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Early View

Original research article

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Salivary detection of COVID-19. Clinical performance of oral sponge sampling for SARS-CoV-2 testing

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ABSTRACT

Background:

The current diagnostic standard for coronavirus 2019 disease (COVID-19) is reverse transcriptase-polymerase chain reaction (RT-PCR) testing with naso-pharyngeal (NP) swabs. The invasiveness and need for trained personnel make the NP technique unsuited for repeated community-based mass screening. We developed a technique to collect saliva in a simple and easy way with the sponges that are usually used for tamponade of epistaxis. This study was carried out to validate the clinical performance of oral sponge (OS) sampling for SARS-CoV-2 testing.

Methods:

Over a period of 22 weeks, we collected prospectively 409 paired NP and OS samples from consecutive subjects presenting to a public community-based free screening center. Subjects were referred by their attending physician because of recent COVID-19 symptoms (n=147) or by the contact tracing staff of the French public health insurance since they were considered as close contacts of a laboratory-confirmed COVID-19 case (n=262).

Results:

In symptomatic subjects, RT-PCR SARS-CoV-2 testing with OS showed a 96.5% (95%CI: 89.6-94.8) concordance with NP testing, and, a 93.2% (95%CI: 89.1 – 97.3)] sensitivity when using the IdyllaTM platform and a sensitivity of 76.3% [69.4 – 83.2] on the Synlab Barla laboratory platform. In close contacts the NP-OS concordance (93.8% [95%CI: 90.9-96.7]) and OS sensitivity (71.9% [95%CI: 66.5-77.3]) were slightly lower.

Conclusion:

These results strongly suggest that OS testing is a straightforward, low-cost and high-throughput sampling method that can be used for frequent RT-PCR testing of COVID-19 patients and mass screening of populations.

Summary of the "take home" message: Oral sampling for SARS-CoV2 RT-PCR is an easy to perform, straightforward self-administered sampling technique, which has a sensitivity of up to 93.2% in symptomatic patients and 71% in close contact subjects.

INTRODUCTION

To date, reverse transcriptase-polymerase chain reaction (RT-PCR) testing of naso-pharyngeal swab specimens (NP) is the gold standard for the detection of SARS-CoV-2 [1–3]. While its specificity can be as high as 100%, its sensitivity ranges sensitivity ranges from 42 to 98% during the first 10 days of coronavirus 2019 disease (COVID-19) [4, 5] and depends on the operator and on the moment the sampling is performed during the course of the infection. RT-PCR on NP, known as being of "high analytical sensitivity", is particularly well suited for symptomatic patients. However, in the context of a pandemic, when mass screening or repeated testing is required, the use of NP for RT-PCR is not appropriate since its acceptability is debated and its implementation requires time and dedicated trained staff. [6, 7]

Antigen-detecting rapid diagnostic tests (Ag-RDT) also called lateral-flow tests are quick, simple, inexpensive and allow the decentralization of testing at the point of care. These tests can be performed on NP but also on anterior nasal or mid-turbinate samplings which require minimal supervision. Their sensitivity is high in symptomatic patients, which is crucial for triage in hospital emergency departments, but is low in asymptomatic subjects [8, 9]. In these latter individuals they can be categorized as high frequency tests with low analytical sensitivity as reported previously by Mina et al [6].

Oral sampling offers a promising alternative to NP [10]. Various pathophysiological pathways explain the presence of the SARS-CoV-2 in saliva of patients with COVID-19 [11–13]. Tests with saliva involve amplification of viral RNA by RT-PCR or by Reverse Transcriptase Loop-Mediated Isothermal Amplification (RT-LAMP). These tests are non-invasive, easily repeatable and can be performed with or even without assistance, leading in the latter case to a lower risk of contamination of nursing staff. RT-PCR on saliva samples has been approved in Japan since June 2020 and in the USA since October 2020. Following the publication of an umbrella review [14] including ten meta-analyses [10] as well as trials meeting predefined quality criteria, the French “Haute Autorité de Santé” [15] recently retained the indication of RT-PCR on saliva in the following three situations: 1 / on symptomatic patients as a second-

line alternative to NP when NP is difficult or impossible (deviation of the nasal septum, very young patients, patients with psychiatric disorders, etc.); 2 / on contact persons as a second-line alternative to NP for whom NP is difficult or impossible; 3 / in the first instance as part of targeted mass screening, particularly if it is repeated regularly such as in schools, universities, for staff in health care establishments, in nursing homes, etc From a practical point of view, saliva sampling should be performed more than 30 minutes after the last drink, food, cigarette/e-cigarette, tooth brushing or mouth rinsing [28]. Saliva spit in a dry and sterile bottle is preferred; otherwise, saliva is collected using a dedicated system. Based on our research on the respiratory epithelium and on COVID-19 [16–19] We aimed to investigate the concordance of RT-PCR SARS-CoV-2 testing using OS as compared to NP testing and its sensitivity for symptomatic subjects and close contacts.

METHODS

Study design and participants

The present study was conducted on a prospective cohort of consecutive volunteers at the Nice-Côte d'Azur Metropolis community-based COVID-19 center (Nice, France), accessible for free screening to the general population.

During the first part of the study (Sept 21, 2020 to Jan 6, 2021) we enrolled adults referred by their attending physician because of recent (≤ 2 weeks) symptoms of COVID-19. During the second part of the study (Feb 6 to March 8, 2021) we enrolled adults referred by the contact tracing staff of the French public health insurance [20], since they were considered as close contacts of a laboratory-confirmed COVID-19 case (figure 1).

Procedures

Specimen Collection

After signing an informed consent to participate, all volunteers were interviewed and underwent paired NP and OS sampling in a random order. Disposable NP nylon swabs (type

A-04. Jiangsu Han-Heng Medical technology Co., Ltd. Changzou, Jiangsu, China) were eluted into a vial containing viral transport medium. Small hydroxylated polyvinyl acetate (PVA) sponges (Merocel® Standard Dressing, réf 400400, Medtronic), usually used for tamponade of epistaxis, were placed in the mouth for one minute and inserted into a sterile collection tube by the subjects themselves. No instructions were given regarding the need to fast, to have chewed gum or smoked a cigarette/e-cigarette. NP and OS were received within 4 hours at the laboratory.

