Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Virus Research 305 (2021) 198575

Contents lists available at ScienceDirect

Virus Research

journal homepage: www.elsevier.com/locate/virusres

A simple pooling salivary test for SARS-CoV-2 diagnosis: A Columbus' egg?

Annamaria Pratelli [a,*], Maria Stella Lucente [a], Viviana Mari [a], Marco Cordisco [a], Alessio Sposato [a], Paolo Capozza [a], Gianvito Lanave [a], Vito Martella [a], Alessio Buonavoglia [b]

[a] Department of Veterinary Medicine, University Aldo Moro of Bari, Valenzano (Ba) 70010, Italy
[b] Dental Surgeon, Bari 70010, Italy

ARTICLE INFO

Keywords:
SARS-CoV-2
saliva
qPCR

ABSTRACT

Saliva is an appropriate specimen for Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) diagnosis. The possibility of pooling samples of saliva, using non-invasive bibula strips for sampling, was explored employing *Bovine coronavirus* (BCoV) spiked saliva. In laboratory, up to 30 saliva-soaked strips were pooled in a single tube with 2 mL of medium. After quick adsorption with the medium and vortexing, the liquid was collected and tested with a quantitative molecular assay to quantify viral RNA genome copies. On testing of single and pooled strips, the difference between the median threshold cycles (Ct) value of test performed on the single positive saliva sample and the median Ct value obtained on the pool of 30 strips, was 3.21 cycles. Saliva pooling with bibula strips could allow monitoring of COVID-19 on a large scale, reducing costs for the health bodies in terms of medical material and skilled personnel. Finally, saliva sampling is noninvasive and less traumatic than nasopharyngeal swabs and can be self-collected.

1. Introduction

The COVID-19 pandemic caused by *severe acute respiratory syndrome coronavirus 2* (SARS-CoV-2) started spreading in the first months of 2020 and was characterized by multiple waves of infections and deaths globally, with critical issues becoming apparent due to inadequate management strategies (Falzone et al., 2021). Despite the optimism deriving from the approval of vaccines by the health authorities, economic disparities and/or political decisions have generated major differences in terms of vaccines coverage and of access to SARS-CoV-2 testing, affecting the control of COVID-19 among the various countries (Rajendrakumar et al., 2021). Since the beginning of the pandemic, health authorities were urged to achieve the most important goal of developing effective diagnostic tests for rapid and accurate identification of SARS-CoV-2 in the infected patients, thus limiting the risk of contagion (Ji et al., 2020). The gold standard test for COVID-19 diagnosis is the collection of deep nasopharyngeal swabs (NPS) followed by molecular testing, such as real-time polymerase chain reaction (qPCR), although samples collection of NPS is invasive chiefly for children. This situation has been addressed using innovative approaches (Callahan et al., 2020), for easier and less invasive sample collection, and for speeding up the screening of group of persons and of large populations (Czumbel et al., 2020). Recent data support the idea that saliva testing is a valid and practicable test with a number of advantages over NPS when multiple samples need to be tested periodically: i) saliva samples can be self-collected with minimal guidance of healthcare personnel; ii) the procedure is less invasive; iii) it is a more feasible option for repeated or systematic testing.

(Azzi et al., 2020). Recently, the FDA in the United States approved the first diagnostic test with the option for saliva sampling for detection of SARS-CoV-2 (https://www.fda.gov/media/136877/download; Herrera et al., 2021). Pooling saliva samples for detection of SARS-CoV-2 proved to be an inexpensive diagnostic procedure suitable for testing a greater number of individuals even at lower prevalence levels. A pooling strategy was developed by SalivaClear Mirimus Clinical to detect/monitor infection in symptomatic and asymptomatic populations (Pasomsub et al., 2021; Watkins et al., 2021). When schools will reopen and commercial and corporate business will resume completely, screening for SARS-CoV-2 must be implemented to increase biosecurity. In this context, for example, it is essential to potentiate the diagnostic activities, considering that the frequent execution of NPS can generate stress in children and it is a time-consuming and expensive strategy. This note describes a procedure developed to perform a biomolecular test on a pool of salivary samples. Sample pooling could be a solution for many laboratories to comply with the increased demand of SARS-CoV-2 testing. For this purpose, we developed a procedure using *Bovine*

* Corresponding author at: Strada provinciale per Casamassima Km 3, 70010 Valenzano (Ba), Italy.
E-mail address: annamaria.pratelli@uniba.it (A. Pratelli).

https://doi.org/10.1016/j.virusres.2021.198575
Received 17 June 2021; Received in revised form 30 August 2021; Accepted 13 September 2021
Available online 21 September 2021

coronavirus (BCoV) as surrogate virus and spiked bovine saliva. BCoV is a member of the same *Betacoronavirus* genus in the *Coronaviridae* family as SARS-CoV-2 but it safe for humans and of easier manipulation in laboratory. The pooling strategy could be easily applied for mass screening of SARS-CoV-2 in companies and schools.

