

**Pandemic printing: Evaluation of a novel 3D printed swab for detection of SARS-CoV-2**

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**ABSTRACT**

*Objectives:* To design and evaluate 3D-printed nasal swabs for the diagnosis of SARS-CoV-2.

*Design:* An iterative design process incorporating regular feedback from clinical and engineering investigators was used to determine a final 3D-printed swab prototype. Laboratory evaluation comprised an *in vitro* study of contrived nasopharyngeal samples spiked with two different concentrations of gamma-irradiated SARS-CoV-2. Recovery of SARS-CoV-2 by RT-PCR from contrived samples using 3D-printed swabs in combination with three different transport media was compared to two swabs routinely used in Australia. Clinical evaluation comprised a prospective study comparing SARS-CoV-2 and human cellular material recovery between mid-nasal 3D-printed swabs and standard of care nasopharyngeal swabs (NPS).

*Setting:* Clinical evaluation took place at the Royal Melbourne Hospital (RMH), a large academic teaching hospital in Melbourne, Victoria, Australia, between the 1<sup>st</sup> and 18<sup>th</sup> of May, 2020.

*Participants:* Clinical evaluation involved participants from two groups: i) staff attending a COVID-19 screening clinic at RMH, and ii) inpatients with laboratory-confirmed COVID-19 at RMH.

*Intervention:* Each participant had a flocked NPS swab taken as the standard of care sample plus a mid-nasal 3D-printed swab taken from the other nostril.

*Results:* There was 100% categorical agreement of SARS-CoV-2 detection from contrived samples at each SARS-CoV-2 concentrations between the 3D-printed swabs in the three different transport media and the two standard of care swabs in the laboratory evaluation. Fifty staff and two inpatients with laboratory-confirmed COVID-19 at RMH were enrolled in the clinical evaluation. There was 100% categorical agreement of RNase P detection between the standard of care NPS and 3D-printed mid-nasal swab, and 100% categorical agreement of SARS-CoV-2 detection between the 3D-printed mid-nasal swab and NPS taken from inpatients with laboratory-confirmed SARS-CoV-2. Most participants preferred the 3D-printed mid-nasal swab (35/53, 67%) compared to the standard of care NPS.

*Conclusions:* We demonstrate the feasibility, acceptability and utility of 3D-printed swabs for the detection of SARS-CoV-2.

**BOX:**

**The known:**

- Rapid upscaling of laboratory testing for SARS-CoV-2 has led to an acute global shortage of nasal swabs.
- Innovative approaches to sustaining diagnostic testing capacity for COVID-19 are required.
- 3D-printed medical devices have been increasingly used over the past decade and may provide a scalable, on-shore manufacturing solution to this critical shortage.

**The new:**

- We describe the design and evaluation of 3D-printed swabs manufactured in Australia.
- 3D-printed swabs were non-inferior to two commercially available swabs when recovering SARS-CoV-2 *in vitro*.
- 3D-printed swabs were able to capture the same quantity of human cellular material in a clinical validation study.

**The implications:**

- Our work provides evidence supporting the use of 3D-printed swabs for the diagnosis of SARS-CoV-2.

## **INTRODUCTION**

The coronavirus disease (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a global public health emergency. Diagnostic testing plays a critical role in detecting COVID-19 cases and ultimately in reducing viral transmission (1, 2). However, the unprecedented upscaling of laboratory testing has placed extraordinary demands on health systems, with a global imbalance relating to supply and demand for laboratory consumables (3). To date, diagnostic testing for SARS-CoV-2 has relied mainly on reverse-transcriptase PCR (RT-PCR), with the conventional testing paradigm of sample collection, nucleic acid extraction and RT-PCR (4). Both the World Health Organisation (WHO) and the US Centers for Disease Control and Prevention (CDC) recommend a nasopharyngeal swab (NPS) as the preferred diagnostic sample, with mid-nasal (also called mid-turbinate) and oropharyngeal swabs considered suitable alternatives (5, 6). However, a major bottleneck in increasing testing capacity is a shortage of appropriate swabs for diagnostic sampling (7). Further, current global travel and transport restrictions that form part of responses to COVID-19 place geographically remote regions such as Australia at an additional disadvantage accessing internationally manufactured swabs.

