A Comparison Efficacy Study of Commercial Nasopharyngeal Swabs versus a Novel 3D Printed Swab for the Detection of SARS-CoV-2

Short Title: Novel 3D Swab Study

Forest W. Arnold[1], DO, MSc; Gerald Grant[2], DMD, MS; Phillip F. Bressoud[3], MD; Stephen Furmanek[1], MS, MPH; Donghoon Chung[4,5], PhD; Nadine Sbaih[3], MD; Dipan Karmali[3], MD; Meredith Cahill[1], BA; George Pantalos[6], PhD

1. Division of Infectious Diseases, Department of Medicine, School of Medicine, University of Louisville, Louisville, KY
2. Department of Rehabilitative and Restorative Dentistry, University of Louisville School of Dentistry, Louisville, KY
3. Department of Medicine, School of Medicine, University of Louisville, Louisville, KY
4. Center for Predictive Medicine, University of Louisville
5. Department of Microbiology and Immunology, School of Medicine, University of Louisville, Louisville, KY
6. Department of Cardiovascular and Thoracic Surgery and Department of Bioengineering, University of Louisville

DRAFT

Corresponding Author:
Forest W. Arnold
Division of Infectious Diseases, UofL
501 E Broadway; Suite 140B
Louisville, KY 40202
f.arnold@louisville.edu

Key Words

Swab, printed 3D, SARS-CoV-2, coronavirus, diagnosis

Abstract

The large volume of diagnostic tests required by the response to the COVID-19 pandemic resulted in a shortage of commercial nasopharyngeal swabs. In an effort to alleviate the shortage, swabs created by 3D printing may be a solution. We designed and produced 3D printed swabs and sought to compare their ability to detect SARS-CoV-2 in patients admitted for COVID-19 or who were suspected of having COVID-19. A total of 31 patients were swabbed with a commercial and printed 3D swab. Results matched in 28 of 31 patients (90%). Two patients were discordant with a positive commercial swab and a negative 3D printed swab and another was discordant because the 3D printed swab was positive and the commercial swab was negative. The sensitivity was 89%, specificity was 94% and Cohen's kappa coefficient was 0.89. The 3D printed swabs performed acceptably compared to the commercial swab and may be considered for use in lieu of a commercial swab.

Introduction

During the coronavirus-19 (COVID-19) pandemic, materials were in short supply including personal protective equipment (PPE), ventilators, viral transport medium and nasopharyngeal swabs. The shortages were compounded by the increased demand in "hotspots" like New York and Louisiana. Creative solutions were approved for PPE and viral transport medium by the Centers for Disease Control and Prevention (CDC).[1] Emergency use authorizations were granted by the Food & Drug Administration for ventilators and the analysis of alternative body fluids for the diagnosis of COVID-19.[2] However, a nasopharyngeal specimen remains the standard diagnostic method for COVID-19, especially in asymptomatic patients.

Working to alleviate the need for an increased demand of nasopharyngeal swabs, and using 3D technology available to make models for dental implants out of resin, we designed and created a 3D printed swab resembling a commercial flocked nasopharyngeal swab. Certain physical and handling parameters were included in the design, but it was unknown if it would actually extract SARS-CoV-2 from a mucus membrane of someone's nasopharynx and elute the virus into the transport media so that it could be detected by reverse transcription-polymerase chain reaction (RT-PCR) analysis. If a certain swab is not specified by a manufacturer to be used with their kit, then a new swab should have in-house validation first.[3] The objective of this study was to compare the detectability of SARS-CoV-2 from the nasopharynx of a commercial swab to our novel 3D printed swab.

Methods

Setting and Population

This was a cohort prospective study of non-consecutive patients and healthcare workers with a recent diagnosis of, or suspicion for, COVID-19. (IRB # 20.0334) It was performed at two acute care hospitals of the University of Louisville Health and required Institutional Review Board approved informed consent. There was a recently implemented policy at the time of the study that was relevant; all patients admitted to the hospital were screened upon admission. Recently identified COVID-19 positive populations, or those who tested negative, but who had signs or symptoms consistent with COVID-19 were candidates for enrollment.

