Open Development and Clinical Validation Of Multiple 3D-Printed Nasopharyngeal Collection Swabs: Rapid Resolution of a Critical COVID-19 Testing Bottleneck

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Abstract

The SARS-CoV-2 pandemic has caused a severe international shortage of the nasopharyngeal swabs that are required for collection of optimal specimens, creating a critical bottleneck blocking clinical laboratories’ ability to perform high-sensitivity virological testing for SARS-CoV-2. To address this crisis, we designed and executed an innovative, cooperative, rapid-response translational-research program that brought together healthcare workers, manufacturers, and scientists to emergently develop and clinically validate new swabs for immediate mass production by 3D printing. We performed a multi-step preclinical evaluation on 160 swab designs and 48 materials from 24 companies, laboratories, and individuals, and shared results and other feedback via a public data repository (http://github.com/rarnaout/CovidSwab). We validated four prototypes through an institutional review board (IRB)-approved clinical trial that involved 276 outpatient volunteers who presented to our hospital’s drive-through testing center with symptoms suspicious for COVID-19. Each participant was swabbed with a reference swab (the control) and a prototype, and SARS-CoV-2 reverse-transcriptase polymerase chain reaction (RT-PCR) results were compared. All prototypes displayed excellent concordance with the control ($\kappa=0.85-0.89$). Cycle-threshold (Ct) values were not significantly different between each prototype and the control, supporting the new swabs’ non-inferiority (Mann-Whitney U [MWU] $p>0.05$). Study staff preferred one of the prototypes over the others and the control swab overall. The total time elapsed between identification of the problem and validation of the first prototype was 22 days. Contact information for ordering can be found at http://printedswabs.org.

Our experience holds lessons for the rapid development, validation, and deployment of new technology for this pandemic and beyond.
Introduction

Since the emergence of the COVID-19 pandemic, more than 2.5 million cases have been diagnosed worldwide (1). These diagnoses were made using material collected from nasopharyngeal swabs, which provide the highest sensitivity for detecting SARS-CoV-2 infection during early infection using commercial RT-PCR-based assays (2). An NP swab is an FDA Class I exempt medical device roughly 15 cm in length and 2-3mm in diameter designed to collect secretions from the posterior nasopharynx (Figs. 1a, left and 1b, top). The head of the swab is generally coated with short synthetic filaments called flock or with spun fibers. The swab is inserted into the nasopharynx, rotated several times to collect material, and then placed into a vial containing a few milliliters of transport media. A breakpoint on the shaft enables detachment and release of the head into the vial, which is then sealed and sent for testing.

The rapid spread of SARS-CoV-2 has resulted in severe shortages of NP swabs, due to both manufacturing stoppages resulting in decreased supply and the spread of the pandemic resulting in unprecedented demand (3). To address the swab shortage, hospitals and other testing centers have repurposed other commercially available swabs (e.g. throat, urogenital) to collect nasal epithelial cells for testing (Fig. 1a, second from left and 1b, second from top).

However, such swabs are suboptimal for swabbing the nasopharynx due to differences in size and flexibility and the possibility that the materials of which they are made may inhibit PCR (4, 5). Material from other anatomical sites is being investigated for its ability to substitute for swabbing the nasopharynx, but reports are still early (6–8).

One solution to the swab crisis is to design and 3D-print swabs. Advantages of 3D printing include simplicity (avoiding the multistep process of applying flock), the widespread availability of 3D printing capacity, and the ability to iterate prototypes rapidly (9). To resolve the swab shortage crisis, we have been coordinating an open collaborative process that has brought together many medical centers, individuals, academic laboratories, and both new and well-established manufacturers (10). As part of this process, we have been testing and continuously providing feedback on prototype swabs in order to proceed rapidly but safely toward the development of swabs that can be used clinically, at volumes equal to the need. The openness of the process was a conscious decision supported by a substantial body of scientific literature, including the previous experience of the present authors, that demonstrates the advantages of openness over closed or hybrid approaches (11–13). At our institution, this process has led to an ongoing clinical trial of several prototype swabs, the first results of which we report here.
Materials and Methods

