several approaches (Fig. 2). We tested collection and recovery of 1) human mRNA in nasal matrix from swabs, 2) mRNA from viral particles added to swabs, and 3) mRNA from viral particles added to swabs coated in nasal matrix (Fig. 2B). Human mRNA was used as a process control to assess successful collection and recovery of cells from swabs. The process control also assesses the efficiency of the reverse transcription (RT) reaction as the primers span two exons to ensure the assay quantifies mRNA rather than DNA (Stevenson et al., 2008). A single volunteer swabbed with 8 different brands of AN swabs in triplicate (Fig. 2B, scheme I) and the eluent was used as direct

input to a RT-qPCR assay for GAPDH mRNA detection to quantify the amount of human mRNA recovered from the swab (Fig. 2C). All 8 swabs performed similarly in this assay and no GAPDH was detected on any of the unused swabs (Fig. 2C). For all evaluations of AN swabs in this work we performed direct RT-qPCR on the swab eluant without RNA purification (Qian et al., 2020; Smyrlaki et al., 2020).

Recovery of viral particles was first assessed on a contrived sample by applying packaged synthetic AccuPlex SARS-CoV-2 viral particles to an unused swab for each of the 8 AN swabs tested (Fig. 2B, scheme II). The packaged synthetic virus was dried onto the swab and eluted into PBS by vortexing. In a similar experiment, we found that elution into PBS by gentle swirling of the swabs releases the virus at equivalent or superior levels to vortexing in the same amount of time (Fig. S2A and B). The level of viral particles released by each swab was quantified by RT-qPCR for the SARS-CoV-2 N gene (Fig. 2D). The Rhinostic performed as well as the other swabs tested, and released an equivalent number of viral particles to the positive control (Fig. 2D). The lower detection of viral RNA for other swabs such as the Puritan foam is likely due to the fact that these swabs absorb significant volumes of liquid (Table 1) making it hard to elute the contents off the swab efficiently, especially given that the maximal recovery of AccuPlex synthetic virus is 10 molecules per reaction. Going forward, due to swab availability, and its common use we used the Puritan foam swab for most comparisons to the Rhinostic.

To test recovery of SARS-CoV-2 RNA from contrived samples from a clinical source in the presence of nasal matrix, volunteers self-swabbed using either the Rhinostic or Puritan foam swab, then SARS-CoV-2 positive remnant clinical swab solution was applied to the used swabs (Fig. 2B, scheme III). After drying, the viral material was recovered by spinning the swabs in PBS. This experiment was performed with both a lower and a higher titer remnant clinical swab sample (Methods), and the presence of both SARS-CoV-2 N gene and GAPDH mRNA was detected by RT-qPCR using the swab eluent as direct input to RT-qPCR (Fig. 2E, Fig. S2C). Additionally, the equivalent performance of the Rhinostic to the positive control demonstrates the robustness of RT-qPCR to nasal matrix. The remnant clinical swab sample titers were determined using an N gene standard curve (Fig. S2D, Supplementary Methods). Rhinostic swabs were not statistically distinguishable from the positive control at either titer, but the Puritan foam swabs showed lower recovery ($P < 0.0001$ by an independent t-test). Replicate Ct values shows the high reproducibility of the RT-qPCR data (Fig. S2E and F).

