In recent time, saliva has been extensively investigated for its potential as a diagnostic aid for detection of SARS-CoV-2 infection. The source of virus in saliva is still controversial and researchers claim that the virus particles possibly come from the respiratory system and infect salivary glands. A significant number of studies reported statistically high sensitivity and specificity of the saliva-based diagnosis of SARS-CoV-2 infection.1 Proposed advantages are highly encouraging such as less-invasive, convenient, associated ease of self-collection and minimal risk of cross-infection.2 Due to the presence of justifiable advantages over the nasopharyngeal swab, saliva-based SARS-CoV-2 testing has been projected as a gold standard methodology, challenging the conventional nasopharyngeal swab testing. Recently conducted research by Pasomsub et al.3 reported staller performance of saliva in COVID-19 diagnosis especially in terms of specificity and sensitivity. The sensitivity and specificity of the saliva sample RT-PCR were 84.2% (95% CI 60.4%-96.6%), and 98.9% (95% CI 96.1%-99.9%), respectively.3 In fact, countries like South Korea, Germany, and Japan successfully adapted the technology for saliva-based diagnosis.4

Although there are many claims made in the literature for the justification of saliva-based COVID-19 testing, we take this opportunity to address some intricacies associated with this proposition.

1. It is quite conceivable that samples from representative areas have fewer chances of false-negative or false-positive results. Thus, for all respiratory tract infections, only a nasopharyngeal swab has been preferred to date. By virtue of abundant angiotensin- converting enzyme 2 (ACE2) receptors, the respiratory tract is the most commonly affected site and regarded as the representative area for COVID-19.5 ACE2 expression, as well as expression of TMPRSS2 and furin, were detected in oral mucosa using immunohistochemistry and western blot.6 However, these methodologies are insufficient to claim the presence or quantification of this protein on tissues of the oral mucosa. The more authentic and sensitive methodology would be flow cytometry and mass cytometry (CyTOF). Till then, viruses in saliva could be just a contamination from the respiratory source and hence future studies are recommended so that justification for the development of salivary diagnostic can be put forth.

2. Thick saliva needs additional processing to reduce the viscosity (e.g. homogenization), which can increase cross-contamination as well as reduce the sensitivity of the test. It is well known that saliva exhibits age-dependent inter-individual-based variability in its consistency. As compared to young individuals, old individuals have thick andropy saliva, which is attributed to the reduced functioning of salivary glands. Moreover, drug-induced xerostomia and certain xerostomia-associated systemic conditions such as dehydration, sialadenosis, sialadenitis, Sjogren's syndrome, etc. also produce saliva with similar consistency.

With these aforementioned aspects in mind, we strongly believe that the saliva-based diagnostic methodology needs two different types of standardization in tune with the consistency of saliva. This involves additional technical expertise for additional intermediate steps required for identification of viscosity and homogenization of saliva samples that will have adverse cost implications. Although per test rise in the expenses will be nominal but we speculate that nationwide testing on population certainly cause a significant financial burden on low- and middle-income countries. We also recommend studies on cost impact analysis of both the testing methodologies for envisaging the practicality in population use.

3. The timing of sample collection is a crucial factor in salivary diagnostics. Collection of saliva samples after smoking, tobacco or quid chewing (a common practice in Asian countries), tooth brushing or mouthwash use, eating food, etc. can potentially affect the inhibition of nucleic acid amplification and also cause degradation of RNA leading to false negativity. This is also applicable for saliva-based antibody testing for SARS-CoV-2 infection. Hence, it is imperative to formulate appropriate guidelines for sample collection such as avoidance of eating or drinking 1 hour before sample collection.

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However, in ‘the self-collection of sample’ strategy, which is one of the prime advantages over nasopharyngeal swabs, judicious following up of these guidelines would be questionable jeopardising the test results (false negative). The exploitation of this limitation for deliberately obtaining negative results (e.g. for air travel, work, etc.) will be disastrous.

4. It has been proposed that nasopharyngeal swab testing could detect historical cases, while saliva might be preferable for gauging active cases. On somewhat similar lines, it is observed that asymptomatic cases are associated with high sensitivity for saliva samples as compared to nasopharyngeal swabs. Intriguingly, a contrasting trend was observed in symptomatic cases wherein sensitivity was high in nasopharyngeal swabs. The proposition put forth judicious use of both techniques for decreasing the incidences of false-negative results. Hence, it is recommended that future studies should report the clinical status of the patients.

In conclusion, saliva has been regarded as a potentially reliable and advantageous tool for the diagnosis of COVID-19 infection. Although, it might be true but there is a dire need to understand the compositional diversity of saliva and its possible impact on the sensitivity of the results. There is also a need to investigate the burden of technical expertise and financial investment in salivary diagnostics.

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