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3D Printed Nasopharyngeal Swabs with Wrapped Rayon Fibers Developed and validated by SCREEN (San Diego Covid19 Research Enterprise Network)

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2 *Works for me* dx.doi.org/10.17504/protocols.io.bemxjc7n

Coronavirus Method Development Community

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ABSTRACT

The global pandemic due to SARS-CoV2 virus, also known as COVID-19, has drastically increased the need for nasopharyngeal-swab-based testing resulting in shortages of commercially available nasopharyngeal (NP) swabs. One solution to overcome the national deficit of swabs is for medical device manufacturers and hospitals to generate NP swabs. Numerous entities are attempting to manufacture a direct from 3D printing NP swab but presented here is a validated two-part swab manufacturing protocol utilizing 3D printing and manual intervention (wrapping of nylon fibers). We recommend material extrusion or powder bed fusion technologies utilizing materials that can be sterilized using high level heat decontamination. Through the application of 3D printing and manual fabrication, we present an NP swab that can be created in a controlled environment. Coupled with CDC published viral transport media, the following swab can be used for COVID-19 testing or for testing for other respiratory viruses (eg, influenza, respiratory syncytial virus). The CDC viral transport medium has only four reagents which are readily available.

ATTACHMENTS

Protocol_for_3D_Printed_Nasopharyngeal_Swabs_with_Wrapped_Rayon_Fibers_NC.pdf

SCREEEN_NPSwab_Rev56 (1).stl

GUIDELINES

CDC guidelines suggest that cotton and wood not be included in NP swabs intended for COVID-19 testing.

THE ROLE OF THE PUBLIC

Other initiatives in the face of the global COVID-19 pandemic have brought the community of engineers together. The result of these initiatives has been to crowd source PPE and 3D printing intended for PPE development. The authors of this protocol strongly urge the public to not create swabs for their local hospital. Lack of process controls can drastically increase chancesespecially for false negative tests.COVID-19 is an RNA virus, and RNA readily degrades if not handled appropriately.The result of which could prolong communal exposure to the virus. Lack of controls could also directly impact the safety of a patient being swabbed, with a risk of brittle materials breaking 8-10cm inside a patient's nose with significant risk for injury and retained foreign body. We instead strongly urge members of the public who wish to help in swab development donatesupplies, money, or timeto hospitals and non-profits facilitating in on-site swab fabrication.

Validation of the effectiveness of printed nasal swabs

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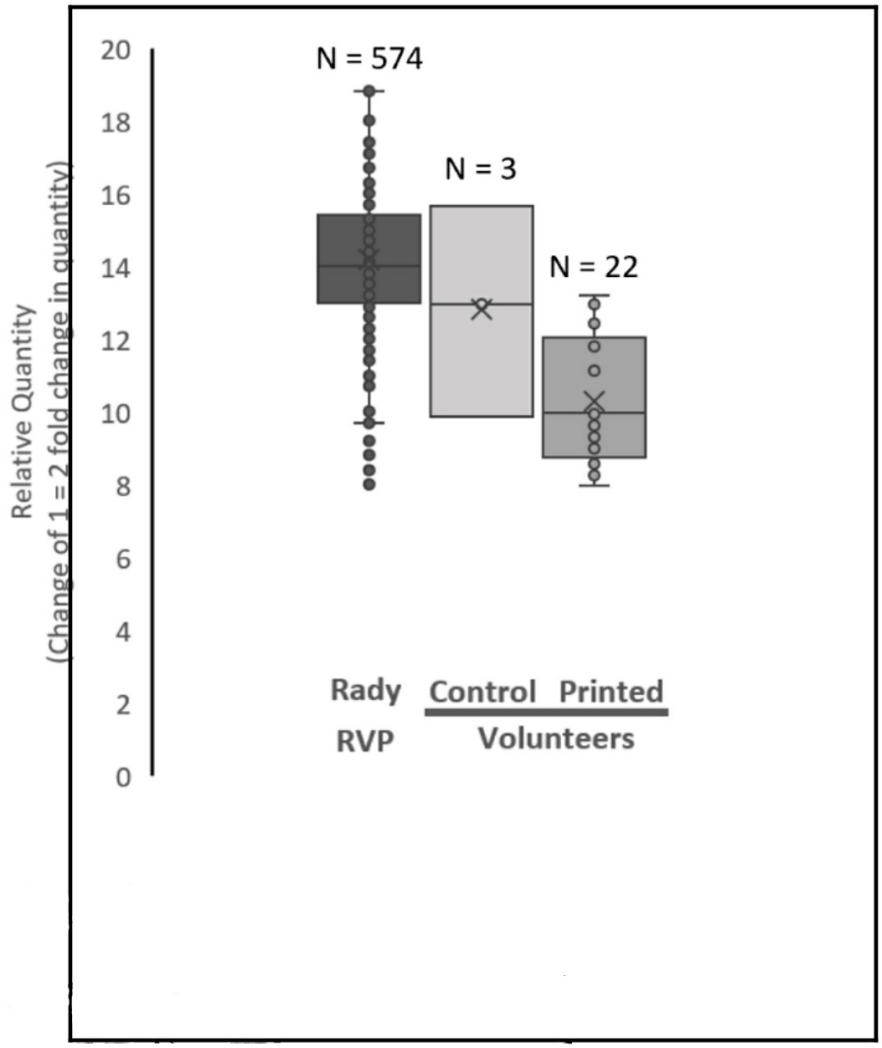