One possible solution to the acute shortage of swabs is the use of 3D-printed swabs. 3D printing is the construction of physical objects from three-dimensional renderings with the use of a printer. 3D-printed medical devices have been increasingly used over the past decade, covering a range of applications such as custom implants and moulds for prosthetic devices (8). Here, we describe the design, and laboratory and clinical evaluation of a locally-manufactured 3D-printed swab. Our work highlights a possible solution to the extreme shortage of swabs, and demonstrates the benefit of rapid partnerships between industry, academia and clinicians in this current public health crisis.

## **METHODS**

### ***Swab design and manufacture***

An iterative design process was employed, incorporating regular feedback from clinical and engineering investigators. Based on initial specifications made publicly available by investigators in the United States (9), four initial prototype designs (Designs A-D; Figure 1) were prepared and 3D-printed by 3DMEDiTech (Port Melbourne, Australia), a specialist in the design and manufacture of 3D-printed medical devices conducted under ISO13485 operating procedures and utilising medical grade material supply chains. Printing was undertaken using SLS (Selective Laser Sintering) technology, which delivers feature resolution down to 80µm. PA2200 medical grade biocompatible Nylon 12 was used as a source material. Through a series of meetings between study investigators, further design iterations were made to: (i) optimize tip geometry for maximum cell collection; (ii) improve shaft geometry for flexibility, clinical safety (break-point position) and ease-of-use; and (iii) ensure overall design

parameters were compatible with patient comfort. The final design ('Design G') is shown in Figure 2 and a link to the STL file is freely available on request (<https://www.3dmeditech.com/contact-us>), along with the design specifications (Supplementary Appendix). Swabs were autoclaved and individually packaged in the Central Sterile Supply Department (CSSD) of the Royal Melbourne Hospital (RMH) before use.

### ***In vitro study***

To assess the ability of the 3DMEDiTech 3D-printed swab to detect SARS-CoV-2, an *in vitro* validation study was conducted. This study: (i) assessed the recovery of SARS-CoV-2 from different transport media using the 'Design G' 3D-printed swabs, and (ii) compared the ability of 3D-printed swabs to recover SARS-CoV-2 with two swabs currently used in Australia to collect specimens for the diagnosis of COVID-19. The swab / transport media combinations were: (1) flocked Copan Eswab with Liquid Amies medium (catalogue no 480CE); (2) flocked Kang Jian swabs with viral transport medium (VTM) (catalogue no KJ502-19); (3) 3DMEDiTech 'Design G' 3D printed swabs with VTM (University of Melbourne Media Preparation Unit, Melbourne, Australia; product no 2512) (4) 3DMEDiTech 'Design G' 3D-printed swab with Liquid Amies medium (University of Melbourne Media Preparation Unit, Melbourne, Australia; product no 2162), and (5) 3DMEDiTech 'Design G' 3D-printed swab with normal saline. Virus stock approximating  $10^7$  copies/ml of SARS-CoV-2 strain VIC001 (10), was prepared in minimum essential medium (MEM) (Sigma, Manheim, German) containing 2% fetal bovine serum (Gibco, Auckland, New Zealand), and gamma-irradiated to allow subsequent handling in PC2 conditions.

A 'mock' sample matrix from nasopharyngeal swabs was constructed, consisting of 20 mL of pooled nasopharyngeal swab samples collected in Liquid Amies from patients who tested negative for SARS CoV-2 by E gene RT-PCR described below. This pooled sample was divided into two 10 mL aliquots, and each aliquot spiked with gamma-irradiated SARS-CoV-2 to achieve two different viral concentrations (16 plaque forming unit (PFU) equivalence /mL and 160 PFU equivalence /mL SARS-CoV-2). Duplicates of each swab/medium combination were 'swizzled' in an individual 500uL aliquot of mock spiked sample at each concentration for five seconds, then immediately placed in 2mL of accompanying medium. Samples underwent SARS-CoV-2 RT-PCR testing at time zero, 24 hours, and 48 hours, and were stored at 4°C in the intervening period. RNA extraction was performed using the QIAamp 96 Virus QIAcube HT Kit (QIAGEN, Hilden, Germany). RT-PCR for the E gene was performed using previously published primers and probes (11).