Detection of SARS-CoV-2 RNA on NP samples

All NP samples were sent to the Synlab Barla laboratory (Nice, France), a private biological analysis laboratory that is under contract with the Nice-Côte d'Azur Metropolis for COVID screening tests. These samples underwent RT-PCR using the ORF1ab and N genes Da An Gene DA0992-Detection Kit for 2019-nCoV (Da An Gene Co., Ltd. Sun Yat-sen University, Guangzhou, Guangdong, China). For logistical reasons, this laboratory switched to the Transcription Mediated Amplification (TMA) technique to detect SARS-CoV-2 RNA on NP samples during the second part of the study (symptom free close contacts). According to the guidelines of the French society for Microbiology [21] for RT-PCR, the result was considered positive when the Ct (cycle threshold) value for the N and/or on Orf1b genes was equal or less than 36. For TMA the result was considered as positive when the RLU was above 850.

Detection of SARS-CoV-2 RNA on OS samples

Upon arrival at the Laboratory of Clinical and Experimental Pathology (LPCE), the sponges were placed in the body of a syringe and the liquid was squeezed out of the sponge by pressing the plunger. 200µl of the OS eluate were pipetted with a sterile tip (D. Dutscher, reference 134000), placed individually into Idylla SARS-CoV-2 test cartridges (Biocartis, reference A1043/6) and underwent fully automated nucleic acid testing including extraction, amplification, and detection in a single-use cartridge asdescribed previously [22]. The residual volume OS eluate was immediately aliquoted. According to the manufacturer, the

Idylla SARS-CoV-2 test provides a qualitative result for the presence or absence of SARS-CoV-2 RNA with a corresponding quality status. The Biocartis SARS-CoV-2 test included 2 genes (N, Orf1b) covered by 5 PCR targets (2 N targets and 3 Orf1b targets). A positive result required at least 2 amplified N targets [by setting a quantification cycle (Cq) of 41.9] and/or at least one or more amplified Orf1b targets. As Orf1b is highly specific no threshold was required for this gene.

External validation of SARS-CoV-2 RNA detection on OS samples

All OS eluates were sent to the Synlab Barla laboratory and underwent RT-PCR using the ORF1ab and N genes Da An Gene DA0992-Detection Kit as described above for NP. No threshold was used to define a positive result for OS. During the first part of the study (symptomatic subjects) this external validation was carried on OS aliquots stored at -80°C in the Nice COVID-19 biobank [17]. During the second part of the study, external validation was carried out on fresh specimens.

The different analyses were processed in a double-blind way: the results of the reference standard test were unavailable to the readers of the index test and vice versa.

Outcomes

The primary outcome was the clinical performance of OS compared to NP for SARS- CoV-2 screening in symptomatic and close contact subjects. For each group (symptomatic vs close contacts) we extracted the number of individuals positive for SARS- CoV-2 in both NP and OS (a), those positive only with NP (b), those positive only with OS (c) and (d) those negative with both NO and OS (Table 1). From these data, we calculated the concordance of the two types of sample (a+d)/(a+b+c+d). We also calculated the sensitivity of the test on each type of sample. The estimation of the sensitivity of a test requires a reference diagnosis. Since NP sampling has been shown to produce false negatives by RT-PCR, sensitivities for NP and OS are defined here respectively as (a+b)/(a+b+c) and (a+c)/(a+b+c), considering as a true positive any individual with a positive result on one or the other sample. This definition of a

positive individual is in agreement with the US-CDC and the ECDC directives on SARS-CoV-2 testing [23–25].

Secondary outcomes included the description of the studied population and the comparison between Ct values measured with NP and OS, an approximate proxy of viral loads.

To calculate the sample size in the absence of data regarding the sensitivity of OS in symptomatic subjects, we calculated the sensitivity of OS once the first 40 paired (NP/OS) samples were obtained. This sensitivity was 85%. Given this estimate and a desired lower bound of the 95% confidence interval for sensitivity of at least 80%, 144 volunteers were needed to complete the study in symptomatic subjects. To deal with the dropout risk we decided to include 149 symptomatic subjects.

Statistics

Continuous variables are presented as means (± SD), and categorical variables as numbers and percentages. Baseline characteristics between patients with and without COVID-19 were compared using the Student's *t*-test or Wilcoxon - Mann Whitney for quantitative variables based on the normality of the distribution of parameters or using the Chi-Square test for qualitative variables. The Shapiro–Wilk test was used to determine normality.

Ethics and regulatory authorizations

The promoter of the study was the Center Hospitalier Universitaire de Nice. The agreement for the study of the Institutional review board Sud Méditerranée V was obtained on April 22, 2020 (registration # 20.04014.35208). SHAM liability insurance (n° 159087). The study is registered in ClinicalTrial.gov (NCT04418206).

Role of the funding sources

The organizations that supported this study played no role in its design, patient selection, data collection, analysis or interpretation, report writing, or decision to submit the document for publication. Authors had full access to all data and responsibility of submission for publication.

RESULTS

Four hundred and twenty five subjects refered to the Nice-Côte d'Azur Metropolis free COVID-19 screening center were approached to participate to the study and 14 declined. Thus 411 subjects underwent COVID-19 diagnostic testing over the 22 weeks of the study, 149 during the first part of the study conducted on symptomatic subjects and 262 during the second part of the study conducted on close contacts. Two symptomatic subjects declined to perform NP once OS had been performed and were excluded from subsequent analyses. One had a positive OS RT-PCR, and the other was negative. The study therefore covers the remaining 409 participants (figure 1). All 409 participants performed the salivary self-sampling with the OS correctly as explained.

The 147 symptomatic subjects were aged 40+/-15 years and predominantly women 86/147 (58.5%). The interval between symptom onset and testing was 3.6 ± 2.6 days and most participants (107/147 [72.8%]) were sampled at the early stage of the disease, *i.e.,* within 4 days of symptom onset. Of these symptomatic subjects, 59 (40.1%) had a positive RT-PCR result with one or both of the sampling techniques and were thus diagnosed COVID-19. No clinical symptoms distinguished between RT-PCR positive and negative subjects (figure 2), with the exception of anosmia and dysgeusia, which were more frequent in RT-PCR positive subjects (42% vs. 10%, $p < 0.001$ and 38 vs. 16%, $p = 0.004$) and sore throat, which was significantly more common in RT-PCR negative subjects (36 vs. 10%, $p = 0.001$).