Figure 1: RNA levels of RNAseP transcript. Relative quantity = 40 – CT value from RT-PCR.

First, volunteers performed nasopharyngeal self-swabs with coaching. Swabs were placed in sterile filtered CDC formulated viral transport medium (VTM). Due to a shortage of commercial swabs, N = 3 for commercial swabs (Control, Fig 1), compared to N = 22 printed swabs. Due to the low availability of commercial swabs, a second comparison was made to clinically obtained samples (N = 574). These samples were from pediatric patients swabbed by trained respiratory therapists. All samples were subjected to RNA extraction and evaluated by reverse transcription followed by quantitative PCR for the relative quantity of Ribonuclease P (RNAseP) as a surrogate for nasal epithelium quantity. RNAseP primers (RPP30) are the CDC/WHO recommended primers (IDT Technologies, Cat#10006626) (Table 1) (https://www.fda.gov/media/136602/download).3D printed swabs had lower RNAseP ($P < 0.05$) as compared to self-swabs.

Secondly, 3D manufactured swabs with CDC formula VTM were utilized in a variety of clinical settings in parallel with commercially available swabs (Copan, USA) (Table2). These comparisons occurred in a variety of patients and settings. N = 25 were utilized at Rady Children's Hospital (San Diego). All patients were swabbed by respiratory therapists. N =22 were run on the GenMark (Carlsbad, CA) ePlex RP panel, which test for a variety of viruses including adenovirus, human metapneumovirus, respiratory syncytial virus (RSV), and several variants of influenza and parainfluenza. One child was positive for multiple viruses (adenovirus and rhinovirus/enterovirus) in both commercial and manufactured swabs. Three children were positive for RSV in both tests. One child each was positive for RSV A and Rhinovirus/enterovirus with the manufactured swab but not with the commercial swab (in one case the result came back "invalid" and in the second negative).

SARS-CoV2 is a new severe respiratory virus with a wide range of symptomatology. For pediatric patients, two COVID negative patients (SARS-CoV2) and one COVID positive patient were run on the DiaSorin Simplexa (Cypress, CA) with comparable results between commercial and 3D manufactured NP swab. Lastly, four adult outpatients who had previously tested positive for COVID-19 on a CAP/CLIA test 3-14 days prior performed self-swabs with commercial and 3D printed swabs. Of these, 50% were positive with

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commercial swab whereas 100% were positive with the 3D printed swab on a research test using the Fluxery One Hour COVID test (FDA approval pending).
We demonstrate here that these 3D printed swabs appear to have similar efficacy as commercial swabs for a variety of viral etiologies including the RNA virus SARS-CoV2. Of note there were no adverse events orNP swab related problems encountered during testing.

Table 1: RNAseP(RPP30) QPCR Primers

Primer/Probe	Sequence
RP-F RNAse P Forward Primer	5'-AGA TTT GGA CCT GCG AGC G-3'
RP-R RNAse P Reverse Primer	5'-GAG CGG CTG TCT CCA CAA GT-3'
RP-P RNAse P Probe	5'-FAM – TTC TGA CCT GAA GGC TCT GCG CG – BHQ-1-3'

Table 2: Comparison of 3D manufactured & commercial NP swabs

	Commercial	3D Printed
Pediatric		
RVP		
Negative	16/16 (100%)	16/16 (100%)
Positive (single)	3/5 (60%)	5/5 (100%)
Positive (multiple)	1/1 (100%)	1/1 (100%)
COVID-	2/2 (100%)	2/2 (100%)
COVID+	1/1 (100%)	1/1 (100%)
Adult		
COVID+	2/4 (50%)	4/4 (100%)

MATERIALS

NAME	CATALOG #	VENDOR
Rayon Fibers		
Powder bed fusion 3D Printing or Nylon-based filament		

MATERIALS TEXT

MATERIALS:
CDC guidelines suggest that cotton and wood not be included in NP swabs intended for COVID-19 testing.