### ***Clinical evaluation***

In order to compare the performance and tolerability of the 3D-printed swab with the swabs currently used as the standard of care in our institution (Copan Eswabs), we undertook a clinical evaluation study.

Participants came from two groups: (i) staff attending a COVID-19 screening clinic at RMH between 1<sup>st</sup> and 5<sup>th</sup> of May and (ii) inpatients with laboratory-confirmed COVID-19 at RMH between 1<sup>st</sup> and 18<sup>th</sup> May 2020. Participants were provided with a study information sheet and provided verbal consent to investigators. A flocked nasopharyngeal swab was taken with the Copan Eswab as the standard of care, followed by a mid-nasal swab in the other nostril, using the 3D-printed swab. Both swabs were placed into individual tubes of 1 mL Liquid Amies transport media. The order in which swabs were collected was randomized 1:1. Participants were asked to complete a brief survey on the levels of discomfort with each swab.

Nucleic acid extraction and SARS-CoV-2 RT-PCR was performed as described above for the *in vitro* validation. In addition, SARS-CoV-2 RT-PCR was performed using the Xpert Xpress SARS-CoV-2 assay (Cepheid, Sunnyvale, USA) for inpatients with laboratory-confirmed COVID-19, according to manufacturer's instructions (12). As a surrogate marker for the amount of cellular material derived from each swab, a semi-quantitative real-time RT-PCR for a human housekeeping gene (RNase P) was used, based on previously published primers and probes (13).

### ***Statistical analysis***

Differences in cycle threshold (Ct) value (lower Ct values reflect higher target concentrations) were compared between control and 3D-printed swabs using the Wilcoxon matched-pairs rank test. All statistical analysis was performed in R (version 3.5.1) and plots were made using GraphPad Prism (version 8.4.2).

### ***Ethics***

Ethical approval for this project was obtained from the Melbourne Health Research Ethics Committee (RMH QA2020059).

## **RESULTS**

### ***In vitro comparison of swabs***

There was 100% categorical agreement of SARS-CoV-2 detection from each concentration of mock sample between the Copan Eswab, Kang Jian swabs and 3DMEDiTech 'Design G' 3D-printed swabs in the three different transport media (Liquid Amies, VTM and normal saline) (Supplementary Table 1). In addition, SARS-CoV-2 was detected at all timepoints, across all swab / media combinations (Supplementary Table 1). There was no difference in the mean Ct value for detection of the E gene between the three swab types at both concentrations, 16 PFU equivalence/mL and 160 PFU equivalence/mL, across the three different time points (Figures 2A and 2B). In addition, there was no difference in Ct values for detection of the E gene for the 3D-printed swab from the three different transport media (Figures 2C and 2D).



***Clinical evaluation and acceptability***

Fifty staff attending a COVID-19 screening clinic at RMH between 1<sup>st</sup> and 5<sup>th</sup> May, 2020 were enrolled in the study. Each participant had a Copan Eswab and 3D-printed swab collected in 1ml Liquid Amies media. No staff members had SARS-CoV-2 detected by RT-PCR on the E gene assay. There was 100% categorical agreement of RNase P detection between the Copan Eswab and 3D-printed swab, with no significant difference in Ct value detected between the two swabs (Supplementary Figure 1).

Two patients with laboratory-confirmed COVID-19 who were admitted to RMH between 1<sup>st</sup> and 18<sup>th</sup> May were enrolled in the study. Three paired swabs were performed on these patients as described above. There was 100% categorical agreement of SARS-CoV-2 detection between the 3D-printed swab and Copan Eswab by RT-PCR on the E gene assay and the Xpert Xpress SARS-CoV-2 assay (Supplementary Table 2).

Study participants described the discomfort of the 3D-printed swab as a median of 5/10 [interquartile range 4-6], compared to 5/10 [IQR 3-6] for the Copan Eswab (Supplementary Table 2). When asked which swab was preferred, 35/53 (67%) participants preferred the 3D-printed swab, 10/53 (19%) preferred the Copan Eswab and 8/53 (13%) had no preference for either (Supplementary Table 3). Healthcare providers described the swabs as easy to use and either preferred them over the Copan Eswab or had no preference for either (Supplementary Table 3).