Process. Our goal was to quickly develop and clinically validate NP swabs that could be mass produced and made available for testing as soon as possible. We created a public repository using GitHub, a company that provides free hosting for collaborative projects on the web, most often used by programmers to share, comment on, and co-develop computer code (http://www.github.com/ramaout/Covidswab) (10). We provided a clear description of the problem and updated the repository with whatever we learned and encouraged others to do the same. By tapping our personal and professional networks, we nucleated an ad hoc network of manufacturers that included companies, academic groups, and individuals. This grew to include other medical centers interested in helping develop and test new swabs. These other groups were given the ability to add to the repository as desired; to date the most contributions have been our own.

We devised a three-phase process consisting of preclinical evaluation (Phase I), production considerations (Phase II), and field testing (Phase III). We described these processes on the repository for all to see. We took high-resolution photographs of all swab prototypes and stored Phase I results in a Microsoft Excel (Microsoft Corporation, Redmond, WA, USA) spreadsheet that remains publicly available in the repository. All contributors could see each others’ designs and our feedback and iterated accordingly.

We made our personal contact information freely available to facilitate communication and speed the delivery of prototypes. We involved representatives of our institution’s nursing, legal, intellectual property, leadership, purchasing, human resources, communications, and contracting teams and the institutional review board early and often in order to facilitate open development, reassign idled staff to our process, and minimize lead times during the rapidly changing situation.

Phase I: Preclinical evaluation. Design. An infectious disease physician, clinical pathologist (clinical microbiologist), and respiratory therapist tested each prototype swab for design and mechanical properties (Fig. 1c-d). These included size measurements of the head, neck, shaft, and breakpoint (requirement of ~15cm to reach the posterior nasopharynx; head diameter of 1-3.2mm to pass into the mid-inferior portion of the inferior turbinate and be able to maneuver appropriately without catching on anatomical variants such as septal spurs or a deviated nasal septum); surface properties such as smoothness (with roughness leading to an unpleasant feel and risk of bleeding); flexibility vs. brittleness of the head, neck, shaft, and breakpoint (to avoid...
fracture during use); durability (e.g. ability to tolerate 20 rough repeated insertions into a 4-mm-inner-diameter clear plastic tube curved back on itself with a curve radius of ~3 centimeters; ability to bend tip and neck 90 degrees without breaking; ability to restore to initial form following bend of 45 degrees; Fig. 1d); strength (resist breakage under rough but reasonable manipulation); and other factors as applicable (e.g. stickiness, smell) (Table 1).

Collection sufficiency. We assessed the ability to collect sufficient material for testing using Gram stain of a swab of the interior cheek smeared onto a standard microscopy slide as a surrogate for NP swabbing and comparison to Gram stain of a swab of the interior cheek using Copan Diagnostics, Inc. (Mantua, Italy) model 501CS01 as the control (“Specimen Collection Swab FLOQSwabs® 80 mm Breakpoint from Tip End Sterile;” Fig. 1c). Cheek swabbing was performed instead of NP swabbing as the least invasive and most readily available source of secretions, making it possible to test head designs even for prototypes that were deemed inappropriate as NP swabs. Slides were heat fixed and Gram stained according to the BD BBL gram stain test kit protocol (14). Slides were examined at 40x magnification for the presence of both epithelial cells and bacteria. Prototypes were passed if the amounts of bacteria and epithelial cells were qualitatively similar to the control (which contained multiple bacteria and epithelial cells per high-power field).

