No One Likes a Stick up Their Nose: Making the Case for Saliva-Based Testing for COVID-19

Farhana Ali, MD[1] and Daniel A. Sweeney MD[2]

[1]Division of Gastroenterology, Department of Pediatrics, University of California San Diego, La Jolla, CA, USA.

[2]Division of Pulmonary, Critical Care and Sleep Medicine, Department of Internal Medicine, University of California, San Diego, La Jolla, CA

Corresponding author:

Daniel A. Sweeney, MD

200 West Arbor Drive #9-928

San Diego, CA 92103

619-543-6777 (office)

Email: dasweeney@health.ucsd.edu

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Curtailing the spread of the SARS-CoV-2 virus is a worldwide challenge. As the COVID-19 pandemic stretches on, some regions are experiencing a decline in cases, prompting governments to search for the means to return to normalcy. Effective strategies to avert a second epidemic wave will certainly entail increased testing, contact tracing, and adherence to isolation for those who test positive or have symptoms [1]. However, the ability to perform large-scale testing remains elusive. The current standard test for COVID-19 involves having a healthcare worker place a swab in the nares of a patient and sample at either the mid-turbinate or nasopharynx [2]. This method of testing is inherently limited in scale due to multiple factors: cost, availability of swabs, and the need for a healthcare worker to administer the test. Sputum-based COVID-19 testing, on the other hand, has been shown in one meta-analysis to have higher sensitivity compared with nasopharyngeal swab testing [3]. However, a majority of patients with COVID-19 present with a dry cough; thus, many persons infected with SARS-CoV-2 may struggle to provide a sputum sample [4]. Alternatively, the minority of COVID-19 patients with a productive cough pose a substantial risk to health care workers when providing a sputum sample.

In this issue of *Clinical Infectious Disease*, Rao and colleagues compare nasopharyngeal (NP) swab testing for COVID-19, versus a saliva sample-based testing strategy [xxx]. They performed a single center prospective study and enrolled 217 asymptomatic male participants in a quarantine center who had tested positive for SARS-CoV-2 infection 8 to 10 days prior. Upon awakening in the morning, participants self-collected saliva by spitting oral fluid into a sterile specimen container. Paired NP swab samples were subsequently collected (presumably by a healthcare worker) from the participants using a sterile flocked swab that was then placed into viral transport medium (VTM). All samples were stored in room air and processed within 5 hours of sample collection. Total nucleic acid extraction was performed on the NP swab-VTM and saliva. For SARS-CoV-2, the primer targets were the E-gene and the RNA-dependent RNA polymerase gene (RdRp). When both E-gene and RdRp primer-probe sets were detected at Ct <38, the samples were classified as positive. Overall, 160 of the 217 (74%) participants tested positive for Covid-19 based on saliva, NP swab, or both testing

methods. The detection rate for SARS-CoV-2 was higher in saliva compared to NP testing (93.1%, 149/160 vs 52.5%, 84/160, p<0.001). The cycle threshold (Ct) values for E and RdRp genes were significantly lower in saliva specimens compared to NP swab specimens.

There are aspects of this study which may limit the generalizability of the results. In particular, the enrollees were remarkably homogeneous and reflect a small portion of patients with COVID-19. All participants were young men (mean age 27), asymptomatic at the time of enrollment, and all were tested during a very specific and narrow time window in the course of their disease (8 to 10 days post diagnosis). Whether saliva-based testing would fare as favorably versus NP swab testing earlier in the course of disease is not addressed in this study, but earlier investigations suggest that viral load in posterior oropharyngeal saliva samples is highest during the first week of symptom onset [5]. Also, the test kit used in this study is somewhat unique in that it targets the envelope (E) gene whereas most other testing kits identify either one of the virus nucleocapsid genes (N1 or N2).

Despite these shortcomings, the work of Rao and colleagues is encouraging and suggests that saliva-based COVID testing may become an option for future testing of populations on a larger scale. There is biologic plausibility for using saliva to test for COVID-19, given that debris from nasopharyngeal epithelium drains into the oral cavity and mixes with saliva. Angiotensin-converting enzyme 2 (ACE2), the entry receptor for SARS-CoV-2, is highly expressed in both the oral mucosa and the base of the tongue [6]. Whether salivary glands are directly infected by SARS-CoV-2 is not known, but a rhesus macaque model of SARS CoV-1 showed that salivary gland duct epithelial cells expressed ACE2 and were an early target of infection [7]. Nonetheless, in prior studies, salivary testing for COVID-19 has not consistently been shown to be equivalent to NP swab testing. Although a recent meta-analysis suggested that the sensitivity of saliva and NP swab testing for SARS-CoV-2 were not different, only 5 trials were included in the analysis and the level heterogeneity was high (I^2=61%) [8]. Also concerning is the fact that most reports have shown a lower viral load in saliva compared to NP samples, although this difference appears to be negligible in the first 7 days of disease. Finally, the variability in performance of saliva-based testing for COVID-19 is likely related to differences in technique used across studies including: sample collection technique, differences in

timing of collection (some studies insisted on a morning sample), whether or not the sample was transported in a storage solution (i.e., a viral transport media or saline) and the differences in viral extraction and Rt-PCR test kits. It is particularly troubling that published studies either describe a wide range of methods by which the participant provides a saliva sample (for example, "drooling technique," "coughing up and clearing the throat" or by "pooling saliva in their mouth") or do not describe the method at all [8].

Currently there are more than 150 US Food and Drug Administration Emergency Use Authorized COVID-19 tests, yet only 6 are saliva-based [9]. The findings by Rao and colleagues are encouraging and suggest that saliva-based testing has the potential to be the basis for the widescale testing that will be needed if schools and businesses are to return to any semblance of normalcy. Future investigations should aim to optimize saliva testing for COVID-19 with attention paid to various technical aspects including: method and timing of collection, specimen transport, viral extraction and PCR testing.

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