**DISCUSSION**

To address the critical shortage of laboratory consumables required for SARS-CoV-2 testing, innovative solutions are required to enable ongoing and sustainable diagnostic capacity. Here, we demonstrate the feasibility, acceptability and utility of 3D-printed swabs for the detection of SARS-CoV-2. We also demonstrate that 3D-printed swabs can be combined with various transport media (including widely available normal saline) with no significant differences in SARS-CoV-2 detection rate by RT-PCR.

Importantly, the widespread availability of 3D-printing capacity may enable many countries to ensure ‘sovereign supply chains’ of swabs, and the scalability of the technology means that, depending on local capacity, thousands of swabs can be produced per day. This may provide onshore manufacturing solutions to swab shortages in an unpredictable international market for both high- and low-income countries. Further, the Nylon 2 material used for swab production is amenable to autoclaving, ensuring a sterile swab supply. However, 3D-printed medical devices, including swabs, are subject to local regulatory guidelines and requirements. As such, appropriate approvals are required prior to

manufacturing or testing 3D-printed medical devices, and the results from this study relating to one specific device should not be assumed to be applicable to devices manufactured to different standards.

Our work builds on recent open work from the United States by Callahan *et al*, where four 3D-printed swab prototypes were evaluated, with no differences in detection of SARS-CoV-2 between ‘control’ swabs and 3D-printed swabs (9). Similar to our study, these authors employed an iterative design process, with close collaboration between academic, clinical, and industry partners. In the US study, healthcare providers and participants preferred the control swab compared to the 3D-printed swabs. This difference is likely to reflect different sampling strategies between the two studies; in our study, nasopharyngeal sampling was used as the ‘control’ method, whereas the 3D-printed swab was a mid-nasal swab. In Australia, both nasopharyngeal and mid-nasal swabs are regarded as acceptable diagnostic sites (14).

The urgent need for laboratory consumables for SARS-CoV-2 testing has necessarily catalysed the development of novel approaches to diagnostic testing. Further study is required to assess the compatibility of 3D-printed swabs with the wide range of available commercial and in-house SARS-CoV-2 RT-PCR platforms. Moreover, there may be additional opportunities for the application of 3D-printed swabs to the diagnosis of other common upper respiratory tract pathogens such as influenza, respiratory syncytial virus, and *Streptococcus pyogenes*.

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#### **FUNDING**

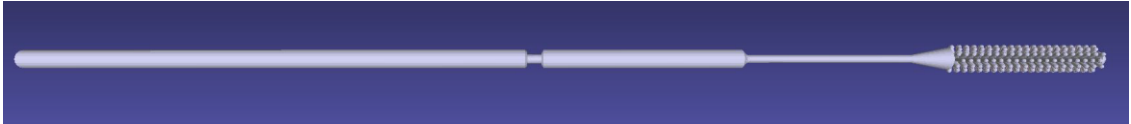
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**Figure 1.** ‘Design G’ 3D-printed swab. (A) Image of side profile of swab. (B) Flexibility of upper part of swab shaft. (C) Break point of swab. (D) Microscopic image of swab head.