2. Materials and methods

2.1. Virus and saliva collection

For experimental test, BCoV, strain 16/20, with a titer of $10^{5.5}$ Tissue Culture Infectious Doses $(TCID)_{50}$/50μL, was employed. BCoV strain 16/20 was isolated from the nasal swabs of a cow and was cultured in *Madin Darby Bovine Kidney* (MDBK) cells. Thirty milliliters of saliva were collected in a 50mL sterile conical tube from five bovines, immediately after suppression at the slaughterhouse. The saliva sample preliminarily tested negative for BCoV by RNA extraction and qPCR (Decaro et al., 2008).

2.2. Salivary samples pooling

In the sample pooling strategy, a set of samples was tested together in a single run. To generate the samples pools, the collected saliva was divided into two aliquots (A and B). The saliva aliquot A was spiked with $1000TCID_{50}$ of BCoV 16/20 strain and the qPCR was performed to quantify viral RNA. Then, bibula paper strips (6cm x 0.4cm; 28mg) (Carta Filtro Labor – Gruppo Cordenons SpA, Milano, Italy) were employed to simulate the collection of individual salivary samples. Two experiments were performed. Experiment #1 was aimed to mimic testing of a positive saliva sample. A bibula strip was dipped into the spiked saliva letting the strip soak until the wetting front reached 2cm (corresponding to about 30μL of adsorbed liquid). The strip was then placed in a 50mL sterile conical tube added with 2mL of *Dulbecco Minimal Essential Medium* (DMEM). The sample was thoroughly mixed until the strip was completely imbibed with the medium and then vortexed for 30s. An aliquot of 200μL was hence collected to carry out qPCR. In experiment #2 the strategy of pooling saliva samples was assessed. The number of pooled samples was established on the basis of the literature (Lohse et al., 2020) and considering the average class size (OECD report, 2014, "How many students are in each classroom?", in Education at a Glance 2014: Highlights, OECD Publishing, Paris. DOI: https://doi.org/10.1787/eag_highlights-2014–24-en). Twenty-nine strips soaked in the aliquot B of saliva (without virus) were placed in a 50mL sterile conical tube, together with a strip dipped into the virus-positive saliva (aliquot A). The pool, encompassing a total of 30 strips (1 virus-positive saliva and 29 virus-negative saliva samples) was added with 2mL of DMEM and thoroughly mixed until all the strips were completely imbibed with the medium and then vortexed for 30s. An aliquot of 200μL was hence collected for qPCR testing. The experiments #1 and #2 were repeated 10 times. The different categories of samples (high, medium and low viral RNA copies) were not tested in our experiments basically because we were not working with clinical samples, but they were based on comparative testing of a single positive sample with a pool of samples containing one positive sample and 29 negative samples.

Preliminary tests were also performed using a pool size of 20 strips (1 virus-positive saliva and 19 virus-negative saliva samples) and 25 strips (1 virus-positive saliva and 24 virus-negative saliva samples) with the same procedures. Because no relevant differences were observed among the three pools, the 30-samples pool was employed. It was not possible to exceed the number of 30 strips due to technical constraints (possibility of recovering the minimum amount of 200μL of medium for extraction, maintaining the standardized conditions of the experiment).

2.3. qPCR

From the 50mL tubes containing the strips, treated as described, 200μL of medium was collected and processed for RNA extraction using the commercial kit QIAamp® cador® Pathogen Mini Kit (Qiagen GmbH, Hilden, Germany). The qPCR, based on TaqMan chemistry, was carried out as described previously (Decaro et al., 2008), using the primers BCoV-F and BCoV-R and the probe BCoV-Pb, that binds selectively to the transmembrane-protein gene M (ORF5) of BCoV. The detection limit of the assay was 20 genome RNA copies (1 log higher than traditional gel-based RT-PCR) with satisfactory reproducibility and repeatability. Each sample was tested in duplicate and the mean value was calculated.