Sample collection

Swabs were inserted into the nasopharynx per standard methods (insertion for 10 seconds with some rotation) and then inserted into a viral transport media tube with a sealed cap for transport to the laboratory on campus. The laboratory was CLIA certified under an emergency use authorization by the FDA and had been used to perform approximately 4,500 samples prior to initiating the present study. Internal validation of the laboratory had been performed with the established CLIA certified University of Louisville Infectious Diseases Research Laboratory. A goal of testing 30 COVID-19 positive people was established per standard recommendations for validation with at least 90% agreement.[4]

Data Collection and Management

All data for this study were de-identified and kept in a Redcap® database. Demographic information was collected for each patient. The primary information collected was the result of each swab used in each subject. Whether the subject was an in-patient or an out-patient was recorded. If a subject had been previously positive, then the time from the previous test to the present test was also recorded.

3D Printed Swab Design and Production

Guidance for the swab design for 3D printing was provided by an on-line research collective that provided insight in to testing and evaluation of different swab designs and materials.[5] A design similar to a typical commercial swab was chosen. (**Figure 1**) Clinicians required that the material be flexible so it would not break off during use, that the collection end be comfortable to the patient and not cause any trauma to the tissues, and that there be a notch between 80-100 mm from the tip in order to break the swab off into the media tube. An open lattice design with a domed tip was selected. (**Figure 2**) Test swabs were produced from Envisiontec (Envisiontec, Inc., Dearborn, MI) E1 guide soft material, and 3d printed using direct light projection with a 3D printer (NewPro3D, North Vancouver, BC). After the printing phase, swabs were washed in 99% alcohol for 10 min, washed again in new 99% isopropyl alcohol, dried for 30 minutes at 38º Celsius, then cured with an UV curing unit at 100% for 10 minutes. All swabs were individually inspected and prepared for sterilization and were subjected to internal testing for collection ability, physical properties, and a UV-Vis spectroscopy was run to ensure that the swab material did not affect the media. The commercially

available FLOQSwab (COPAN Diagnostics, Brescia, Italy) was used as a reference swab for comparison to the 3D printed swabs.

Definitions

The CDC has defined that a positive result means that two viral RNA portions (N1 and N2) were identified from the sample.[6] They were determined with less than 38 cycles of the PCR equipment expressed as cycle threshold (Ct) values. If neither were identified, then the result was negative. If only one was identified, then the result was 'inconclusive' and the test was run again from the same sample. A certain human DNA fragment is also part of each test. If no human or viral fragments were identified, then the result was deemed 'invalid' and recollecting another specimen is advised. A recollection was not performed for the present study and so if a sample was invalid, the matched pair were excluded.

Statistics

Comparative proportional analyses were performed to assess sensitivity, specificity, positive predictive value, and negative predictive value. Because only two values may be compared with these tests – positive or negative – and because indeterminate values were not positive, patients with two indeterminate tests were considered negative. Additionally, Cohen's Kappa Statistic was used to measure agreement of diagnostic results between the 3D printed and commercial swab groups.[7] Ct values for N1 and N2 were plotted against each to produce paired plots.

Results

There were a total of 34 subjects enrolled into the study. Three subjects had an invalid swab, so the matched pairs were excluded leaving 31 patients. Demographic and clinical information is included in **Table 1**. All subjects had already been diagnosed with COVID-19 except one who had tested negative but who had bilateral infiltrates with a right pleural effusion and was ultimately diagnosed with metastatic cancer. The median time from the admission surveillance swab to enrollment for all subjects was 2 days.

Results matched in 28 of 31 patients (90%). Among the patients included, 16 matched as positive and 12 matched as negative. Two were discordant because the commercial swabs were positive (considered true positives) and the 3D swabs were negative (considered false negatives). Another one was discordant because the commercial swab was negative (considered true negative) and the 3D swab was positive (considered false positive). The sensitivity was 94%, specificity was 86%, positive predictive value was 89% and the negative predictive value was 90%. Cohen's kappa coefficient was 0.80.

For subjects whose swabs were both positive or who had one swab test positive, the Ct values were plotted against each other. (**Figure 3**) For indeterminate pairs with only one Ct value, the complement sample was attributed the least positive value of 39. Values closer to the identity line (45º one-to-one line) corresponds to more similarity between the swabs.

