Review of: "Pooling saliva samples as an excellent option to increase the surveillance for SARS-CoV-2 when re-opening community settings"

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The current COVID-19 pandemic has highlighted the need to implement fast, cheap, and high-quality massive testing, particularly in the peaks of the different waves of SARS-CoV-2 infection. During these high-demand periods, testing became a fundamental tool both for the diagnosis of infection and for the evaluation of the extent of viral spread within specific populations[1].

However, this massive testing exerted different types of pressure on the whole chain involved in the application of diagnostic tests, including manufacturing and distribution of reagents, plastics and equipment, human resources available to collect samples properly, standardize and perform tests in a diagnostic setting in a timely and quality assured manner.

A fundamental part of the diagnostic procedure is sample collection. The standard for SARS-CoV-2 detection is the collection of samples with a nasopharyngeal swab, which is deposited in viral transport media for RNA extraction and RT-PCR. During the peaks of the pandemic, even these fundamental items became scarce. Also, swab collection requires dedicated trained staff, as well as personal protective equipment and environmental safety management. All these items impact the total cost and safety of the test, and failure in one of these requirements can halt the whole diagnostic procedure[2,3].

In these scenarios, the development and validation of cheaper, easier, and reliable options for sample collection became evident. Since the main anatomical site of sample collection, the nasopharynx, is in close contact with the saliva, this fluid turns out to be a convenient matrix for the potential detection of SARS-CoV-2[4,5].

The paper by Moreno-Contreras, *et.al* [6]., corroborates that saliva is an excellent option to increase surveillance for SARS-CoV-2 infection, the authors show the advantage of self-collection of 2-3 ml of saliva without preservatives, which is stable up to 48 h at 4°C. All samples were obtained from ambulatory patients, they reference a direct lysis procedure of the saliva sample developed by their group. Viral detection by RT-qPCR was performed with the Charité-Berlin protocol, reporting the detection of the E gene.

Our experience using saliva as an alternative matrix for SARS-CoV-2 detection, which was initially reported in 2021, indicates that saliva is a feasible option for virus detection by RT-qPCR[7]; we showed that saliva samples are stable

without the use of any preservative, and a positive SARS-CoV-2 sample can be detected even 15 days after collection when the sample is stored at 4°C. Up to April 2022, we have performed 6400 saliva-NPS paired samples getting a concordance of 98%. A total of 238,000 saliva samples have been obtained in surveillance programs of public and private settings during the pandemic, which included symptomatic and asymptomatic individuals, with an average positivity of 2.1%.

Moreno-Contreras *et al*[6]., also tested pools of 5 and 10 saliva samples, considering threshold cycle values (Ct) of 38 and 41, respectively, to ponder the pool as positive. The results show that using pooling of 10 samples decreases the Ct value of the original positive sample by 2.7 units, while for the pool of 5 samples, the difference in Ct was 1.8 units. Nevertheless, they demonstrate that their detection method allows the identification of positive samples with Ct values higher than 35 when pooling 5 samples.

However, the use of a 10-sample pool shows that it is only detectable as positive up to a threshold cycle of 37 in the RT-qPCR, losing 3 Ct in some of the 10 individuals that might be at an early onset of infection, subjects whose detection is important in a setting aming for the return to work and academic activities.

Finally, the authors corroborate the validity of a sample pooling strategy to diminish the associated costs of the test to 77.9%, with a prevalence of 0.6%. In conclusion, a combination of direct saliva lysis, and pooling would facilitate sampling and testing in community settings[6].

Direct cost savings is an important advantage of using saliva and pooling for the detection of SARS-CoV-2, nevertheless, this aspect remained to be seen in the paper by Moreno-Contreras, *et. al*. In our two-year experience, we have observed that pooling of 5 samples is the most appropriate option as it allows to maintain the RT-qPCR sensitivity. The cost savings depend on the moment in which the pooling strategy is implemented during the pandemic. For example, if we pool 5 samples, when the prevalence of positive cases is 1% to 14%, the savings are 75% to 27%, respectively. This prevalence occurred in the interweave period of infection with a duration of 3-4 months. On the other hand, when the prevalence is higher than 15%, usually during waves of infection, the economic advantage of the pooling strategy is dramatically reduced since the probability that a pool results positive increases. In addition, we should consider that as the number of positive pools increases in a tested population, the need of reprocessing samples to determine the positive individual(s) also augments, which enlarges not only the resources used but also the time to inform the results. Therefore, the implementation of a pooling strategy is effective only during the interweaves periods and should not be considered during the peaks of the pandemic.

In conclusion, the COVID-19 pandemic prompted scientific research for cheaper detection tests, the use of saliva as a matrix for the detection of SARS-CoV-2, and the possibility of pooling samples have proven useful in several studies. Specifically, the pooling strategy should be used based on the prevalence of positive cases as this is the basis for cost savings in testing.

This research broadens the possibility of transferring this knowledge to be used in the detection of other diseases caused by microorganisms, even where resources are limited. It also prepares us to respond more efficiently to the next pandemics.

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