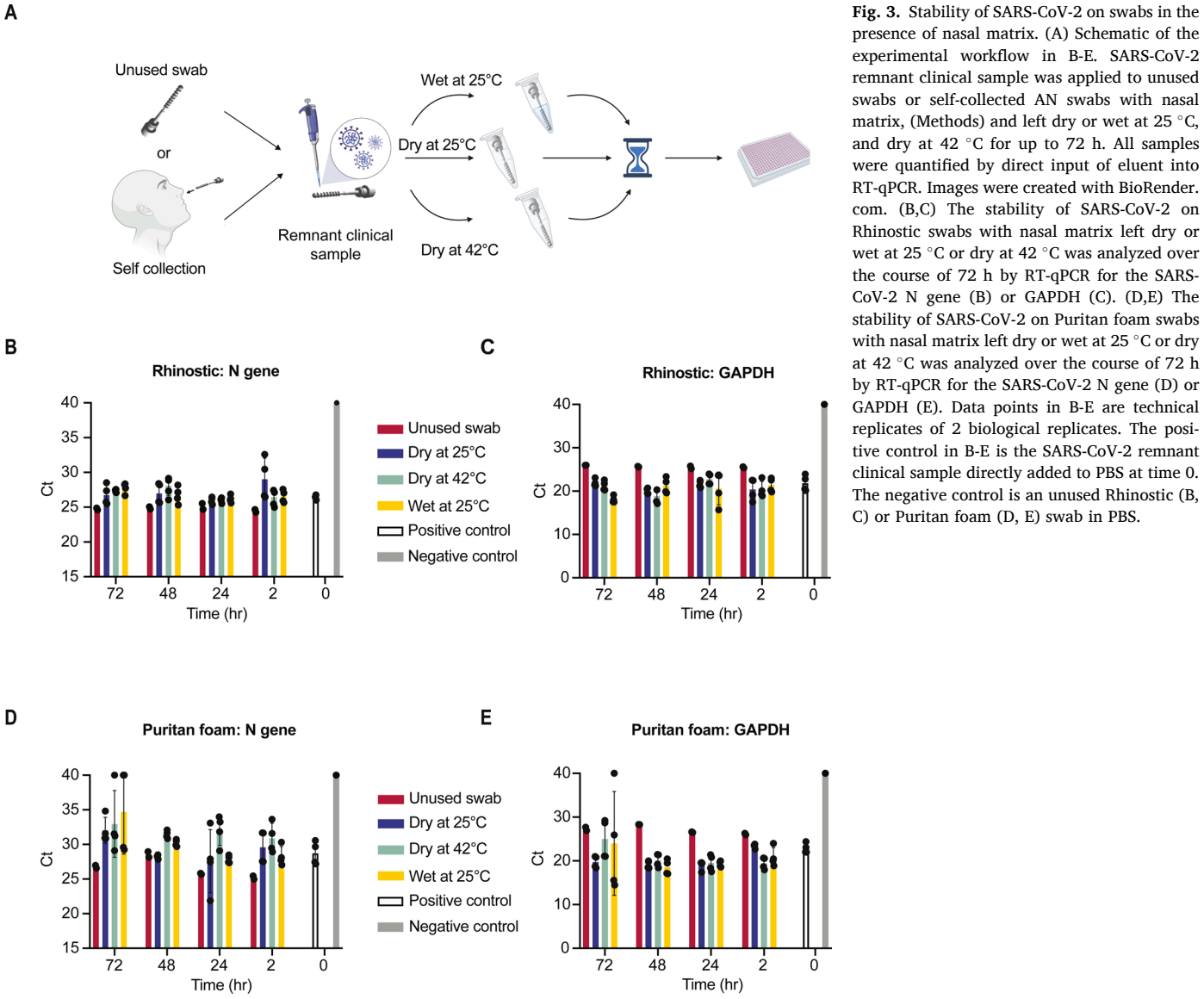

Fig. 3. Stability of SARS-CoV-2 on swabs in the presence of nasal matrix. (A) Schematic of the experimental workflow in B-E. SARS-CoV-2 remnant clinical sample was applied to unused swabs or self-collected AN swabs with nasal matrix, (Methods) and left dry or wet at 25 °C, and dry at 42 °C for up to 72 h. All samples were quantified by direct input of eluent into RT-qPCR. Images were created with BioRender.com. (B,C) The stability of SARS-CoV-2 on Rhinostic swabs with nasal matrix left dry or wet at 25 °C or dry at 42 °C was analyzed over the course of 72 h by RT-qPCR for the SARS-CoV-2 N gene (B) or GAPDH (C). (D,E) The stability of SARS-CoV-2 on Puritan foam swabs with nasal matrix left dry or wet at 25 °C or dry at 42 °C was analyzed over the course of 72 h by RT-qPCR for the SARS-CoV-2 N gene (D) or GAPDH (E). Data points in B-E are technical replicates of 2 biological replicates. The positive control in B-E is the SARS-CoV-2 remnant clinical sample directly added to PBS at time 0. The negative control is an unused Rhinostic (B, C) or Puritan foam (D, E) swab in PBS.

3.3. Virus stability on swabs

A key issue with swabs is the stability of viral particles on the swabs during transport from the collection site to the clinical lab. To test the stability of SARS-CoV-2 on swabs over time we added SARS-CoV-2 from remnant clinical swab samples to Rhinostic and Puritan foam swabs containing nasal matrix (Fig. 3A). The contrived samples were left wet or dry at 25 °C as well as dry at 42 °C, to simulate storage and transport conditions, for up to 72 h before elution into PBS. The presence of both SARS-CoV-2 N gene RNA and GAPDH mRNA was detected by using the swab eluent as direct input into RT-qPCR (Fig. 3 and S3). SARS-CoV-2 viral particles on the Rhinostic swabs were stable under all conditions tested both in the presence and absence of nasal matrix (Fig. 3B and S3A) whereas the Puritan foam swabs showed much greater variation in N gene detection when in the presence of nasal matrix, particularly when the sample was left out for 72 h (Fig. 3D and S3A). Overall, GAPDH detection was more consistent than N gene for both the Rhinostic and Puritan foam swabs (Fig. 3B–E, S3B-D) across all conditions in the time course. The variability in the N gene as well as GAPDH data collected from Puritan foam swabs during the time course is also observed when comparing the Ct's between two technical replicates in the RT-qPCR data (Fig. S3E and F).

