SALIVA IS THE KEY ELEMENT FOR SARS-CoV-2 MASS SCREENING

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The current COVID-19 pandemic has shown clinicians and researchers the fundamental role played by asymptomatic carriers and pre-symptomatic individuals in the infectious outbreak, a feature that distinguishes SARS-CoV-2 from SARS-CoV and MERS-CoV [1].

Recent findings have pointed out how the viral load in COVID-19 is high at the very onset of the disease and then decreases over time, underlining a probably high load also in the pre-symptomatic phase [2].

Within this frame, identifying those subjects who unwittingly can spread the infection when coming into contact with their family members, or other people in social gathering spaces, is the only way to prevent new pandemic outbreaks and avert future national lockdown measures.

However, we must be aware that mass-screening programs of the population pose a strong challenge, if compared with the diagnostic procedures performed in a hospital setting. Precisely, a symptomatic COVID-19 patient can be diagnosed by clinical signs, laboratory tests and radiological imaging [3], while an asymptomatic individual can be identified only by testing for the presence of SARS-CoV-2 infection [4]. There is great concern over this issue, which needs to be quickly addressed to draw up an effective and feasible mass screening strategy [5].

If the diagnosis of asymptomatic infections relies only on tests, it means that the diagnostic technologies adopted should guarantee very high accuracy parameters. A high sensitivity is required to prevent false negative subjects from going undetected and spreading the infection, while a high specificity is desirable to save false positive subjects from being unnecessarily quarantined, with following economic and social consequences [6].

A diagnostic test which provides 100% sensitivity and 100% specificity values has not been identified yet, thus a combination of more than one test is necessary to establish the right steps of the diagnostic chain “from the street to the hospital” [7].

The key diagnostic technology for front line use is represented by a test able to screen the general population before entering a social gathering space, i.e., a workplace, a shopping center, a cinema, a school or a sport facility.

To achieve this aim, a diagnostic test should have a widespread delivery on the territory, should be performed also by non-specialized personnel, its use should be easy, rapid and at a fair price [8].

The diagnostic protocol for SARS-CoV-2 infection, approved by the World Health Organization, encompasses the use of real time reverse transcription Polymerase Chain Reaction (rRT-PCR) to detect viral RNA in respiratory samples, especially those collected through a nasopharyngeal swab (NPS) [9].

This procedure, however, is not suitable for a mass screening context, because it requires specialized healthcare professionals, expensive equipment and reagents, centralized laboratories and is associated with a raised risk of viral transmission for the operator and discomfort for the person undergoing the test [10].

In contrast, saliva has recently gained attention in the international literature and public opinion because it is a suitable sample to be used for mass screening [11;12].

The advantages of the use of saliva are numerous: it can be easily and non-invasively self-collected by the subject, avoiding thus the employment of skilled staff and the risk of viral transmission during the procedure, it is more comfortable for the patient if compared with the nasopharyngeal swab, and for this reason it is more frequently repeatable with a good compliance [13].

Starting from the first reports at the beginning of the pandemic, several other papers have analyzed the diagnostic performance of saliva in comparison with the NPS, achieving discordant results [14-16].

Most of these papers reported that saliva has a sensitivity in detecting SARS-CoV-2 similar to the sensitivity shown by the NPS [17], sometimes slightly lower [18], and other times higher [19].

These results should be confirmed with larger studies by adopting a standardized procedure, since in these papers there are often low-sized cohorts of patients, different collection and processing methods and clinical settings.

The study of Yokota et al., published in this issue of the journal [20], is a milestone in the validation of the use of saliva as a diagnostic tool to detect SARS-CoV-2 in population-wide testing.

This study recruited a total number of 1,924 asymptomatic subjects, and for this reason it is the first study reporting the recruitment of such a large-sized cohort.

The authors analyzed two groups of subjects: the *contact tracing cohort* represented by asymptomatic individuals, who had been in contact with a laboratory-confirmed infected person, and the *airport quarantine cohort*, composed of travelers arriving at the Japanese airports from foreign countries. This feature represents another key point of this study, which not only focused on asymptomatic individuals, but also set up their recruitment in two of the most worrisome social contexts for the pandemic diffusion, family clusters and airports**.**

As an example, the second wave of contagion that started in Italy at the end of August was mainly due to people returning from their holidays spent abroad; this increase in positive cases has promptly led our health authorities to establish an airport-based screening campaign for travelers.

In this study, each subject underwent both NPS and salivary self-collection simultaneously.

The authors reported a salivary sensitivity higher than 90% and a specificity greater than 99.9%, accounting for a high concordance between the NPS and saliva when analyzed by molecular-based tests. Interestingly, sensitivity values were higher in the saliva samples than in the NPSs (i.e., 92% vs 86%) when the analysis was conducted by standard rRT-PCR, underlining the value of this fluid in

detecting also false negatives in the NPS, a feature that also other papers had previously reported [21].

Another issue that the authors pointed out in their paper is the use of a *point-of-care* technology, i.e., the *reverse transcription loop-mediated isothermal amplification* (*RT-LAMP*) that, when compared with standard rRT-PCR, provided concordant results.

The use of point-of-care technology has been gaining more and more attention during the last weeks in expectation of re-opening schools and workplaces, since the analysis can be performed directly when and where the subject is tested, i.e., the occupational medicine office, the GP's surgery, airports, schools, universities, cinemas [22]. With these technologies, results can be provided within 30-60 minutes and allow a faster diagnosis without referring to a centralized laboratory, which in contrast requires 24-48 hours to have results [23]. Besides, during the pandemic the overcrowding of those centers appointed to analyze respiratory specimens made it difficult to maintain essential diagnostic services and caused the disruption of many medical procedures, and only symptomatic people could get a nasopharyngeal swab, which was not sufficient for testing the entire population.

In conclusion, the use of saliva as a diagnostic sample in a point-of-care technology is a winning strategy within the context of a mass screening program, since it combines two benefits: the use of an organic fluid that can be self-collected by the subject without any risk for the operator, and the rapid molecular-based diagnosis at the time and place of specimen collection, avoiding thus the preventive quarantine of potential infected individuals, a necessary measure when waiting for the NPS analysis carried out in hospital/private medical laboratories.

The next stage of salivary screening will be the introduction of *rapid saliva-based antigen tests* able to detect the presence of the virus by identifying the Spike protein in the fluid with a lateral flow assay device, which works similarly to the pregnancy test or the rapid serologic test.

This technology will be very useful as first-line screening of the general population in an everyday context, such as companies, factories, offices or social gathering spaces (*point-of-need* devices), to select only those who require second-line diagnosis by molecular-based testing in a laboratory. A paper has been published regarding this issue [24], followed by other companies that have announced the development of similar technologies to gain certification and enter the market. Nevertheless, more in-depth and thorough studies should be carried out and published, because sensitivity and specificity values may vary depending on the antibodies employed in the nitrocellulose membrane of the test device.

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