In these symptomatic subjects, the Ct values for the Orf 1b and N genes with OS showed a more than 3-point increase compared with NP; the magnitude of this difference indicates a more than 10-fold lower quantity of viral genetic material in OS samples [26].

The 262 close contacts with a laboratory-confirmed COVID-19 case were predominantly women 145/262 (55.3%). The mean age was 41 ± 15 years. The contact was identified in the household (n=41; 15.6%), in the family (n=56; 21.4%); in the close environment (n=40; 15.3%), at work (n=65; 24.8%), or was not known by the subject (n=60; 22.9%). Of these close contact subjects, 57 (21.7%) had a positive RT-PCR result with one or both of the sampling techniques. Differences in viral load between the NP and the OS could not be assessed in close contact subjects since viral RNA detection with NP relied on TMA and not on RT-PCR in this part of the study. The viral strains identified during the study were almost exclusively beta and gamma variants.

In the symptomatic subjects, the NP-OS concordance was 96.5% (95%CI: 93.5 – 99.5) and the OS sensitivity was 93.2% (95%CI: 89.1 – 97.3) with the Idylla platform. With the Synlab Barla platform, the NP-OS concordance was 89.0% (95%CI: 83.9 – 94.1) and the OS sensitivity was 76.3% (95%CI: 69.4 – 83.2) (table 1).

In the close contact subjects, the NP-OS concordance was 93.8% (95%CI: 90.9 – 96.7) and the OS sensitivity was 71.9% (95%CI: 66.5 – 77.3) with the Idylla platform. With the Synlab barla platform, the NP-OS concordance was 94.2% (95%CI: 91.4 – 97.0) and the OS sensitivity was 73.7% (95%CI: 68.4 – 79.0) (table 1).

DISCUSSION

In this study, RT-PCR SARS-CoV-2 testing with OS showed a high level of concordance with NP testing, 96.5% (95%CI: 89.6-94.8) and a high sensitivity, 93.2 (95%CI: 89.1-97.3) for symptomatic subjects and a slightly lower level of concordance, 93.8% (95%CI: 90.9-96.7) and a slightly lower sensitivity, 71.9% (95%CI: 66.5-77.3) in asymptomatic close contacts of a laboratory-confirmed COVID-19 case. These results obtained in ambulatory subjects presenting to a public community-based screening center, strongly suggest that OS, a straightforward, low-cost and high-throughput sampling material can be used for frequent RT-PCR testing of COVID-19 patients and mass screening of populations. To our knowledge, this is the first trial of oral sponge sampling for SARS-CoV2.

During the early phase of the COVID-19 pandemic, many molecular tests, immunoassays and sampling methods were rapidly developed and validated using archived biological samples of known virological status, albeit many still await clinical validation [27]. To evaluate the clinical performance of OS, compared to NP, the present study was carried out according to the standards recommended by the French “Haute Autorité de Santé” [28]; i.e., a prospective comparative clinical study relating to a series of individuals of unknown COVID-19 status, recruited consecutively; a salivary test including at least two molecular targets; and by the US and European CDC directives; i.e. definition of a positive individual when comparing two diagnostic SARS-Cov-2 testing methods [25,37,38].

In published studies that compared salivary and NP testing, symptomatic subjects were the dominant inclusion profile (85%), especially in the majority of short series of hospitalized patients [14]. Only 6 cohorts of equal or larger size than ours reported comparative test results obtained in a community-based settings [29–34].

Given the particularly high incidence of COVID-19 in the Nice metropolitan area throughout our study period (incidence between 330 and 720/100,000), the high rate of positivity (21% in asymptomatic contacts and 41% in symptomatic subjects) observed in the present study, was higher than the rates observed in these 6 published cohorts (1.5% to 10.7%) [28-33].

Despite this difference, our NP-OS concordance and the sensitivity of RT-PCR on NP and on OS are in agreement with the results reported for these cohorts [25].

RT-PCR with OS performed at the Synlab Barla platform showed a significantly lower sensitivity than Idylla platform in symptomatic subjects. This discrepancy may be due to 2 factors: the Idylla platform uses 3 Orf1 and 2 N targets while the Synlab Barla platform uses only one Orf1 and one N target; preservation at -80°C for several weeks may have altered the nucleic acids. The discrepancy disappeared in the second part of the study in which the external validation of OS testing at the Synlab Barla platform was done on fresh samples in real time.

The significant difference in Cts, an approximate proxy for viral load, observed between paired samples from the nasopharyngeal and oral cavity in symptomatic subjects can be due to several parameters, notably endogenous to the laboratory [(i.e. total volume of sample collection buffer/medium, sample preparation method (heat, lysis methods)] and laboratory reagent volumes used in each step of the RT-PCR process. However, such a large difference is hardly explained by the experimental conditions and has also been observed by others [2, 35–37]. This is why the diagnostic yield of NP is considered to be higher than that of throat swabs [36, 37]. This difference most likely also has a pathophysiological basis, related to the fact that the mouth squamous cells act as a viral reservoir to a lesser degree than the nasopharynx ciliated cells [18, 19]. As a consequence, the interpretative cut-offs for NP Ct [21] cannot apply to saliva samples.

The OS sampling method is well standardized. It does not need sialagogic drugs, nor clearing the throat or spitting effort, nor particular constraints such as early morning saliva sampling before tooth brushing and breakfast, avoiding eating, drinking, gum chewing, smoking, or vaping. [38, 39]. It does not need dedicated trained nursing staff and its acceptability makes it possible to consider repeating the test even in institutionalized elderly people or in children.