In order to verify the absence of RNA losses during the extraction step and the presence of RT-PCR inhibitors in the RNA templates, an internal control (IC), consisting of an RNA synthetic transcript containing the M gene of canine coronavirus (CCoV) type II (Decaro et al., 2005), was added to the lysis buffer at a concentration of 10^4 RNA copies mL^{-1} of buffer prior to nucleic acid extraction, as described previously (Decaro et al., 2008)

Table 1
qPCR quantitation (Ct values and numbers of viral copies per reaction) on the single strip and the 30-strip pool. The experiment was repeated 10 times. .

Experiment	Single strip		Pool of 30 strips	
	Ct value	Viral RNA copies/mL	Ct value	Viral RNA copies/mL
1	30.49	1.13×10^4	32.22	3.15×10^3
2	31.62	4.90×10^3	33.06	1.69×10^3
3	33.65	1.09×10^3	36.23	1.62×10^2
4	34.33	6.61×10^2	36.50	1.33×10^2
5	30.99	7.81×10^3	39.31	1.67×10^1
6	38.87	2.31×10^1	40.23	$8.45\times10^{\circ}$
7	29.59	2.20×10^4	30.65	1.00×10^4
8	30.66	9.97×10^3	35.52	2.74×10^2
9	30.63	1.02×10^4	35.80	2.23×10^2
10	29.89	1.76×10^4	33.31	1.41×10^3

2.4. Data analysis

Statistical analyses were performed using the software R version 4.0.2 (https://www.R-project.org/) setting a statistical significance of p-value < 0.05. Shapiro-Wilk normality test was used to evaluate the distribution of normal distribution of the threshold cycles (Ct) obtained in the two different experiments. Single sample *t*-test was used to evaluate means of Ct obtained in pool and in single positive spiked strip compared to a means of Ct of 40 which is considered the cut-off of negativity (Decaro et al., 2008).

3. Results

To develop the saliva pooling approaches that can meet bulk testing demands, a pool of salivary samples was prepared to test the effects of pool sizes of 30 for the detection of BCoV, and its sensitivity was compared with the test on single saliva sample. The salivary pool consists of 29 strips dipped with saliva collected from slaughtered cattle, plus an additional strip soaked with $1000TCID_{50}$ of spiked saliva (average Ct values of 26.32). The potential value of pooling saliva testing was monitored in the 30-strips pools, and then compared with the single positive spiked strip. The experiments were repeated 10 times. In qPCR, the numbers of viral copies per reaction and the Ct values were determined on the single positive strip and on the 30-samples pool (Table 1), with the genome copies varying from 2.31×10^1 to 2.20×10^4 and from $8.45\times10^{\circ}$ to 1.00×10^4, respectively. The difference between the median Ct value of test performed on the single positive saliva sample and the median Ct value obtained on the 30-strips pool was 3.21 cycles with a standard deviation of 2.298 and 95% CI of 1.565–4.855.

Preliminary data obtained testing smaller pools of 20 and 25 samples did not display relevant differences with respect to the 30-strips pool, showing average Ct values of 34.32, 33.74 and 35.28, respectively.

4. Discussion

In this study we evaluated a sample pooling strategy for mass screening of groups of individuals, using saliva as sampling material instead of NPSs. The rational for this study relied in the fact that sampling of saliva is considered as reliable as, but easier than sampling with NPS, chiefly for some categories of patients/individuals (Pasomsub et al., 2021; Watkins et al., 2021 Takeuchi et al., 2020). Also, pooling of samples is a strategy that decreases costs and times for laboratory analysis, and proved helpful to screen large numbers of asymptomatic people (Lohse et al., 2020). Finally, using strips of a costless and common matrix as bibula paper could be an advantage for laboratories in low-income countries and could help decreasing sample processing.

The potential advantage of pooling saliva samples was explored with a safe laboratory surrogate model to assess the application of this diagnostic approach to the epidemiological monitoring of SARS-CoV-2 infection in closed environments (offices, schools, kindergartens, airplanes, etc.). Our strategy represents the simplest form of sample pooling, known as the Dorfman pooling, where a set of samples get tested together in a single run (McMahan et al., 2012).