Discussion

The primary finding of this study was that the sensitivity and specificity were acceptable for the 3D swab with respect to a commercial swab. Swabbing patients and identifying the virus requires a series of steps performed in the present study including capturing the virus from someone's nasopharynx on to a swab, transferring it in media, and identifying it with PCR analysis. All results are important, positive or negative, thus it is important to be as accurate as possible. A false negative may result in a patient being taken out of isolation and exposing others, while a false positive may, and likely would, result in someone staying in quarantine or isolation inappropriately, and even going on to die alone.

When calculating sensitivity, an experiment (*e.g.*, 3D printed swab) is measured against a reference standard (*e.g.*, commercial swab). A commercial swab is certainly not 100% accurate as false negatives do occur. In the present study, one discordant sample was positive with the 3D printed swab, but negative with the commercial swab. This was labeled as a false positive 3D printed swab result because the commercial swab was the reference, but in actuality it was a false negative commercial swab result because it occurred in a patient who had tested positive prior to being enrolled. False negative tests during the COVID-19 pandemic were ignored in patients with consistent signs, symptoms and radiographical evidence of COVID-19. False negative results may

be related to swab technique, transport or laboratory error. The implication of the sensitivity and specificity generated with the present data are either to proceed with mass production to fill the void created by the COVID-19 pandemic or redesign a better 3D swab.

Nasopharyngeal swabs are considered a class I device, which is not regulated by the FDA, but using a swab that is not specified by a manufacturer to be used with their kit is not recommended if it has not been shown to be accurate. Our data were comparable to another comparison study between a commercial nasopharyngeal, a repurposed urogenital cleaning swab and four printed 3D prototypes with Cohen's kappa coefficients ranging from 0.85 to 0.90.[8] There are other prototypes of printed 3D swabs being tested. Manufacturing companies (Carbon, Redwood City, CA; Formlabs, Somerville, MA; Markforged, Watertown, MA;) currently manufacture and distribute 3D printed nasopharyngeal swabs for the purpose of detecting COVID-19, but efficiency data were not provided.

Limitations of the study include the commercial swab serving as a gold standard reference, which it is certainly not in actual practice. Potential reasons for discordant results may have been the variability of collection between samplings. Although swabbing once should not preclude virus from being collected by a subsequent swab, the depth that a patient allows a swab inserted does vary. Sometimes, swabs have blood on them and in differing amounts, which may interfere with PCR analysis. The

study was strengthened by consistency in the swab printing process, and consistency in swab inspection criteria after printing and before steam autoclave sterilization. It was also strengthened by matching swab technique, using similar viral transport media and running pairs together in the same batch for RT-PCR analysis.

Future studies may address testing a different swab design, a different body site (*e.g.*, nasal versus nasopharyngeal) or a different body fluid (*e.g.*, nasopharyngeal versus saliva). Advantages of each would be higher viral capture, more comfort and more convenience, respectively.

Conclusion

A 3D manufactured swab with locally designed specifications was found to have a sensitivity and specificity of 89% and 94%, respectively. The 3D swab tested may be considered appropriate for use independently now but future optimization studies would be beneficial.

Acknowledgements

The authors graciously appreciate counsel from Ramy Arnaout, Assistant Professor, Department of Pathology, Associate Director, Clinical Microbiology, Faculty, Division of Clinical Informatics, Department of Medicine, Beth Israel Deaconess Medical Center Faculty, Department of Systems Biology, Harvard Medical School; and assistance form Leah Oppy, MPH, Data Analyst for Department of Infection Prevention and Control, University of Louisville (UofL) Hospital; Rachel A. Sheppard, Clinical Regulatory Director, Clinical Trials Unit, UofL; Dr. Prajakta Kulkarni and Dr. Kristen Eguren, prosthodontic residents, UofL; and Justin Gillham, Sienna Shacklette and Clara Jones, bioengineering students, UofL.