3.4. Clinical validation

Within two days of a valid positive or negative SARS-CoV-2 test result from an independent CLIA lab 119 patient samples were collected with the Rhinostic. The SARS-CoV-2 status of all 119 clinical patient samples tested using the Rhinostic were 100 % concordant with results from an independent CLIA test (Fig. 4A). To quantitively compare the performance of the Rhinostic, a subset of those individuals self-swabbed using both a Rhinostic and a CLIA use approved swab (Fig. 4A and B). In the majority of samples there was both qualitative and quantitative agreement. Qualitatively, the Rhinostic was 86 % concordant with a CLIA use approved swab for N gene detection (Fig. 4B); all swabs were positive for the GAPDH process control (Fig. 4C). The 2 discordant measurements were from confirmed positive patients based on independent swabs assayed by a CLIA lab, thus these are false negatives by a CLIA use approved swabs, one with the US Cotton swab and one with a FLOQ swab (Fig. 4B). Quantitatively, for samples that had qualitative concordance for the N gene, the Ct values were within an average of 3 Cts for all measurements. While the CLIA use approved swabs work well for the standard RT-qPCR SARS-CoV-2 detection from purified RNA, the Rhinostic had better performance in the direct RT-qPCR assay used in this study.

4. Discussion

Our improved AN swab is comfortable to use, allows patients to perform self-collection, and enables rapid accessioning and processing. The Rhinostic performs comparably to currently available swabs, releasing similar amounts of human and viral material into solution after use (Fig. 2). We found that Rhinostic and Puritan foam swabs detected similar levels of GAPDH mRNA (Fig. 3), while SARS-CoV-2 was detected more consistently from the Rhinostic swab with lower titer contrived samples (Fig. 2D and E) or after long periods of storage (Fig. 3). Clinical patient samples collected with the Rhinostic were 100 % concordant with results from a variety of CLIA use approved swabs assayed by RT-qPCR at an independent CLIA lab for 15 SARS-CoV-2 positive patient samples and 104 negative patient samples (Fig. 4A, Table S2). All RT-qPCR reactions performed in this study used direct input of swab eluant to the reaction mix without any RNA extraction and we were able to detect as low as 10 molecules per assay (Fig. 2D). It is important to note that results from swabs can be highly dependent not just on the swab but based on the sample collection procedure. The same swabbing technique was given as instruction for use for all swabs in this study.

SARS-CoV-2 viral particles on the Rhinostic swab proved to be very stable with no statistically significant loss of Ct under all the conditions tested (Fig. 3). One of the key design elements of the Rhinostic swab is the ability for a patient to self-collect their AN swab for sample processing. To best use this feature, it is preferred to use dry swabs in which the swab is put into the collection tube after self-collection in the absence of any buffer. This swab may then be mailed in or collected at a central location without the need for concern over sample leakage in transport. The stability of SARS-CoV-2 on the Rhinostic swab for up to 72 h before processing (Fig. 3B and C) demonstrates the feasibility of the dry swab method which is consistent with other studies (Moore et al., 2008; Srivatsan et al., 2020). An additional advantage of the new swab design is the ability to elute the sample in a low volume of liquid (200 µL), potentially increasing the sensitivity of the direct RT-qPCR method by 5–15 fold compared to standard methods. Most commercial swabs cannot be used with this low elution volume, due to the high volume of liquid absorbed by the swab (Table 1).