PCR compatibility. We tested PCR compatibility by placing the swab, head-downward after breaking off at breakpoint when present (as in a typical NP swab collection), in 3 mL of modified CDC VTM (Hank’s balanced salt solution containing: 2% heat inactivated FBS, 100μg/mL gentamicin, 0.5μg/mL fungizone, and 10mg/L Phenol red (15)) overnight to allow any PCR-inhibitory material to leach into the medium, spiking 1.5mL with 200 copies/mL of control SARS-CoV-2 amplicon target (representing 2 times the limit of detection on our system), vortexing, and testing using the Abbott RealTime SARS-CoV-2 Assay on an Abbott m2000 RealTime System platform (16), following the same protocol as for clinical testing (37 cycles, with Ct ≤31.50 being reported as positive). PCR-positive prototypes passed.

Phase II: Production considerations. We considered stability to autoclaving by repeating Phase I testing on post-autoclaved materials; manufacturers’ short-term strategies for individual packaging; and manufacturers’ stated ability to produce at least 10,000 swabs per day (at the time roughly a week’s worth of swabs for a mid-sized testing center) within a week’s notice. We considered differences in supply chain to minimize the risk of future crises.

Phase III: Field testing. Trial design and oversight. COVIDSwab is an adaptive trial for evaluating the performance of prototypes compared to the control (see above). Participants
Participants. Participants were individuals clinically suspected of COVID-19 who were brought to the drive-through/walk-up ("drive-through") COVID-19 testing site at BIDMC. Adults over 18 years of age were given a participant information sheet by study staff and asked whether they would agree to being swabbed with a prototype swab performed by a trained nurse or respiratory therapist in addition to the control swab required for testing. Individuals with known thrombocytopenia of <50,000 platelets/µl were excluded from the study to avoid risk of mild bleeding.

Trial procedures. Prototype swabs were individually packaged and autoclaved at BIDMC for sterilization according to manufacturer protocols. Swabbing was performed per standard protocol. Participants were first swabbed with the control swab, then the prototype. Choice of naris for each swab was left to study staff and the participant. Approximately half of all drive-through arrivals participated. Control and prototype swabs were placed in separate vials of VTM and transported to the BIDMC Clinical Microbiology Laboratories where each sample was tested on the Abbott m2000 SARS-CoV-2 RT-PCR platform as per standard clinical protocol.

Statistical analyses. RT-PCR results are reported categorically as either positive or negative. We tested categorical concordance using Cohen’s kappa(18). For each positive test, the Ct value (the RT-PCR cycle number at which the sample first turns positive) was obtained from the Clinical Microbiology Laboratories. Higher values reflect lower viral load in the sample.

We tested for systematic bias in Ct values by comparing values for controls vs. prototypes using the Mann-Whitney U test (MWU) (19). This tested the null hypothesis that values for controls and prototypes are drawn from the same underlying distribution; p>0.05 was interpreted as no bias. For discordant (positive control/negative prototype or vice versa) samples, the negative was assigned a Ct value of 37, the total number of cycles run. As a second test for bias, we
compared (again by MWU) the distribution of differences in Ct values between control and
prototype swabs to the distribution of differences between two control swabs taken within 24
hours (quality-control data independent of our study). This tested the null hypothesis that the
differences between control and prototype swabs and the differences between two control
swabs are drawn from the same underlying distribution; $p > 0.05$ was interpreted as no bias.

To quantify relative preferences among the prototypes, we performed round-robin A/B
testing(20, 21). (In A/B testing, two variants of a single variable are compared and a test subject
chooses their preference; in a round robin, a test subject has the opportunity sequentially to
consider every possible pair). Specifically, we gave each study staff member a printout of all six
possible pairs of swabs, in randomized order, and for each pair asked them to circle their
preference. We collated the results and assessed preferences.

**Results**

*Open process.* In the first days of the development effort GitHub repository (10) was established
to serve a public resource and knowledge base. We updated the repository continuously with
design information and test results. These updates included high-resolution images of
prototypes submitted to us for testing(10), a public database of results of our Phase I testing,
and periodic updates and guidance based on our experiences. Open communication facilitated
rapid design iteration by providing anyone interested with a way to quickly understand the
required specifications and to learn from each other’s experiences.