References

1. Preparation of viral transport medium. Centers for Disease Control and Prevention Web site. https://www.cdc.gov/coronavirus/2019-ncov/downloads/Viral-Transport-Medium.pdf. Accessed April 19, 2020.
2. Emergency use authorizations. U.S. Food & Drug Web site https://www.fda.gov/medical-devices/emergency-situations-medical-devices/emergency-use-authorizations#covid19ivd. Updated April 17, 2020. Accessed April 18, 2020
3. Michael Miller J, Campbell S, Loeffelholz M. Changing Swabs: To Validate or Not To Validate? J Clin Micro 2013;51:3910. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3889799/
4. Clark RB, Lewinski MA, Loeffelholz MJ, Tibbetts RJ. Verification and validation of procedures in the clinical micromicrobiology laboratory cumitech 31B. In Press
5. Manufacturing NP swabs. GitHub Web site. https://github.com/rarnaout/Covidswab. Updated April 22, 2020. Accessed April 22, 2020.
6. CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel. Centers for Disease Control and Prevention. Web site https://www.fda.gov/media/134922/download. Accessed April 22, 2020. Updated March 30, 2020.
7. Kwiecien R, Kopp-Schneider A, Blettner M. Concordance Analysis. Dtsch Ärztebl Int 2011;108(30):515–21.

8. Callahan CJ, Lee R, Zulauf KE, Tamburello L, Smith KP, Previtera J, Cheng A, Green A, Azim AA, Yano A, Doraiswami N, Kirby JE, Arnaout R. Rapid open development and clinical validation of multiple 3d-printed np swabs for the COVID-19 pandemic. J Clin Micro 2020 *In Press*

Figure legends

Figure 1

The comparison of design of a commercially available, flocked, nasopharyngeal swab (above) to a 3D printed one (below).

Figure 2

The tip of a nasopharyngeal commercial swab (2a) and a 3D printed swab (2b).

Figure 3

A scatterplot of Ct values for N1 (3a) and N2 (3b) for all samples with at least one swab in a pair that was positive. For discordant pairs, the swab without a Ct value was attributed a value of 39 – the closest value to negative.

Table

Demographic and clinical information for patients diagnosed with or suspected for COVID-19 who were swabbed with a commercial nasopharyngeal swab and a 3D printed swab.

Variable	Subjects (No. = 31)
Age (median [IQR])	59 [43, 72]
Female (%)	17 (55)
History of Pulmonary Disease	
COPD (%)	5 (16)
Asthma (%)	5 (16)
Other (%)	4 (13)
Days between Symptoms Starting and Admission (median [IQR])	1 [1, 3]
Days between Admission and Study Swab (median [IQR])	2 [2, 4]
ICU Required (%)	17 (55)
Ventilator Required (%)	16 (52)
Absolute Lymphocyte Count (mean (SD))	1.23 (1.02)
Ferritin ng/mL (median [IQR])	17 [10, 24]
D-dimer µg/mL FEU (median [IQR])	14 [6, 22]