The question as to whether the 72% sensitivity of OS we showed in a symptomatic contact subjects is good enough to identify SARS-CoV-2 infected people in the setting of mass screening can be debated. As a comparison, in non-symptomatic subjects, the lateral-flow tests tests that are currently widely used because they can be performed with minimal supervision have a 35% sensitivity; 95%CI (27%-44%) for anterior nasal sampling and 51% sensitivity; 95%CI (22%-80%) for mid-turbinate sampling [8]. In a landmark position paper [6], Mina *et al*. distinguished between low frequency tests with high analytical sensitivity and high frequency tests with low analytical sensitivity. According to this classification, RT-PCR on NP specimens belongs to the former category for symptomatic and non-symptomatic subjects. Importantly, RT-PCR on OS specimens would be considered as a high frequency test with high analytical sensitivity in symptomatic subjects and as a high frequency test with intermediate analytical sensitivity in non-symptomatic subjects.

We now recently industrialized the OS process in the form of an "all-in-one" kit simplifying extraction of the biological liquid (figure 4).

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REFERENCES

1. Hanson KE, Caliendo AM, Arias CA, Englund JA, Lee MJ, Patel R, Alayli AE, Kalot MA, Falck-Ytter Y, Morgan RL, Murad MH, Sultan S, Bhimraj A, Mustafa RA. Infectious Diseases Society of America Guidelines on the Diagnosis of COVID-19. : 72.

2. Zou L, Ruan F, Huang M, Liang L, Huang H, Hong Z, Yu J, Kang M, Song Y, Xia J, Guo Q, Song T, He J, Yen H-L, Peiris M, Wu J. SARS-CoV-2 Viral Load in Upper Respiratory Specimens of Infected Patients. *New England Journal of Medicine* Massachusetts Medical Society; 2020; 382: 1177–1179.

3. Sullivan PS, Sailey C, Guest JL, Guarner J, Kelley C, Siegler AJ, Valentine-Graves M, Gravens L, Del Rio C, Sanchez TH. Detection of SARS-CoV-2 RNA and Antibodies in Diverse Samples: Protocol to Validate the Sufficiency of Provider-Observed, Home-Collected Blood, Saliva, and Oropharyngeal Samples. *JMIR Public Health Surveill* 2020; 6: e19054.

4. Kucirka LM, Lauer SA, Laeyendecker O, Boon D, Lessler J. Variation in False-Negative Rate of Reverse Transcriptase Polymerase Chain Reaction–Based SARS-CoV-2 Tests by Time Since Exposure. *Ann Intern Med* American College of Physicians; 2020; 173: 262–267.

5. Arevalo-Rodriguez I, Buitrago-Garcia D, Simancas-Racines D, Zambrano-Achig P, Campo RD, Ciapponi A, Sued O, Martinez-García L, Rutjes AW, Low N, Bossuyt PM, Perez-Molina JA, Zamora J. False-negative results of initial RT-PCR assays for COVID-19: A systematic review. *PLOS ONE* Public Library of Science; 2020; 15: e0242958.

6. Mina MJ, Parker R, Larremore DB. Rethinking Covid-19 Test Sensitivity — A Strategy for Containment. *New England Journal of Medicine* Massachusetts Medical Society; 2020; 383: e120.

7. Goldfarb DM, Tilley P, Al-Rawahi GN, Srigley JA, Ford G, Pedersen H, Pabbi A, Hannam-Clark S, Charles M, Dittrick M, Gadkar VJ, Pernica JM, Hoang LMN. Self-Collected Saline Gargle Samples as an Alternative to Health Care Worker-Collected Nasopharyngeal Swabs for COVID-19 Diagnosis in Outpatients. *J Clin Microbiol* 2021; 59.

8. Haute Autorité de Santé. Évaluation de l'intérêt des tests antigéniques rapides (TDR/TROD) sur prélèvement nasal pour la détection du virus SARS-CoV-2 (Méta-analyse). 2021. https://www.has-sante.fr/jcms/p_3269794/fr/evaluation-de-l-interet-des-tests-antigeniques-rapides-tdr/trod-sur-prelevement-nasal-pour-la-detection-du-virus-sars-cov-2-meta-analyse. .

9. World Health Organization. (2020). Antigen-detection in the diagnosis of SARS-CoV-2 infection using rapid immunoassays: interim guidance, 11 September 2020. World Health Organization. https://apps.who.int/iris/handle/10665/334253. License: CC BY-NC-SA 3.0 IGO [Internet]. [cited 2021 Jan 22].Available from: https://www.who.int/publications-detail-redirect/antigen-detection-in-the-diagnosis-of-sars-cov-2infection-using-rapid-immunoassays.

10. Butler-Laporte G, Lawandi A, Schiller I, Yao M, Dendukuri N, McDonald EG, Lee TC. Comparison of Saliva and Nasopharyngeal Swab Nucleic Acid Amplification Testing for Detection of SARS-CoV-2: A Systematic Review and Meta-analysis. *JAMA Intern Med* 2021; 181: 353–360.

11. Liu L, Wei Q, Alvarez X, Wang H, Du Y, Zhu H, Jiang H, Zhou J, Lam P, Zhang L, Lackner A, Qin C, Chen Z. Epithelial cells lining salivary gland ducts are early target cells of severe acute

respiratory syndrome coronavirus infection in the upper respiratory tracts of rhesus macaques. *J Virol* 2011; 85: 4025–4030.

12. Lu B, Huang Y, Huang L, Li B, Zheng Z, Chen Z, Chen J, Hu Q, Wang H. Effect of mucosal and systemic immunization with virus-like particles of severe acute respiratory syndrome coronavirus in mice. *Immunology* 2010; 130: 254–261.

13. Xu H, Zhong L, Deng J, Peng J, Dan H, Zeng X, Li T, Chen Q. High expression of ACE2 receptor of 2019-nCoV on the epithelial cells of oral mucosa. *International Journal of Oral Science* Nature Publishing Group; 2020; 12: 1–5.

14. Haute Autorité de Santé. Méta-analyse de l'intérêt diagnostique des tests RT-PCR salivaires de détection du SARS-CoV-2. https://www.has-sante.fr/jcms/p_3237008/fr/meta-analyse-de-l-interet-diagnostique-des-tests-rt-pcr-salivaires-de-detection-du-sars-cov-2 (accessed Apr. 10, 2021)]. .