*Phase I testing.* To date we have evaluated 48 materials and 160 designs submitted to us for
testing by 4 individuals, 2 laboratories, and 18 companies, for a total of 24 manufacturers.
Prototypes from seven manufacturers have passed Phase I testing. Most failures were either for
inappropriate materials (too brittle, too stiff or not stiff enough; sticky; too rough), or for
inappropriate designs, including those with heads that were too sharp, too flimsy, or too
topologically bland (leading to relatively low surface area). Prototypes from 19 manufacturers
got through at least two iterations, with a maximum of 28 prototypes from one manufacturer
(Prototype 4 below; Fig. 1). The rate-limiting steps were receipt of new prototypes, with slow
mail delivery during the pandemic being a major contributor, and PCR-compatibility testing, as
testing patient samples took priority over testing prototypes. Communication with and
responsiveness by manufacturers were considered outstanding.
Phase II and III prototypes. Prototypes from four manufacturers have passed Phase II testing (two of the seven that passed Phase I are still being evaluated; one could not be manufactured at high volume), all of which have completed our Phase III clinical trial: these are prototypes from the 3D-printing manufacturers Resolution Medical (with technology from Carbon3D), EnvisionTec, Origin.io, and HP Inc. (Prototypes 1-4, respectively; Fig. 1a). Like control swabs, the prototypes were 15-16 cm in length with 1-3 cm length radially symmetric heads 2-3 mm in diameter, a thin neck 4-7 cm long and 1-2 mm in diameter, and a thicker shaft 2-4 mm in diameter, with a breakpoint most often 7-8 cm from the tip of the head. The materials for the four Phase III prototypes were Keysplint Soft Clear, E Guide Soft, methyl-acrylate photopolymer resin, and PA11 for Prototypes 1-4 respectively. Head design evolved over many iterations to increase surface area. Designs generally featured either a polygonal matrix connected to a central, tapered strut with multiple branch points or else some form of spiral (Fig. 1b). Manufacturers were able to balance sample collection (Fig. 1c), stiffness, and surface texture. Variations of a longitudinal central strut allowed for varying degrees of stability, flexibility, and impact cushioning (Fig. 1d).

Sample and data acquisition. We collected and tested control and prototype swab pairs from 276 participants. VTM was used in all cases. Approximately half of the patients tested at our drive-through testing center participated. Because testing runs were batched and the COVID-19 status of participants was not known prior to testing, the number of control-positives usually exceeded the minimum requirement of 10 (range, 10-19). Total time required for collecting all specimens for a given prototype was 2-3 days per prototype, and RT-PCR testing on test samples was run along with the clinical sample. Typically the test sample and clinical sample were run on the same Abbott m2000 machine as part of the same batch; occasionally a test sample ran on a different machine or subsequent batch, with temporary storage at 4°C. The frequency of control-positive tests was 18%, generally increasing by prototype as the pandemic worsened in and around Boston.

Comparison. All four prototypes exhibited a high degree of concordance with the control swab, with kappas of 0.88, 0.85, 0.89, and 0.88, respectively (Fig. 2a). For convenience we use the terminology of true positives, true negatives, false positives, and false negatives, with the control swab result considered the provisional gold standard. Prototypes exhibited 0-1 false positives and 1-2 false negatives. However, since control swabs are known to be an imperfect gold standard (<100% sensitivity) and because PCR positives are more likely to reflect true infection than error, false positives were interpreted as identifying missed infections; indeed,
false positives were referred to clinical care teams as clinically actionable, as per IRB protocol.

Of note, discordant cases were always associated with high Ct values, reflecting low viral load (Fig. 2b). For example, for Prototype 4, the control swab for one of the two false negatives had a Ct of 31.47, just shy of 31.50, the manufacturer’s reporting cutoff (corresponding approximately to a single virion per mL of VTM); in addition, testing of this false negative was delayed by 16 hours because of prioritizing patient samples, which can result in decreased signal.