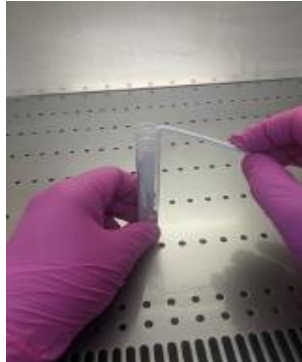
**A.**



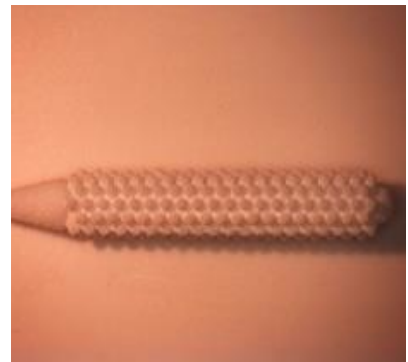
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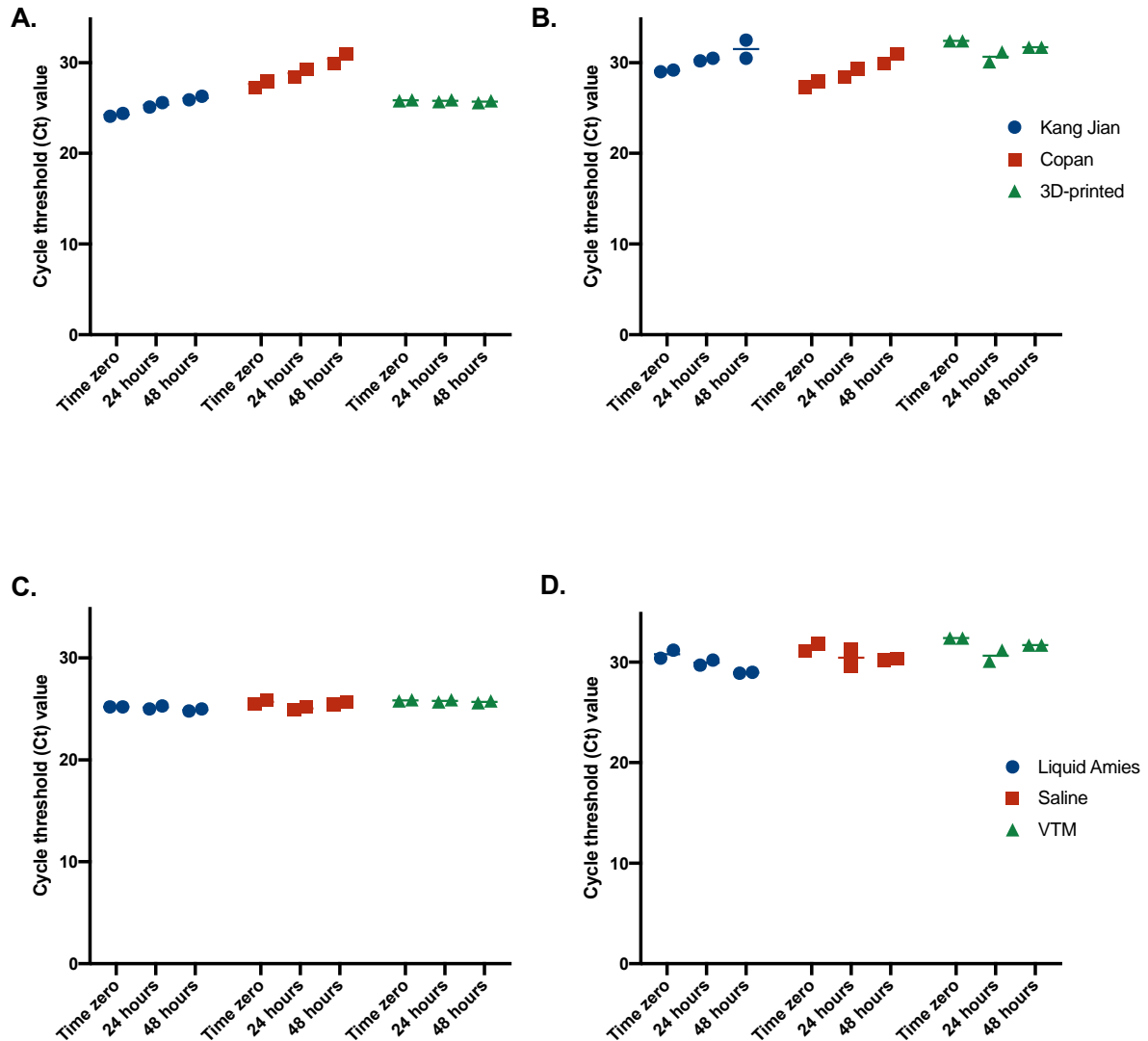
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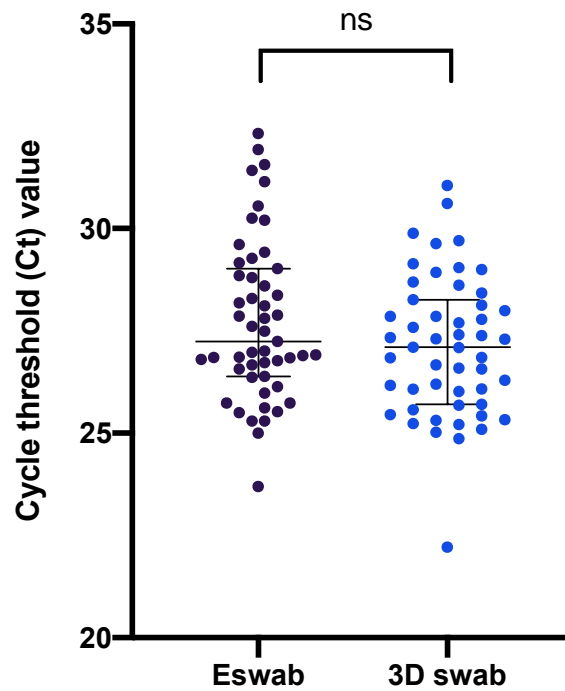
**D.**



