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SHORT COMMUNICATION

Comparison of nasopharyngeal and saliva swabs for the detection of RNA SARS-CoV-2 during mass screening (SALICOV study)

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SUMMARY

In the context of a second wave of SARS-CoV-2 transmission, the use of saliva sampling has become an issue of real importance. SARS-CoV-2 RNA screening was performed on nasopharyngeal and saliva swabs collected from 501 individuals from residential homes for the elderly. The saliva samples were collected at the same time as the nasopharyngeal samples. Nasopharyngeal samples yielded positive results for 26 individuals, only two of whom also tested positive with saliva swabs. In this context, saliva collected by swabbing the fluid is not an ideal sample.

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Since December 2019, the world has been in the grip of a pandemic of COVID-19, an infectious disease caused by SARS-CoV-2, a virus that emerged in China (Wu *et al.*, 2020). The reference first-line diagnosis method involves searching for the SARS-CoV-2 genome in a nasopharyngeal sample. Competent trained staffs are required to obtain this sample, and the sampling technique is uncomfortable for the person screened. The collection of saliva is simple, non-invasive, painless and cheap, and can be performed by staff with little training; as such, it could be a useful alternative to the reference technique based on a nasopharyngeal sample (Azzi *et al.*, 2020).

In the context of a second wave of transmission, the use of saliva sampling has become an issue of real importance, as it would provide greater access to easier screening on a larger scale. Saliva sampling has already proved effective in other infectious and non-infectious diagnostic contexts (Sri Santosh *et al.*, 2020).

Samples for mass screening must be collectable without constraints, at any time of the day, and regardless of the individual's prior ingestion of liquid or solid food, or smoking (To *et al.*, 2020). Several studies have reported major benefits of saliva sampling, mostly in symptomatic patients (Williams *et al.*, 2020; Pasomsub *et al.*, 2020; Wyllie *et al.*, 2020).

The objective of this study was to determine whether the sensitivity of saliva samples was similar to that of nasopharyngeal samples in asymptomatic individuals screening for SARS-CoV-2 RNA.

In total, 501 individuals from residential care homes for the elderly (193 residents, 308 caregivers and administrative staff) participated in screening for SARS-CoV-2 RNA between May 6 and 13, 2020. For each individual, we collected a swab from the nasopharynx and a swab from the saliva (Kaufman *et al.*, 2002). The saliva sample was collected by swabbing the fluid in the groove at the base of the lower jaw. The two swabs were then placed in separate tubes containing viral transport medium and were sent to the virology laboratory of Amiens Picardie University Hospital.

This study was approved by the French personal protection committee CPP EST IV (CPP 20/40 (COVID)/SI 20.04.23.40207) and registered with ClinicalTrials.gov under reference number SALICOV NCT04386551.

All samples were analyzed with the Abbott RealTime SARS-CoV-2 assay, in an automated Abbott *m2000sp/rt* System (Abbott Molecular Inc., Des Plaines, IL). The Abbott m2000sp Analyzer performed automated sample preparation using magnetic microparticles (Abbott mSample Preparation System DNA) to test 0.5 ml samples of nasopharyngeal swabs previously inactivated by 0.5 ml of lysis buffer from the supplier. An eluate

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volume of 40 µL was utilized in the PCR reaction. The *m2000* System can process up to 96 samples simultaneously. The Abbott RealTime SARS-CoV-2 assay amplifies target regions of the SARS-CoV-2 RNA-dependent RNA polymerase (RdRp) gene and the N gene within a single well. The stated limit of detection in the published instructions for use is 100 RNA copies/ml (Degli Angeli *et al.*, 2020). Intergroup comparisons were performed with non-parametric Mann-Whitney *U* tests for continuous variables, and Fisher's exact test for categorical variables. Two-tailed tests were performed, with values of p≤0.05 considered significant.

In total, 501 individuals were included in the protocol distributed among 193 residents and 308 caregivers/administrative staff, between May 6 and 13, 2020 (*Table 1*). The median age of those included was 53 years (range: 19 to 99 years). We took samples from 399 women and 102 men. At inclusion, 104 (20.7%) patients were symptomatic but none of them presented hospitalization criteria (*Table 1*). In the context of regional massive epidemic, the samples were taken to identify individuals who were potentially infected. The saliva samples were collected at the same time as the nasopharyngeal samples, by the same healthcare professional.