To better assess possible performance differences between control and prototype swabs, we compared Ct values for control-prototype pairs for which at least one was positive (assigning the maximum-possible Ct to negatives; see Methods). Specifically, we asked whether the Ct values for the prototype swabs were systematically different from those of the control swabs. Systematically higher values for prototype swabs would suggest that they may underperform control swabs, notwithstanding the high kappa values. A p-value of >0.05 indicates no statistical difference. Although there were more data points below the 1:1 line than above it (Fig. 2b), statistical testing revealed no evidence for underperformance, with MWU p-values of 0.36, 0.26, 0.42, and 0.31 for Prototypes 1-4, respectively (Fig. 2b). This result supports the conclusion that the prototypes are non-inferior to the control.

As an additional assessment of non-inferiority, we compared the difference in Ct values observed between control and prototype swabs to the differences between replicates of control swabs. Independent of our clinical trial, there were 88 cases in which a patient, in the course of clinical care, was swabbed twice within 24 hours (mean±stdev, 15±7 hours), during the time period of our study. In 11 of these cases, at least one of the two swabs was positive for SARS-CoV-2. There were two disagreements between replicate swab tests, resulting in a kappa of 0.90, similar to what was observed in our study for each prototype (kappa=0.85-0.89). Also as in our study, the Ct values for the first swab and second swab were not significantly different (MWU p-value of 0.18). Finally, the differences between Ct values for the first and second control swabs were comparable to the differences between control and prototype swabs (MWU p-values of 0.31, 0.26, 0.47, and 0.44 for Prototypes 1-4; Fig. 2b).

Staff and participant preferences. A written staff survey (see Methods) showed a preference for Prototype 4, then Prototypes 2 and 3, then Prototype 1. There was a slight preference for the control swab over Prototype 4 (Fig. 3a). In narrative feedback, Prototype 4, which underwent the largest number of revisions through our process (28), was described as comparable to the control swab (Fig. 3b).
Availability. Swabs are available to order. Several million have been used across the United States as of this writing. Details can be found on the GitHub repository in the updates at https://github.com/rarnaout/Covidswab/tree/master/BIDMC.

Discussion

The COVID-19 pandemic has forced healthcare providers to seek alternative sources of critical materials affected by supply-chain disruptions and increases in demand. The situation has forced providers to innovate under extraordinary time-pressure. Over the course of our study we received numerous anecdotal reports of swab shortages at hospitals across the United States and in Europe, necessitating urgent stopgap solutions. Scientific literature on time-sensitive innovation suggests that open, collaborative, decentralized processes outperform closed or proprietary ones (11–13). Here we report the success of such a process, going from the identification of the swab crisis to multiple clinically validated prototypes capable of high-volume manufacture beginning at 22 days. Notably, none of the prototypes tested were flocked, yet their performance was statistically indistinguishable from the flocked control swab.

The urgency of the situation, the configuration of the manufacturing ecosystem, and human nature contributed to several observations and shortcomings worth mentioning. First, 3D printing has important advantages in a crisis, including the ability to iterate designs and output swabs rapidly. It remains to be seen how complementary manufacturing techniques, each with advantages and disadvantages relative to 3D printing, will contribute in a more mature market and less urgent setting. Second, in any cooperative process there is a temptation to "defect," i.e. taking without giving back. Individuals and manufacturers may well exploit open knowledge for competitive advantage (22). This is a known price of openness that can disincentivize cooperation, absent social or structural mechanisms to enforce norms; managing this temptation took considerable effort by all. Third, ideally the study size would have been larger, and there would have been a better null model than replicates separated by many hours, to which to compare our results. Possible sources of variance in our study include differences in secretions or viral burden between nares and the possibility that the first (control) swab left less material for the second (prototype) when the same naris was used for both swabs. Despite these potential issues, our statistical tests supported analytical non-inferiority for all four prototypes. And fourth, we note our “round-robin” A/B testing survey was useful in summarizing the direction of preferences, although a tally of the narrative comments added useful detail regarding the strengths of the various preferences. A possible explanation is that the control
swab was preferred in large part simply due to its being familiar, and preferred only narrowly (if often).