**Figure 2.** Cycle threshold (Ct) values for detection of the SARS-CoV-2 E gene using: (A) Three swab types at a viral concentration 160 PFU equivalence/mL. (B) Three swab types at a viral concentration 16 PFU equivalence/mL. (C) Three transport media used with ‘Design G’ 3D-printed swab at a viral concentration 160 PFU equivalence/mL. (D) Three transport media used with ‘Design G’ 3D-printed swab at a viral concentration 16 PFU equivalence/mL. Lines represent the mean Ct value. Abbreviations: VTM, viral transport medium; PFU, plaque forming units.



**Supplementary Figure 1.** Cycle threshold (Ct) values for RNaseP detection when collected using a Copan Eswab or ‘Design G’ 3D-printed swab in fifty participants in a clinical evaluation study. The lines represent the median and interquartile range Ct values. Abbreviations: ns, not significant.



**Supplementary Table 1. In vitro validation study SARS-CoV-2 results for contrived nasopharyngeal samples using various swabs and transport media.**

Concentration SARS-CoV-2	Swab (N)	Media	Storage Temp (°C)	E gene Ct value		
				Day 0	Day 1	Day 2
16 PFU equivalence/ml	Kang Jian (2)	VTM	4°C	29.0	30.5	32.5
				29.2	30.2	30.5
16 PFU equivalence/ml	Copan Eswab (2)	Liquid	4°C	28.0	29.3	31.0
		Amies		27.3	28.4	29.9
16 PFU equivalence/ml	'Design G' 3D-printed swab (2)	Liquid	4°C	30.4	30.2	28.9
		Amies		31.2	29.7	29.0
16 PFU equivalence/ml	'Design G' 3D-printed swab (2)	VTM	4°C	32.4	31.2	31.7
				32.4	30.1	32.0
16 PFU equivalence/ml	'Design G' 3D-printed swab (2)	Normal saline	4°C	31.8	29.6	30.2
				31.1	31.3	30.4
160 PFU equivalence/ml	Kang Jian (2)	VTM	4°C	24.4	25.6	26.3
				24.1	25.1	25.9
160 PFU equivalence/ml	Copan Eswab (2)	Liquid	4°C	23.7	24.3	25.2
		Amies		23.8	24.3	25.5
160 PFU equivalence/ml	'Design G' 3D-printed swab (2)	Liquid	4°C	25.2	25.0	25.0
		Amies		25.2	25.3	24.8
160 PFU equivalence/ml	'Design G' 3D-printed swab (2)	VTM	4°C	25.8	25.7	25.6
				25.9	25.9	25.8
160 PFU equivalence/ml	'Design G' 3D-printed swab (2)	Normal saline	4°C	25.5	24.9	25.4
				25.9	25.2	25.7

°C, degrees Celsius; Ct, cycle threshold; E, envelope target; N, number of samples tested; PFU, plaque forming units; VTM, viral transport medium