SARS-CoV-2 was detected in 26 nasopharyngeal swabs and two salivary swabs (*Table 1*). Each of the positive saliva samples was associated with a positive nasopharyngeal sample. No saliva sample was found positive in the absence of a positive nasopharyngeal sample. The prevalence in our study was therefore 5.18%.

The mean cycle threshold (Ct) value for RT-PCR for the positive nasopharyngeal swabs was 22.9±5.37. The Ct values for the two nasopharyngeal swabs for which the paired saliva sample also tested positive were 11.3 and 22.6, respectively. The Ct values for the positive saliva swab were 25.2 and 28.8, respectively. The individuals tested positive for both samples were 49 and 91 years old. They were symptomatic at the time of sampling, and both had presented signs suggestive of COVID-19 seven days before. Among the 26 individuals tested positive, 8 were asymptomatic and 3 presented symptoms at inclusion. Unfortunately, data were not available for 15 of them.

We show here that saliva swabs have a much lower sensitivity than nasopharyngeal swabs ($p<0.001$). We decided to use the same collection kit for swabbing the two sites, to ensure that the conditions were identical, particularly as concerned the transport medium. Although collection by sputum or aspiration of saliva has proven its effectiveness in the diagnosis of SARS-CoV-2, we have chosen to swab the flow of saliva fluid in the groove at the base of the lower jaw. Indeed, the objective was to perform a saliva sample that did not require suitable pre-treatment.

The analysis of viral load based on Ct values showed the Ct to be about 23 for nasopharyngeal samples. However, it should be noted that viral load was high for one of the two positive saliva samples (Ct=11.3), whereas the other was in the same range as for the other positive samples (Ct=22.6). Thus, the lack of detection in the other saliva samples cannot be attributed to the Ct value.

The age of the participants may also be a limiting factor for salivary sample screening. Indeed, the elderly are more likely to suffer a decrease in salivary secre-

Table 1 - *Demographic characteristics of patients undergoing SARS-CoV-2 screening.*

	Global screening	*Resident*	*Caregivers / administrative staff*	*p value*
	N=501	*N=193*	*N=308*	
Age (mean years ± SD)	56.3±22.2	80.8±10.6	41.2±11.4	<0.0001
Gender				<0.0001
Male, n (%)	102 (20.4)	66 (34.2)	36 (11.7)	
Female, n (%)	399 (79.6)	127 (65.8)	272 (88.3)	
Clinical characteristics*				
Asymptomatic before and at inclusion, n (%)	169 (33.7)	59 (30.6)	110 (35.7)	ns#
Symptomatic** before and at inclusion, n (%)	94 (18.7)	27 (14.0)	67 (21.7)	ns
Asymptomatic before and symptomatic at inclusion, n (%)	10 (2.0)	2 (1.0)	8 (2.6)	ns
Symptomatic before and asymptomatic at inclusion, n (%)	182 (36.3)	79 (40.9)	103 (33.4)	ns
Positive Nasopharyngeal swab, n (%)	26 (5.2)	17 (65.4)	9 (34.6)	0.006
Positive Saliva swab, n (%)	2 (0.4)	1 (50.0)	1 (50.0)	ns

*data not available for 46 individuals.
**Symptomatic: symptoms at presentation (fever; cough, sore throat; runny nose; sneezing; diarrhea).
ns: non significant.

tion (De Rossi *et al.*, 2020). However, the two positive saliva samples were obtained from individuals aged 49 and 91 years. Age does not, therefore, seem to have affected the sensitivity of the saliva test in this study. Finally, it should be noted that no saliva sample tested positive in the absence of a positive result for the paired nasopharyngeal sample. Our results are very different from previously published studies showing equivalent sensitivities between nasopharyngeal swab and saliva samples for symptomatic hospitalized patients (Azzi *et al.*, 2020; Wyllie *et al.*, 2020) or slightly lower for asymptomatic persons or outpatients (Williams *et al.*, 2020; Pasomsub *et al.*, 2020). The major difference is that the saliva sample was taken each time by sputum in a suitable container. It highlights the lower sensitivity of the saliva sample using this specific collection method.

In conclusion, we show here that, in a context of screening in asymptomatic or paucisymptomatic adults, saliva collected by swabbing the fluid is not an ideal sample. The method used to collect saliva must be designed to ensure sensitivity as close as possible to that of the nasopharyngeal sample.

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