15. Avis n° 2021.0007/AC/SEAP du 10 février 2021 du collège de la HAS relatif aux modifications des conditions d'inscription sur la LAP mentionnée à l'article L. 162-1-7 du CSS, à la détection du génome du virus SARS-CoV-2 par technique de transcription inverse suivie d'une amplification (RT-PCR) sur prélèvement salivaire [Internet]. Haute Autorité de Santé [cited 2021 Apr 7].Available from: https://www.has-sante.fr/jcms/p_3236975/fr/avis-n-2021-0007/ac/seap-du-10-fevrier-2021-du-college-de-la-has-relatif-aux-modifications-des-conditions-d-inscription-sur-la-lap-mentionnee-a-l-article-l-162-1-7-du-css-a-la-detection-du-genome-du-virus-sars-cov-2-par-technique-de-transcription-inverse-suivie-d-une-amplification-rt-pcr-sur-prelevement-salivaire.

16. Deprez M, Zaragosi L-E, Truchi M, Becavin C, Ruiz García S, Arguel M-J, Plaisant M, Magnone V, Lebrigand K, Abelanet S, Brau F, Paquet A, Pe'er D, Marquette C-H, Leroy S, Barbry P. A Single-Cell Atlas of the Human Healthy Airways. *Am J Respir Crit Care Med* 2020; 202: 1636–1645.

17. Tanga V, Leroy S, Fayada J, Hamila M, Allegra M, Messaoudi Z, Bonnetaud C, Lespinet V, Bordone O, Washetine K, Griffonnet J, Maniel C, Philibert L, Selva E, Benzaquen J, Ilie M, Long E, Lassalle S, Lantéri E, Marquette C-H, Hofman V, Hofman P. Establishment of a Collection of Blood-Derived Products from COVID-19 Patients for Translational Research: Experience of the LPCE Biobank (Nice, France). *Biopreservation and Biobanking* Mary Ann Liebert, Inc., publishers; 2020; 18: 517–524.

18. Sungnak W, Huang N, Bécavin C, Berg M, Queen R, Litvinukova M, Talavera-López C, Maatz H, Reichart D, Sampaziotis F, Worlock KB, Yoshida M, Barnes JL. SARS-CoV-2 entry factors are highly expressed in nasal epithelial cells together with innate immune genes. *Nature Medicine* Nature Publishing Group; 2020; 26: 681–687.

19. Ziegler CGK, Allon SJ, Nyquist SK, Mbano IM, Miao VN, Tzouanas CN, Cao Y, Yousif AS, Bals J, Hauser BM, Feldman J, Muus C, Wadsworth MH, Kazer SW, Hughes TK, Doran B, Gatter GJ, Vukovic M, Taliaferro F, Mead BE, Guo Z, Wang JP, Gras D, Plaisant M, Ansari M, Angelidis I, Adler H, Sucre JMS, Taylor CJ, Lin B, et al. SARS-CoV-2 Receptor ACE2 Is an Interferon-Stimulated Gene in Human Airway Epithelial Cells and Is Detected in Specific Cell Subsets across Tissues. *Cell* 2020; 181: 1016-1035.e19.

20. « Contact tracing » : arrêter les chaînes de transmission [Internet]. [cited 2021 Apr 13].Available from: https://www.ameli.fr/assure/covid-19/tester-alerter-proteger-comprendre-la-strategie-pour-stopper-lepidemie/contact-tracing-arreter-les-chaines-de-transmission.

21. Avis du 25 septembre 2020 de la Société Française de Microbiologie (SFM) relatif à l'interprétation de la valeur de Ct (estimation de la charge virale) obtenue en cas de RT-PCR SARS-CoV-2 positive sur les prélèvements cliniques réalisés à des fins diagnostiques ou de dépistage. Version 3 [Internet]. 2020 [cited 2021 Jan 22].Available from: https://www.sfm-microbiologie.org/wp-content/uploads/2020/10/Avis-SFM-valeur-Ct-excre%CC%81tion-virale-_-Version-Finale-07102020-V3.pdf.

22. Hofman P, Boutros J, Benchetrit D, Benzaquen J, Leroy S, Tanga V, Bordone O, Allégra M, Lespinet V, Fayada J, Maniel C, Griffonnet J, Selva E, Troncone G, Portella G, Lavrut T, Chemla R, Carles M, Ilié M, Marquette C. A rapid near-patient RT-PCR test for suspected COVID-19: a study of the diagnostic accuracy. *Annals of Translational Medicine* AME Publishing Company; 2021; 9: 921–921.

23. CDC. Healthcare Workers [Internet]. Centers for Disease Control and Prevention 2020 [cited 2021 Apr 12].Available from: https://www.cdc.gov/coronavirus/2019-ncov/hcp/testing-overview.html.

24. Diagnostic testing and screening for SARS-CoV-2 [Internet]. European Centre for Disease Prevention and Control [cited 2021 Apr 12].Available from: https://www.ecdc.europa.eu/en/covid-19/latest-evidence/diagnostic-testing.

25. Ibrahimi N, Delaunay-Moisan A, Hill C, Le Teuff G, Rupprecht J-F, Thuret J-Y, Chaltiel D, Potier M-C. Screening for SARS-CoV-2 by RT-PCR: Saliva or nasopharyngeal swab? Rapid review and meta-analysis. Darlix J-LE, editor. *PLoS ONE* 2021; 16: e0253007.

26. Understanding cycle threshold (Ct) in SARS-CoV-2 RT-PCR. [Internet] Public Health England. Available from: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/926410/Understanding_Cycle_Threshold__Ct__in_SARS-CoV-2_RT-PCR_.pdf. : 12.

27. Vandenberg O, Martiny D, Rochas O, van Belkum A, Kozlakidis Z. Considerations for diagnostic COVID-19 tests. *Nat Rev Microbiol* 2020; : 1–13.

28. Haute Autorité de Santé. Revue rapide sur les tests de détection antigénique du virus SARS-CoV-2. 8 octobre 2020 (https://www.has-sante.fr/upload/docs/application/pdf/2020-10/synthese_tests_antigeniques_vd.pdf). .

29. Caulley L, Corsten M, Eapen L, Whelan J, Angel JB, Antonation K, Bastien N, Poliquin G, Johnson-Obaseki S. Salivary Detection of COVID-19. *Ann Intern Med* American College of Physicians; 2020; 174: 131–133.

