Saliva as a Potential Diagnostic Specimen for COVID-19 Testing

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Abstract: The current outbreak of the highly contagious, animal origin SARS-CoV-2 virus causes the disease COVID-19. The disease is globally pandemic and as per World Health Organization (WHO) has spread to 235 countries. There is global lockdown for containment of the virus transmission. Testing of symptomatic patients, healthcare workers and suspected individuals and mass screening is vital. WHO recommends nasopharyngeal (NP) and oropharyngeal (OP) swab for the quantitative assessment of SARS-CoV-2 RNA level through real-time reverse transcription polymerase chain reaction (rRT-PCR). The virus is shown to be consistently present in saliva and rRT-PCR of saliva specimens and have advantages over NP and OP swabs such as self-collection of saliva, avoided iatrogenic errors, and direct collection from suspected individuals, and planning for mass screening.

Salivary diagnostics mostly involve “Salivaomics” representing the identification of the various “omics” constituents of saliva like the salivary proteome, transcriptome, microRNA, metabolome and microbiome. Rapid, economic and high sensitivity analytical techniques using salivary samples to detect oral diseases, viral infections and human stress tests were reported. Thus, saliva is suggested as a diagnostic fluid for COVID-19 and the relevant current literature is explored and discussed.

Key Words: COVID-19, nasopharyngeal swab, oropharyngeal swab, RT-PCR, saliva, SARS-CoV-2

In December 2019, an outbreak of a highly contagious pathogen happened in Huanan Seafood Market in Wuhan, China.1 The pathogen was identified in early January 2020 as a novel beta-coronavirus named initially as 2019-nCoV and later as SARS-CoV-2, and the disease called COVID-19. The disease is globally pandemic and as per World Health Organization (WHO) on April 29th, COVID-19 has spread to 235 countries. The virus is of zoonotic origin from bats, with an intermediate host still unclear regarding transmission to humans.2 There is global lockdown phenomena for containment of transmission of the virus and testing of symptomatic patients, healthcare workers and suspected individuals, and planning for mass screening.

Various clinical specimens like blood, pharyngeal swabs, saliva, anal swabs and urine showed the presence of the virus in infected patients. WHO recommends specimens from upper respiratory tract like nasopharyngeal (NP), oropharyngeal (OP) swab or wash in ambulatory patients, and lower respiratory specimens like sputum, endotracheal aspirate or bronchoalveolar lavage in patients with more severe respiratory disease for the quantitative assessment of SARS-CoV-2 RNA level through real-time reverse transcription polymerase chain reaction (rRT-PCR).3 At present the rRT-PCR is considered as the gold standard for SARS-CoV-2 detection.4 Currently all rRT-PCR tests are performed using the forward (5'-ACCTTTCCAGTTAACAACCA-3') and reverse (5'-TTACCTTCTGTCACCCCG-3') primers targeting the 5' UTR region of SARS-CoV-2. WHO also claims that 2019-nCoV spreads primarily through saliva droplets or discharge from the nose.5

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Saliva: The Diagnostic Biofluid for COVID-19

The SARS-CoV-2 virus may enter humans through respiratory tract or conjunctival mucosa and has a preferential tropism to human airway epithelial cells and cellular receptor for angiotensin-converting enzyme 2 (ACE2).8 The expression of ACE2 is higher in minor salivary glands than lungs suggesting that salivary glands could be potential target for COVID-19.9 It is suggested that there are 3 different pathways of SARS-CoV-2 entry in the saliva: first, from the lower and upper respiratory tract, secondly from the blood into the gingival crevicular fluid and thirdly by major and minor salivary gland infection.10 Currently, 3 approaches have been reported in saliva collection which are coughing out, saliva swabs and directly from salivary gland duct.11 To et al demonstrated in their study that SARS-CoV-2 could be detected in the saliva specimens of 11 out of 12 patients. Serial saliva specimens showed declines in salivary SARS-CoV-2 RNA levels after hospitalization. Viral culture demonstrated that live viruses were present in the saliva of 3 patients. Also, in all the patients whose NP specimens tested negative for 2019-nCoV, all saliva specimens also tested negative. The use of saliva is preferred over NP or OP specimens for serial viral load monitoring because this would reduce the discomfort to the patient and reduce the health hazards to healthcare workers during repeated sampling. This suggests that COVID-19 may originate from infected saliva.12 Another study used self-collected saliva (mixed with nasopharyngeal and bronchopulmonary secretions from deep throat by coughing out in the morning) from confirmed COVID-19 patients. Among 23 COVID-19 patients, 20 patient’s saliva specimen showed detectable SARS-CoV-2 RNA. In the temporal profile of viral load, saliva reached the peak viral load during the first week of symptom onset and then declined.13