Like the control swab, the prototype swabs we tested can be improved upon, and manufacturers are currently doing so. The same is true for other prototypes we may test through our ongoing clinical trial. Especially in a crisis, perfect is the enemy of good enough. The pandemic continues to change quickly, and bottlenecks will likely continue to appear unpredictably. The constant requirement is the ability to respond in a timely fashion under this extraordinary pressure. We hope our experience, based on past scientific work on cooperation and innovation, will provide a useful case study for how to iterate and produce a clinically validated medical device under the pressure of an ongoing pandemic, work on which others will hopefully improve as we continue to fight COVID-19 together.

Availability

Contact information for ordering can be found at http://printedswabs.org, a website set up by a consortium of academic, medical and commercial enterprises to deliver clinically tested, FDA-registered, 3D-printed COVID-19 nasopharyngeal test swabs designed at scale. Please note this site may list manufacturers whose products were not validated in this study.

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**Figure Legends**

**Table 1:** Preclinical evaluation testing parameters and acceptance criteria. Swab properties tested during Phase I.

**Figure 1:** Control and prototype swabs. (a) From left to right: the control swab (“C;” Copan 501CS01), a repurposed urogenital cleaning swab approved for NP testing through our process (“R”), Prototype 1 (Resolution Medical), Prototype 2 (EnvisionTec), Prototype 3 (Origin.io), and Prototype 4 (HP, Inc.). (b) From top to bottom, close-ups of the heads of the swabs in (a). Scale bars, 1cm. (c) Examples of Gram stain of cheek swab using control (top) and prototype swabs. Scale bar, 10µm. (d) Examples of materials testing. Clockwise from top left: head flexibility and robustness to fracture, neck flexibility and robustness to fracture, robustness to repeat insertion into and removal from a tortuous canal (diameter 3cm), and breakpoint evaluation.

**Figure 2:** Categorical concordance vs. control swab. (a) 2x2 tables giving counts for each prototype vs. the control swab (first four panels) and for control vs. replicate control obtained within 24 hours on the same individual. Discordant results in gray; totals for each swab below and to the right of each box; total number of pairs in bold; $K$=Cohen’s kappa. (b) Scatterplot of Ct values for pairs of swabs for which at least one swab was SARS-CoV-2 positive.
discordant pairs, the negative swab was assigned a Ct value of 37 (the maximum number of cycles run).

**Figure 3: Subjective feedback.** (a) Round-robin A-B testing of net preferences among Prototypes 1-3 (large bold numbers) and the control (“C”). Each arrow points from the less preferred to the more preferred swab. Arrow weight indicates strength of relative preference. Preferences were unanimous except where noted with numbers separated by a slash: the first number denotes the number of responses for the direction indicated by the arrowhead, while the second number denotes the number of responses that had the opposite preference. The weight of the arrow is proportional to the difference (e.g. 7-3=a net preference of 4). Unless noted, each arrow denotes 12-15 separate responses. (b) Number of positive and negative comments received from study staff who administered the swabs, tabulated by category. In each plot, negative feedback is to the left of the zero, while positive feedback is to the right. Bars on both the positive and negative sides of zero reflect differing opinions among study staff. n, total number of comments received about each prototype from study staff.
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Table 1: Preclinical evaluation testing parameters and acceptance criteria

| Measurements       | Description/comments                                                                 | Acceptance criteria |
|-------------------|----------------------------------------------------------------------------------------|---------------------|
| Total length      | Length of NP swab from end to end                                                       | 15-16cm             |
| Head length       | Length of NP swab head used for collection of secretions and cellular material from posterior nasopharynx | 1.5-3.5cm          |
| Head diameter     | Diameter of NP head allowing for passage into posterior nasopharynx. Must be sufficiently small for passage beyond inferior turbinate without catching on abnormal anatomy such as septal spurs or a deviated nasal septum, but otherwise maximize surface area for specimen collection | 1-4mm               |
| Neck diameter     | A neck thinner than the head and shaft allows flexibility, easing manipulation of the swab in the posterior nasopharynx | 1-2mm               |
| Neck length       | Length of neck of NP swab following the head tip prior to the shaft                      | 3-3.5cm             |
| Breakpoint location | A breakpoint is a scoring or narrowing that allows user to break the head off into the viral transport tube. This must be sufficiently easy that breaking can occur without need of e.g. scissors or excessive infection risk, but not so easy as to risk breaking | 7-10cm              |
During insertion into patient, distance from head tip to breakpoint must be less than the length of the tube (Fig. 1d, bottom left).