30. Barat B, Das S, Giorgi VD, Henderson DK, Kopka S, Lau AF, Miller T, Moriarty T, Palmore TN, Sawney S, Spalding C, Tanjutco P, Wortmann G, Zelazny AM, Frank KM. Pooled Saliva Specimens for SARS-CoV-2 Testing. *Journal of Clinical Microbiology* [Internet] American Society for Microbiology Journals; 2021 [cited 2021 Apr 12]; 59Available from: https://jcm.asm.org/content/59/3/e02486-20.

31. Kandel C, Zheng J, McCready J, Serbanescu MA, Racher H, Desaulnier M, Powis JE, Vojdani K, Finlay L, Sheldrake E, Vermeiren C, Katz K, McGeer A, Kozak R, Goneau LW. Detection of SARS-CoV-2 from Saliva as Compared to Nasopharyngeal Swabs in Outpatients. *Viruses* Multidisciplinary Digital Publishing Institute; 2020; 12: 1314.

32. Senok A, Alsuwaidi H, Atrah Y, Ayedi OA, Zahid JA, Han A, Marzooqi AA, Heialy SA, Altrabulsi B, AbdelWareth L, Idaghdour Y, Ali R, Loney T, Alsheikh-Ali A. Saliva as an Alternative Specimen for Molecular COVID-19 Testing in Community Settings and Population-Based Screening . *IDR* Dove Press; 2020; 13: 3393–3399.

33. Vogels CBF, Watkins AE, Harden CA, Brackney DE, Shafer J, Wang J, Caraballo C, Kalinich CC, Ott IM, Fauver JR, Kudo E, Lu P, Venkataraman A, Tokuyama M, Moore AJ, Muenker MC, Casanovas-Massana A, Fournier J, Bermejo S, Campbell M, Datta R, Nelson A, Anastasio K, Askenase MH, Batsu M, Bickerton S, Brower K, Bucklin ML, Cahill S, Cao Y, et al. SalivaDirect: A simplified and flexible platform to enhance SARS-CoV-2 testing capacity. *Med* 2021; 2: 263-280.e6.

34. Yokota I, Shane PY, Okada K, Unoki Y, Yang Y, Inao T, Sakamaki K, Iwasaki S, Hayasaka K, Sugita J, Nishida M, Fujisawa S, Teshima T. Mass screening of asymptomatic persons for SARS-CoV-2 using saliva. *Clinical Infectious Diseases* [Internet] 2020 [cited 2021 Apr 12]; Available from: https://doi.org/10.1093/cid/ciaa1388.

35. Vlek ALM, Wesselius TS, Achterberg R, Thijsen SFT. Combined throat/nasal swab sampling for SARS-CoV-2 is equivalent to nasopharyngeal sampling. *Eur J Clin Microbiol Infect Dis* 2021; 40: 193–195.

36. Wang X, Tan L, Wang X, Liu W, Lu Y, Cheng L, Sun Z. Comparison of nasopharyngeal and oropharyngeal swabs for SARS-CoV-2 detection in 353 patients received tests with both specimens simultaneously. *International Journal of Infectious Diseases* 2020; 94: 107–109.

37. Patel MR, Carroll D, Ussery E, Whitham H, Elkins CA, Noble-Wang J, Rasheed JK, Lu X, Lindstrom S, Bowen V, Waller J, Armstrong G, Gerber S, Brooks JT. Performance of Oropharyngeal Swab Testing Compared With Nasopharyngeal Swab Testing for Diagnosis of Coronavirus Disease 2019—United States, January 2020–February 2020. *Clin Infect Dis* [Internet] 2020 [cited 2021 Feb 8]; Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7337670/.

38. L'Helgouach N, Champigneux P, Schneider FS, Molina L, Espeut J, Alali M, Baptiste J, Cardeur L, Dubuc B, Foulongne V, Galtier F, Makinson A, Marin G, Picot M-C, Prieux-Lejeune A, Quenot M, Robles FC, Salvetat N, Vetter D, Reynes J, Molina F. EasyCOV : LAMP based rapid detection of SARS-CoV-2 in saliva. *medRxiv* Cold Spring Harbor Laboratory Press; 2020; : 2020.05.30.20117291.

39. Lai CKC, Chen Z, Lui G, Ling L, Li T, Wong MCS, Ng RWY, Tso EYK, Ho T, Fung KSC, Ng ST, Wong BKC, Boon SS, Hui DSC, Chan PKS. Prospective Study Comparing Deep Throat Saliva With Other Respiratory Tract Specimens in the Diagnosis of Novel Coronavirus Disease 2019. *The Journal of Infectious Diseases* 2020; 222: 1612–1619.

TABLES AND FIGURES

Table 1a: Clinical performance of oral sponges (OS) compared to naso-pharyngeal swabs (NP) for SARS-CoV-2 screening. LPCE, Idylla™ platform.

	NP + OS +	NP + OS -	NP - OS +	NP - OS -	Total	Concordance	Sensitivity of NP	Sensitivity of OS
	a	b	c	d	n = a+b+c+d	(a+d)/n [95% CI]	(a+b)/(a+b+c) [95% CI]	(a+c)/(a+b+c) [95% CI]
Symptomatic subjects	54	4	1	88	147	96.5% [93.5 – 99.5]	98.3% [96.2 - 100]	93.2%* [89.1 - 97.3]
Close contact subjects	41	16	0	205	262	93.8% [90.9 – 96.7]	100%	71.9% [66.5 – 77.3]

Table 1b: Clinical performance of oral sponges (OS) compared to naso-pharyngeal swabs (NP) for SARS- CoV-2 screening. External validation at the Synlab Barla laboratory platform

	NP + OS +	NP + OS -	NP - OS +	NP - OS -	Total	Concordance	Sensitivity of NP	Sensitivity of OS
	a	b	c	d	n = a+b+c+d	(a+d)/n [95% CI]	(a+b)/(a+b+c) [95% CI]	(a+c)/(a+b+c) [95% CI]
Symptomatic subjects	43	14	2	87	146	89.0% [83.9 – 94.1]	96.6% [93.7 – 99.5]	76.3%* [69.4 – 83.2]
Close contact subjects	42	15	0	205	262	94.2% [91.4 – 97.0]	100%	73.7% [68.4 – 79.0]

* the 16,9% difference in OS sensitivity between the Idylla and the Synlab Barla laboratory platforms was statistically significant.

