Saliva sampling as a source for SARS-COVID 19 PCR detection
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Abstract
The diagnosis of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) infection relies on the detection of viral RNA by RT-qPCR conducted with respiratory specimens such as nasopharyngeal swabs. However, this procedure requires specialized personnel, centralized laboratory facilities, and time to provide the results. Saliva has been increasingly used over the last few decades for evaluating human health. In recent days, a test for assessing the RNA in saliva samples was approved by the US Food and Drug Administration. The use of saliva as a diagnostic specimen has advantages such as it is easily self-collected by the patient with no discomfort, and it reduces the risks for the operator.

Objective: The objective of this project is to substantiate the use of saliva sampling as a noninvasive analysis of Covid-19 infection.

Methods: SARS-RNA 5x107/equivalent (1:1000, 1:10,000 and 1:100,000), saliva-8,1µL (only), saliva plus SARS-CoV-2-RNA were mixed with oligonucleotides primers detecting nucleocapsid gene (N1 or N2), and saliva plus SARS-RNA RNase P primers to detect human RNase P (to show that adequate sample was collected for control) were mixed with Reaction Master Mix for detection of COVID-19 virus and amplified by RT-PCR equipment (QuantStudio 3 – Thermo Fisher).

Results and Conclusions: The initial experiments provided positive results for the 3 SARS-RNA dilutions and Saliva plus SARS-RNA. The highest dilution (1:100,000) showed 2.75 genome copies could be detected with both primers N1 and N2. Saliva plus primers for RNaseP gave a positive result, and saliva only plus SARS-CoV primers were negative. The Ct values were between 21 to 40 (≤40) as requested by CDC for detection of COVID-19. With this data it was concluded that the role of salivary diagnostics is promising for direct testing for SARS-CoV19 as well as being easy, fast, and inexpensive. Additionally, the sensitivity of the salivary sample is comparable to that of respiratory samples.

Discussion
This study demonstrates that the use of saliva without RNA extraction is a viable alternative for SARS-CoV-2 detection. The positive and negative controls presented results as expected, also the human RP RNA was detected in each sample proving that the RNA was accessible in the samples. The RT-qPCRs were conducted blinded to avoid biased response and the results with even weak signals for primers N1 and N2 were compared with those obtained from NP samples collected by specialists at the same day. The low, inconclusive or negatives results for COVID can be explained by the fact the saliva samples tested were positive a month or two before the saliva was collected.

Conclusions
- The role of salivary diagnostics is promising for direct testing for SARS-CoV2 as well as being easy, fast, and inexpensive.
- Saliva sampling can provide diagnostic without viral RNA extraction.

References

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