| Surface properties | Requirements                                                                                     | Acceptable                                      |
|--------------------|--------------------------------------------------------------------------------------------------|-------------------------------------------------|
| Smoothness         | Swabs should be sufficiently smooth to touch and minimally abrasive for patient comfort and safety. In particular, the tip should not be sharp, so as to prevent puncture injuries and minimize epistaxis risk. | Sufficient smoothness                           |
| Adhesiveness or residue | Swabs should not feel sticky, tacky, or leave a residue behind with handling, as such residue could in principle have unwanted effects. | Not sticky                                      |
| Odor               | Swabs should not have an unusual chemical or metallic odor that could be an allergen or safety hazard to patients. | Must have a tolerable odor (e.g. no odor or very faint “plastic smell” acceptable; strong/acrid/glue smell unacceptable) |

| Mechanical properties | Requirements                                                                                     | Acceptable                                      |
|-----------------------|--------------------------------------------------------------------------------------------------|-------------------------------------------------|
| Head and neck flexibility | Must be flexible enough to be maneuvered into the posterior nasopharynx. Ability to bend head and, separately, neck at least 90 degrees without detachment. Ability of swab neck to restore to initial form following repetitive bending to 45° in both directions 45 times (Fig. 1d, top). | Ability to tolerate 20 rough repeated insertions into a 4 mm-inner diameter clear plastic tubing curved |
| Durability/Strength   | Must be durable enough to not break with reasonable manipulation. | Ability to tolerate 20 rough repeated insertions into a 4 mm-inner diameter clear plastic tubing curved |
with a radius of 3cm (Fig. 1d, lower left)

| Additional factors | Swabs must be able to collect sufficient material for detection of viral nucleic acid. Collection sufficiency was approximated by Gram stain procedure of interior cheek swab compared to standard Copan swab model 501CS01 as a control | At least 10 clusters of bacteria/cells at 40x magnification (Fig. 1c) |
|--------------------|---------------------------------------------------------------------------------|-------------------------------------------------------------------|
| Collection sufficiency | Swabs must not inhibit PCR reaction | Swab material was incubated in standard viral transport media overnight and spiked with 2x the limit of detection (200 copies/mL) SARS-CoV-2 amplicon target and tested using the Abbott RealTime SARS-CoV-2 Assay on the Abbott m2000 platform |
### a

|       | Prototype 1 | Prototype 2 | Prototype 3 |
|-------|-------------|-------------|-------------|
| Control | + | 9  1  10 | + | 10  2  12 | + | 17  2  19 |
|        | - | 1  63  64| - | 1  66  67| - | 1  57  58 |
|        |   | 10  64  74|   | 11  66  79|   | 18  59  77 |

K = 0.88  
K = 0.85  
K = 0.89

|       | Prototype 4 | Replicate control |
|-------|-------------|------------------|
| Control | + | 10  2  12 | + | 10  1  11 |
|        | - | 0  34  34| - | 1  88  89 |
|        |   | 10  36  46|   | 11  89 100 |

K = 0.88  
K = 0.90

### b

![Graph showing Ct values for different prototypes and replicates](http://jcm.asm.org/)
Relative preferences

More negative
Insufficient sample
Too thick
Uncomfortable
Too rigid
Hard to advance

More positive
Plentiful sample
Strong material
Comfortable
Good flexibility
Easy to insert

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