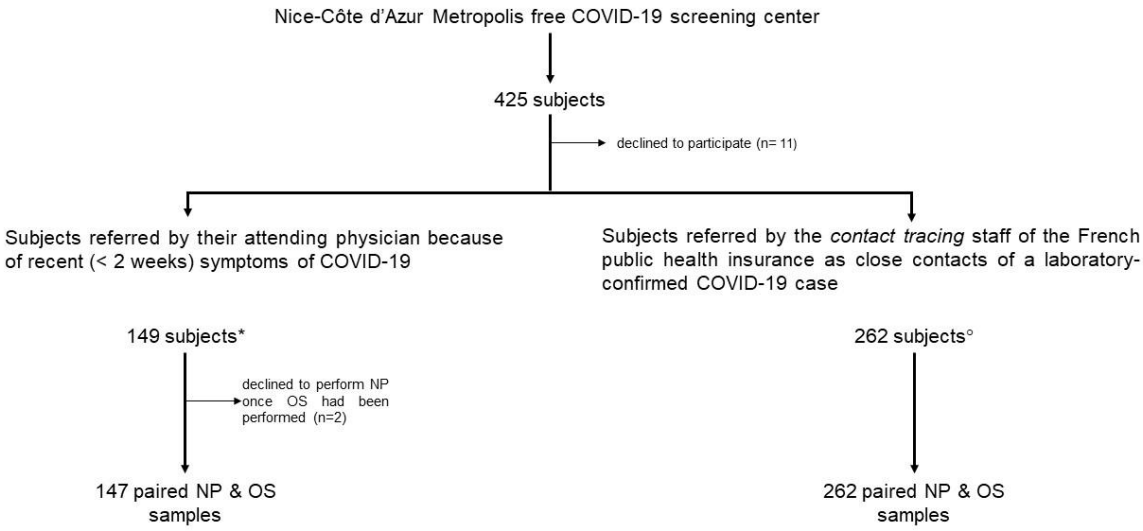

Figure 1: Trial profile

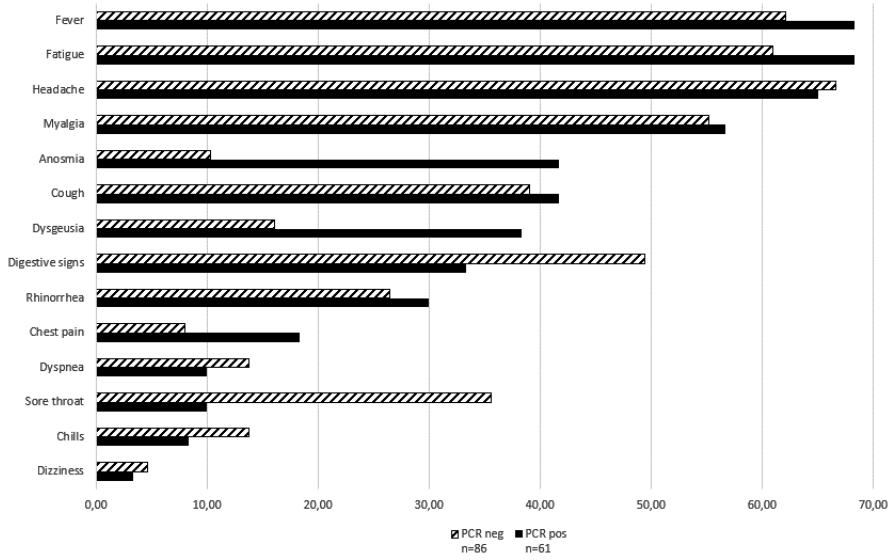

Figure 2: Self-reported symptoms (%)