1. 3D Print Nylon-based filament for material extrusion (commonly known as FFF or FDM) or powder bed fusion 3D Printing
   Approximately 408 mm^3 per swab – not accounting for any purge
2. Rayon fibers
   Less than 0.1g used per swab
   Ensure no cotton is present in the rayon media

SAFETY WARNINGS

For hazard information and safety warnings, please refer to the SDS (Safety Data Sheets).

BEFORE STARTING

Ensure that gloves are worn during the assembly of 3D printed Nasopharyngeal Swabs should be performed and that the environment is clean of exposed wood and cotton fibers.

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3D Printing

1 Print the SCREEN design in a nylon-based media and ensure additional adhesives or binding agents are not used unless additional validation is performed.

Swab Procedure

2 While wearing gloves, open the supplied 3D print file.

SCREEEN_NPSwab_Rev56 (1).stl

Note the following:

- The file was designed to not need support structures in the case of material extrusion
- Specific printers may still benefit from added support structures
- Adhesives should not be used on the build bed unless further validations with the adhesive have taken place

3 Send the job to print.

4 After printing, inspect print for deviations in the print process such as delamination and burrs.

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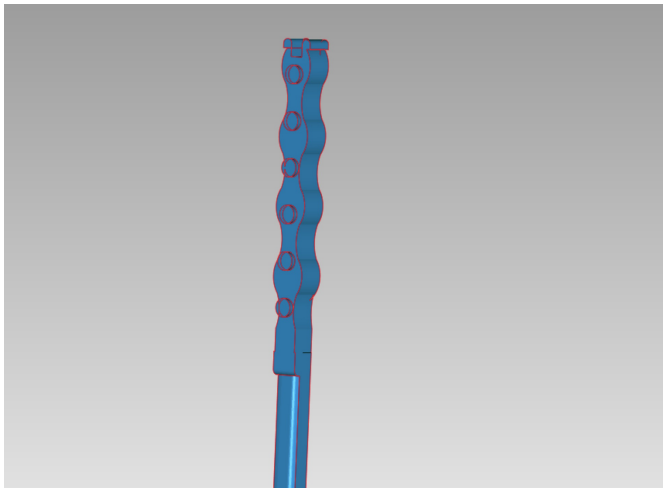
5 Take a quarter-sized amount of rayon fibers.

6 Tightly wrap the fibers onto the tip of the swab.

- The tip has clear ridges that separate it from the shaft of the swab



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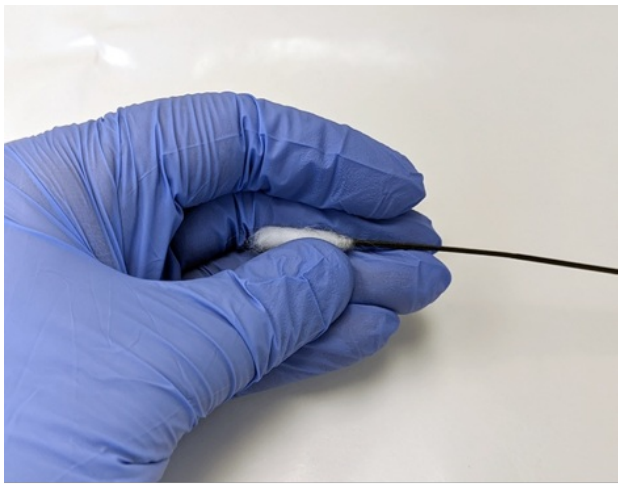
6.1 Extra care should be given to ensure the end of the tip is covered.

- Using a non-white print media can help to see any exposed regions.

6.2 The swab tip should be 3-4mm in diameter following the tight wrapping procedure.

- Excess rayon should be removed or additional rayon should be added to achieve this diameter.