**Supplementary Table 2. SARS-CoV-2 results for samples collected from patients with laboratory-confirmed COVID-19.**

Paired sample	Swab	RNase P assay Ct value	SARS-CoV-2 E gene assay Ct value	Xpert Xpress SARS-CoV-2 Ct value	
				E gene	N2 gene
Sample 1	3D-printed swab	26.8	33.1	30.8	34.5
	Copan Eswab	26.7	45.0	35.1	37.0
Sample 2	3D-printed swab	22.1	20.4	20.2	22.9
	Copan Eswab	22.7	19.1	18.6	21.2
Sample 3	3D-printed swab	24.3	32.0	31.0	34.2
	Copan Eswab	24.2	24.8	27.4	30.1

Ct, cycle threshold

**Supplementary Table 3. Participant and Healthcare Provider Questionnaire Responses**

Respondent	Preferred swab	Discomfort Copan Eswab NPS (median [IQR])	Discomfort 3D-printed swab MNS (median [IQR])
Participant (N = 53)	3D-printed swab 35/53 (67%) Copan Eswab 10/53 (19%) Either 8/53 (13%)	5/10 [3-6]	5/10 [4-6]
Healthcare provider (N = 4)	3D-printed swab 2/4 (50%) Copan Eswab 0/4 (0%) Either 2/4 (50%)	-	-

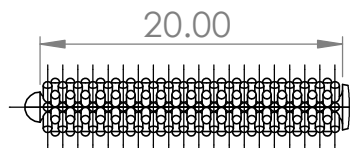
IQR, inter-quartile range; N, number of respondents; NPS, nasopharyngeal swab; MNS, mid-nasal swab



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F

F

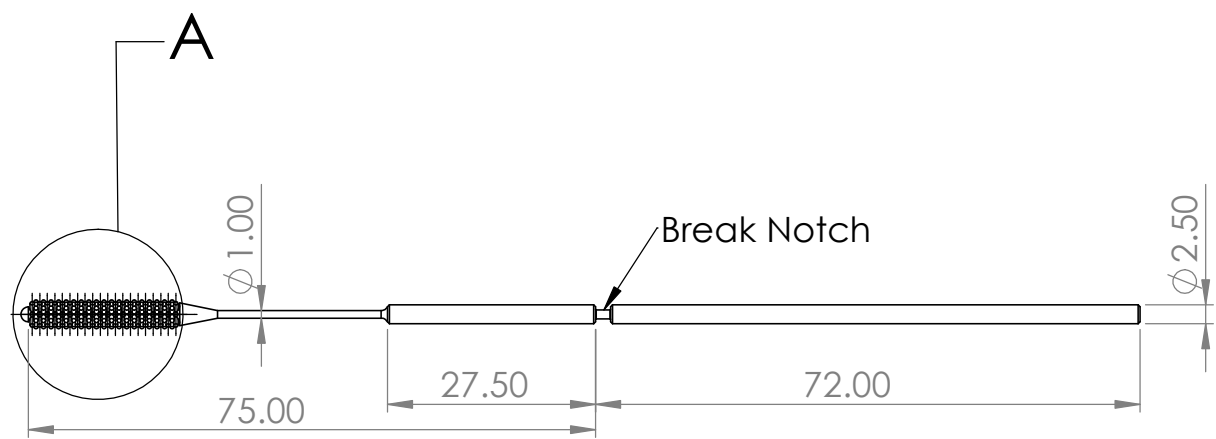


# DETAIL A

SCALE 2 : 1

E

E



D

D

C

C

B

B

UNLESS OTHERWISE SPECIFIED: DIMENSIONS ARE IN MILLIMETERS SURFACE FINISH: TOLERANCES: LINEAR: N/A ANGULAR:	FINISH:	DEBURR AND BREAK SHARP EDGES	DO NOT SCALE DRAWING	REVISION	G
	As Printed				

NAME	SIGNATURE	DATE	TITLE:
DRAWN S Logsdail		13/04/20	3DMEDItech Swab
CHK'D N/A			
APPV'D E Bert		16/04/20	
MFG N/A			
Q.A N/A			
MATERIAL: PA2200 Nylon 12			DWG NO. 1005-G
WEIGHT: N/A			A4
SCALE: 1:1			SHEET 1 OF 1

A

A

4 3 2 1