It has also been shown that saliva has a high consistency rate of greater than 90% compared to NP aspirate specimens in the detection of respiratory viruses. Saliva specimens have high sensitivity and specificity by an automated multiplex clinical laboratory improvement amendments-waived point-of-care molecular assay when compared with NP aspirate specimens.14 Lower respiratory tract sputum is produced by only 28% of COVID-19 patients, which indicates a strong limitation of pharyngeal specimen for diagnostic evaluation.15 Zhang et al reported that in a group of all positive swabs, most of the positive results at early stage were from oral swabs, while more positive came from anal swabs at late stage of COVID-19, suggesting that oral swabs may indicate early infection of 2019-nCoV but cannot be used as a discharge criteria.16 Wyllie et al tested self-collected saliva samples from COVID-19 wards and when compared to SARS-CoV-2 detection from patient-matched NP and saliva samples, they found that saliva yielded greater detection sensitivity and consistency throughout the course of infection. They showed that the geometric mean virus titers from

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saliva were about 5-fold higher than NP swabs. Also, saliva of 2 asymptomatic healthcare workers showed presence of SARS-CoV-2 compared to their negative matched NP swabs, suggesting that saliva may also be a viable alternative for identifying mild or subclinical infections. Furthermore, they report less variability in self-sample collection of saliva.17

Azzi et al collected saliva from 25 COVID-19 patients (confirmed by NP swabs) through the drooling technique and all were tested positive for the presence of SARS-CoV-2, while there was an inverse association between lactate dehydrogenase (LDH) and Cycle threshold (Ct) values (considered as semiquantitative indicators of viral load). Two patients showed positive salivary results on the same day when their pharyngeal or respiratory swabs showed conversion. Thus, they conclude that saliva is not only a reliable tool to detect SARS-CoV-2, but also may provide information about the clinical evolution of the disease.18

As saliva is emerging as a diagnostic fluid for detection of SARS-CoV-2, recently researchers from Rutgers University Cell and DNA Repository (RUCDR) Infinite Biologies in partnership with accurate diagnostic labs have successfully validated saliva as being a viable biosample source for COVID-19 detection when compared to NP or OP swabs, which is also approved by the Food and Drug Administration (FDA).19 According to them, the utilization of saliva to extract viral RNA was in fact a robust source for COVID-19 detection and equals in performance to the approved swab-based collection samples.

Technical Advantages of Salivary Specimen

Rapid and accurate diagnosis of COVID-19 is vital in containment of the disease in both the hospital and community set-up. NP and OP swabs are the recommended specimen types for COVID-19 diagnostic testing. The collection of these specimen types requires close contact with the patients, trained healthcare workers, sufficient time and thus a “high risk job.” In NP swabbing patients need to tilt the head up to 70 degrees to access the NP region and also may face difficulty if nasal obstructions are present (Fig. 1A). The swab in kept in place for several seconds to absorb the secretions which increases the risk of sneezing (Fig. 1B). OP swabbing needs wide mouth opening with tongue depression and chances of patients gagging and coughing during the procedure is high (Fig. 1C). Both NP and OP swab procedure can cause bleeding, especially in patients with bleeding diathesis. There is also a huge need for the amount of swab kits and personal protective equipments (PPE) which can be preserved to be used in critical COVID-19 care wards. Advantages of salivary specimens are that, it can be self-collected easily by adults and children, as the patient spits into a sterile container. Thus, saliva collection will eliminate the need for health care professionals and reduce nosocomial infection. This reduces the time and cost associated with the specimen collection will thus will help in increased testing of patients and in mass screening. Specimen collection can be done where negative ventilation chambers are not available such as in outpatient clinics, community or household areas.

CONCLUSION

Salivary specimen collection is non-invasive and can be self-collected at any time and repeatedly and greatly minimizes the exposure of healthcare workers to COVID-19. Furthermore, saliva may also be an appropriate and sensitive biofluid, alternative to NP and OP swabs for screening asymptomatic or pre-symptomatic SARS-CoV-2 infections and also desirable for sequential monitoring of viral load. Hence, its concluded that with all the advantages discussed, saliva will emerge as a potential diagnostic specimen for COVID-19 testing.

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FIGURE 1. (A) Sagittal section of pharynx, (B) nasopharyngeal swab and (C) oropharyngeal swab locations.
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