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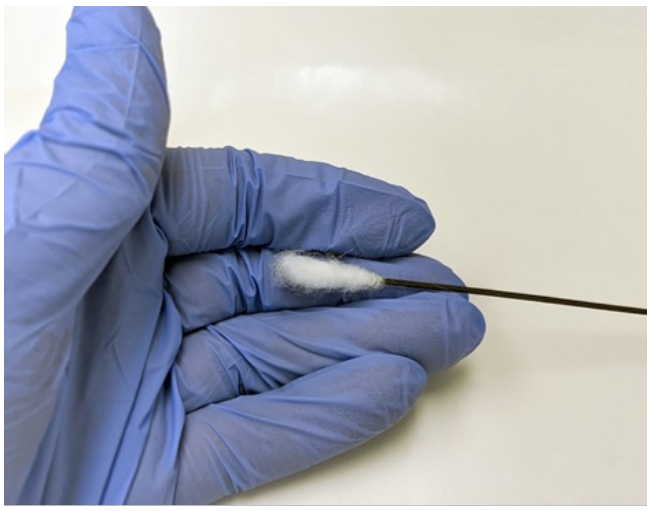
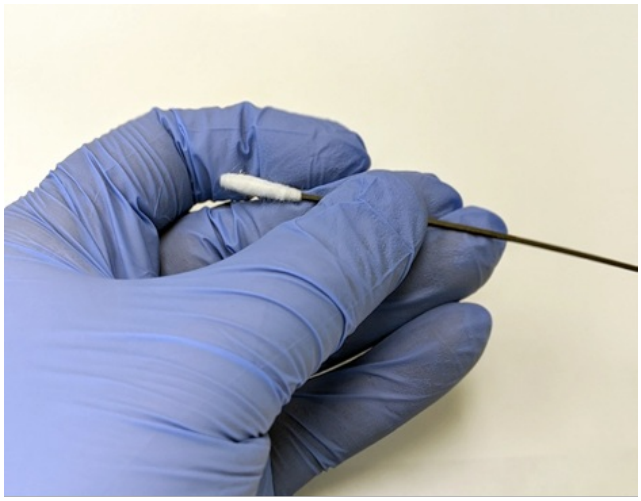
7 Perform non-radial motions to help the fibers intertwine and reduce the chances of the fibers becoming unwound.



8 Pull on the collective rayon fibers to ensure that the fibers do not become dislodged from the swab.

- If fibers do become dislodged, ensure wrapping is tight enough



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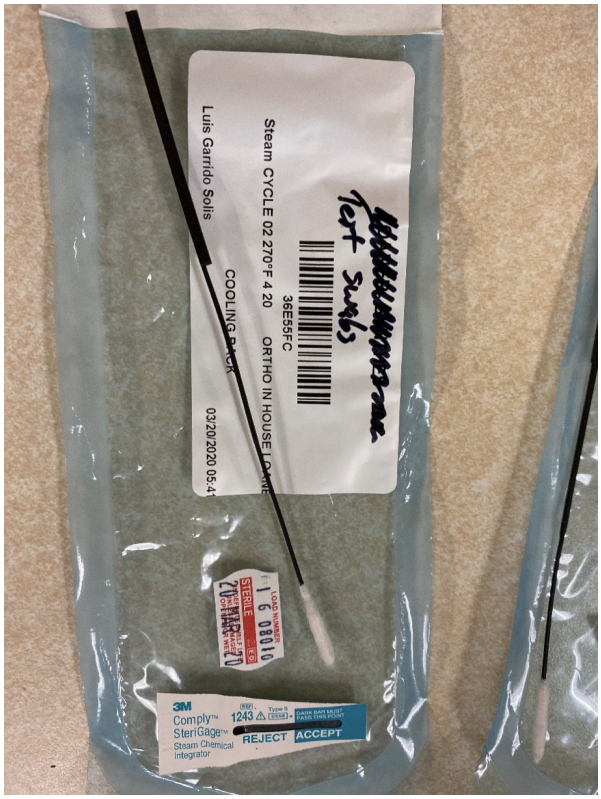
9 Sterilize the swabs utilizing hospital grade high level decontamination at **270 °F** for **00:04:00** in individual packages, preferably with a temperature indicator to indicate appropriate temperature has been attained.

- Prototypes were autoclaved at **121 °C** for **00:20:00** without complications.



10

Validation of the effectiveness of printed nasal swabs can be found in the Guidelines Section.

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