Due to the COVID-19 pandemic the need for fast, scalable testing methods has become an immediate and widespread need from traditional clinical laboratories to universities. We envision the Rhinostic swabs being used in the following workflow: the patient will scan the barcode on the side of the tube using a cellphone app, phone-accessed

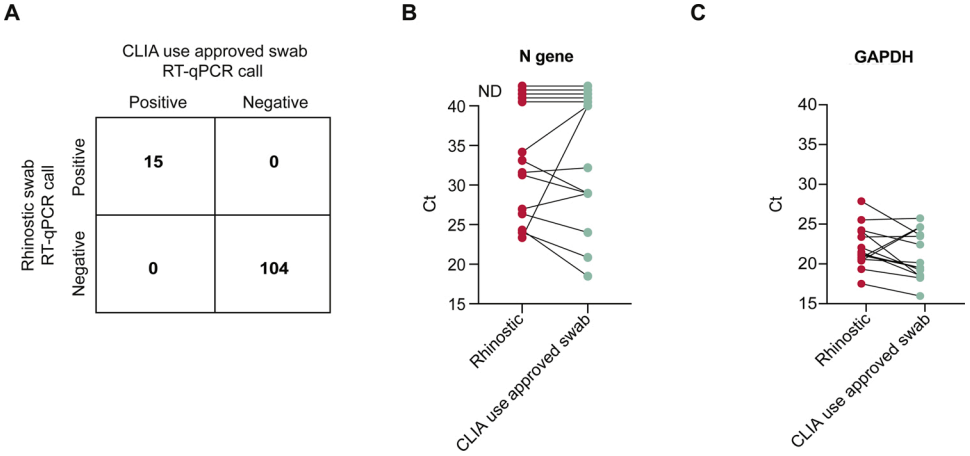

Fig. 4. Rhinostic concordance with control swabs. (A) Determination of SARS-CoV-2 status (positive or negative) based on a Rhinostic swab or a CLIA use approved swab. Rhinostic swabs were used by each patient for a total of 15 positive patient samples and 104 negative patient samples. The CLIA use approved swabs were performed at an independent CLIA lab for RT-qPCR determination; the Rhinostic swabs were self-collected by the patient and assayed by direct RT-qPCR in our lab. Swabs were not taken at the same time; instead Rhinostic swabs were taken within 2 days of CLIA SARS-CoV-2 status determination. (B,C) Fourteen paired clinical patient samples were collected with a Rhinostic swab and a CLIA use approved swab and tested for N gene and GAPDH by direct input of swab eluent into RT-qPCR. Presented are the average of two or three technical replicates for each paired sample tested for N gene (B) or GAPDH (C). Samples that were negative for N gene detection are identified as not determined (ND) in (B).

website, or scanner and an ID card at the collection site to link the patient and sample together. After swabbing with the Rhinostic swab, the patient would screw the swab into the barcoded tube. The sample would then be packaged for Category B compliant transport. In an unsupervised self-collection setting, the tube could be rescanned at the sample deposition site to help track sample custody. The tubes would be deposited in a lockbox at the site, which would periodically be sent to the testing center. All swabs would be stored and transported dry avoiding the risk of liquid leakage. In the testing facility, the samples would be received and loaded into 96-well racks by hand (Fig. 1B). Each rack of tubes would then be put onto a robot that scans the 2D matrix codes on the bottom of the tubes thereby linking the sample ID to each plate and plate location in seconds. After accessioning, the samples can pass to a de-capping robot which removes the caps and the samples can then be eluted, inactivated, and processed for viral quantitation.

5. Conclusions

Here we demonstrate that the Rhinostic, a newly designed injection molded polypropylene swab with a screw cap integrated into the swab handle, performs equivalently to several commonly used AN swabs on the market at capturing and releasing SARS-CoV-2 viral particles from AN swabs with 100 % concordance to results obtained using a CLIA use approved swab from a clinical lab. This AN swab design has the potential to expedite SARS-CoV-2 diagnostic testing through the use of automation while significantly reducing costs. We anticipate that these swabs will be generally useful for pathogen testing in large clinical laboratories.

CRediT authorship contribution statement

Richard Novak and Michael Springer conceived the study. **Mary E. Pettit** and **Sarah A. Boswell** performed most key experiments and data analysis (supervised by **Michael Springer**). Jason Qian assisted with several experiments. **Sarah A. Boswell**, **Mary E. Pettit**, and **Michael Springer** wrote the paper. All authors reviewed the manuscript.

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Ethical approval

This study was approved by the Institutional Review Board from Harvard Medical School.

Data statement

The authors declare that all data generated or analyzed during this study are included in this published article and its Supplementary Information files. A minimum of two biological and technical replicates were performed for each condition.

Declaration of Competing Interest

Michael Springer and Richard Novak are co-founders of a company, Rhinostics Inc., that is commercializing the Rhinostic swab. After submission of the paper, Rhinostics Inc. began conversations with P&G and acquired rights to the P&G blue swab. The remaining authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jviromet.2021.114153.

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