Figure 1

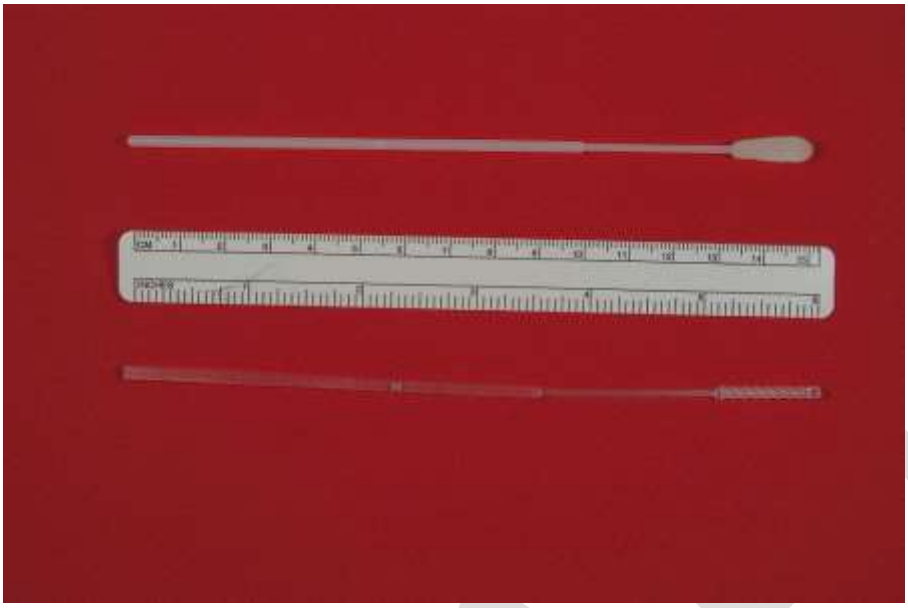


Figure 2

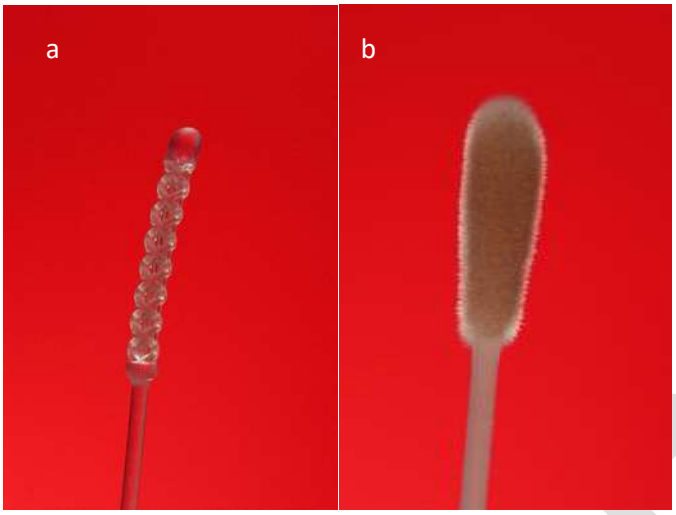
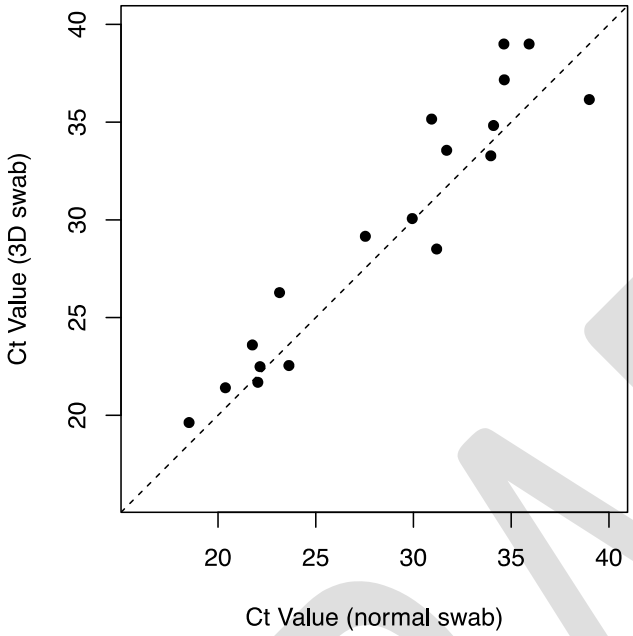
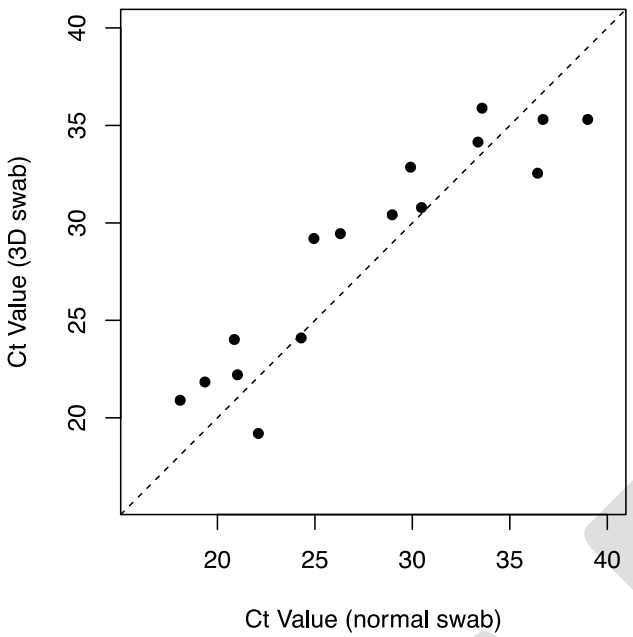


Figure 3

a N1



b N2


Ct Value (3D swab)
20
25
30
35
40
Ct Value (normal swab)
20
25
30
35
40

DRAFT