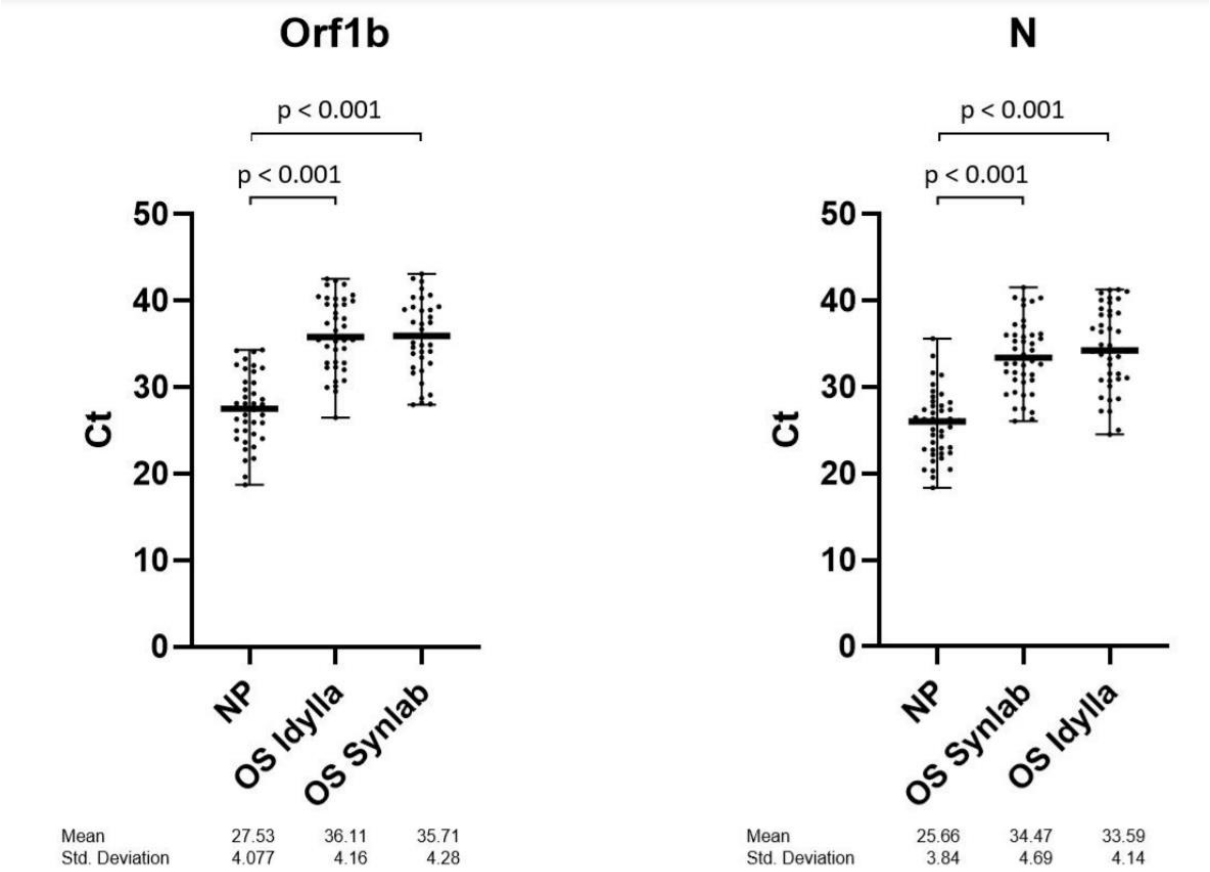

Figure 3: Viral load of NP and OS as indirectly assessed by the Ct for the Orf1b and N genes

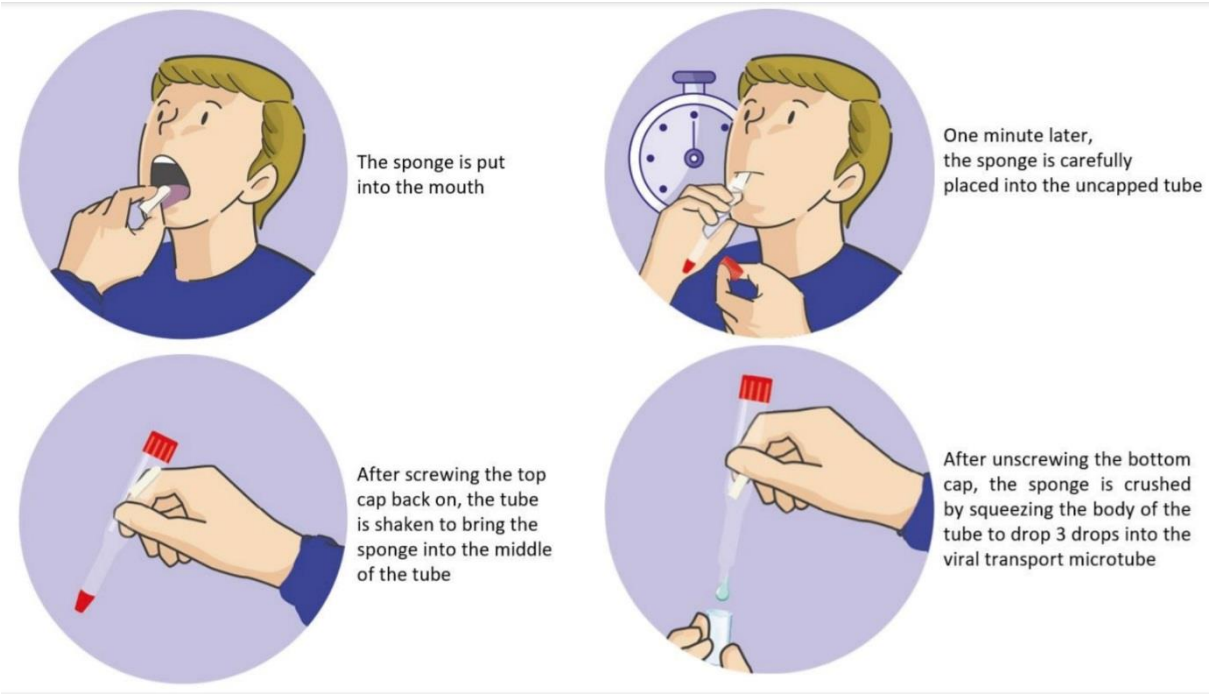

Figure 4: All-in-on saliva sampling technique with a PVA sponge and a flexible, double-capped plastic tube.

PROCEDURES

Detection of SARS-CoV-2 RNA with naso-pharyngeal swab specimens

During the first study period (September 21, 2020 to January 6, 2021; symptomatic patients), naso-pharyngeal (NP) swab specimens were analyzed by RT-PCR using the ORF1ab and N genes Da An Gene DA0992-Detection Kit for 2019-nCoV (Da An Gene Co., Ltd. Sun Yat-sen University, Guangzhou, Guangdong, China). NP swabs were eluted into a vial containing 400 µl of viral RNA extraction buffer (RNA/DNA purification kit, Da An Gene, ref DA0940); and 5 µl was then processed on an AGS 4800 Thermocycler (Hangzhou, Zhejiang, China). According to the guidelines of the French Society for Microbiology [reference 30 Avis du 25 septembre 2020 de la Société Française de Microbiologie (SFM) relatif à l'interprétation de la valeur de Ct] the result was considered as positive when the Ct (cycle threshold) value for the N and/or Orf1b genes was equal or less than 36.

During the second study period (February 6 to March 6, 2021; close contacts) NP samples were analyses with the Aptima® SARS-CoV-2 assay (Hologic, Inc. 10210 Genetic Center Drive San Diego, CA 92121 USA), which combines Transcription Mediated Amplification (TMA), and Dual Kinetic Assay (DKA) that amplifies and detects two conserved regions of the ORF1ab gene. NP swabs were eluted into a vial containing 710 µl of medium (Hologic Specimen Lysis Tubes, ref PDD-06554) before being tested on the Panther system. Assay results were determined with a cut-off based on the total Relative Light Units (RLU). According to the guidelines of the French Society for Microbiology the results were considered as positive when the RLU was above 850.

Detection of SARS-CoV-2 RNA with oral sponge (OS) specimens at the Synlab Barla laboratory

25 µl of the OS eluates transferred fresh from the Nice COVID-19 biobank were put into a vial containing 400 µl of viral RNA extraction buffer (RNA/DNA purification kit, Da An Gene, ref

DA0940); and 5 μl was then processed on an AGS 4800 Thermocycler (Hangzhou, Zhejiang, China).