The goal of the saliva sample pooling strategy is the identification of SARS-CoV-2 infections optimizing the number of tested samples for repeated and systematic monitoring of the infection at group or community level, at the same time reducing the discomfort of NPS execution. Nowadays saliva is considered a suitable and appropriate specimen for SARS-CoV-2 detection and performs similarly to or even better than NPS (Savela et al., 2021). NPS collection followed by qPCR is largely considered the gold standard for SARS-CoV-2 diagnosis, despite recent studies have demonstrated variation in the accuracy of this test. However, saliva is considered more suitable and sensitive than NPS with no significant temporal variation in viral load. Also, a high concordance has been demonstrated between NPS and saliva (Herrera et al., 2021; Wong et al., 2020; Wyllie et al., 2020). Recent reports showed that SARS-CoV-2 can be still detected in saliva in a third of patients 20 days or longer after initial diagnosis (Hung et al., 2020). SARS-CoV-2 can be detected in more than 95% of saliva samples of COVID-19 patients, with the highest viral load during the first week after the onset of symptoms (To et al., 2020a, 2020b). The Italian Ministry of Health on 14 May 2021 approved the salivary test as a possible option for SARS-CoV-2 screening, since it is less invasive and it does not require skilled personnel for its execution. Noteworthy, highly sensitive assays based on saliva can detect viral RNA 1.5 to 4.5 days earlier than low-sensitive tests based on NPS, thus emphasizing that both the sampling site and test sensitivity must be considered to ensure early detection of SARS-CoV-2 infection (Savela et al., 2021).

In the present study we assessed the strategy of pooling saliva collected with bibula strips (See note for *Salivary sampling proposal*). In qPCR, the difference between the median Ct value of test performed on the single positive saliva sample and the median Ct value obtained on the pool of 30 strips was 3.21 cycles, thus confirming that saliva pooling strategy is at least as sensitive as testing individual samples. The means of Ct obtained from the pooled strips and from the single positive strip was significantly lower (p value=3.72×10^{12} and 2.68×10^{6}) than the cut-off (40 ct). Even in the presence of one sample with low viral load (high Ct value), the loss of sensibility of the pool of 30 saliva samples did not affect substantially the results.

The pool size (30 strips) was based on similar studies in the literature as this pool size does not affect substantially the molecular results (Lohse et al., 2020). Increasing the number of samples was not possible in our protocol, as this affected the execution of our simple procedure. Also, the average class size in OECD countries, usually does not exceed the number of 30 students (OECD report, 2014).

The benefits of pool sampling of saliva are several for SARS-CoV-2 systematic and repeated monitoring: i) saliva samples can be self-collected by individuals, even by children, without any stimulation and without rinsing the mouth before sample collection, simply placing the bibula strip inside the buccal cavity for a few seconds; ii) bibula strips ensures an adequate adorbption of saliva in a few seconds, preventing the use of invasive swabs and decreasing the sampling times, and guarantees processing of up to 30 salivary samples in few mL of medium; iii) no restriction on timing or food intake are required for saliva collection (Nagura-Ikeda et al., 2020). Recent studies have already evaluated the saliva pooling strategy, replacing the use of the NPS for sampling (Lohse et al., 2020; Pasomsub et al., 2021; Watkins et al., 2021).

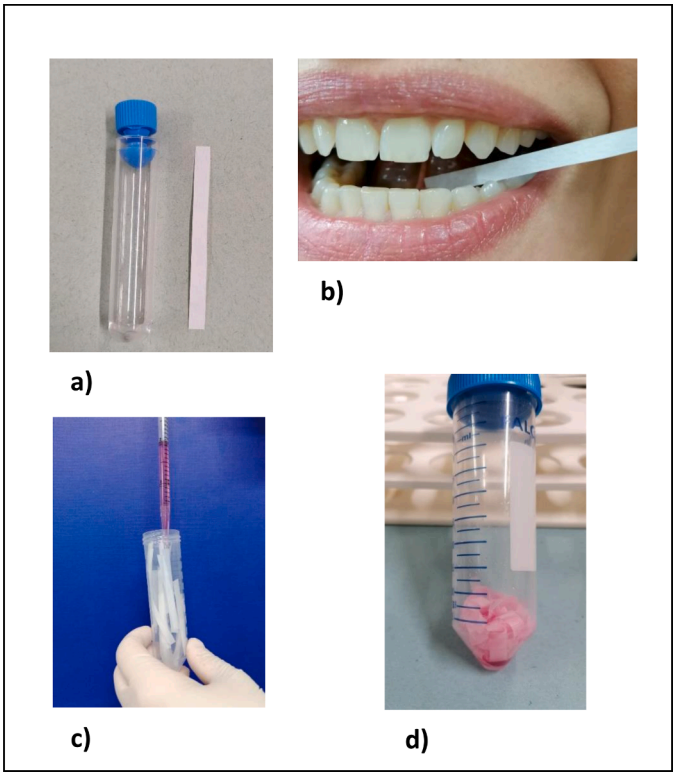

Fig. 1. Executive steps of salivary sampling and testing. a) Individual test tube and bibula strip. b) Saliva self-sampling. c) Pool of individual bibula strips collected in a single-50 mL sterile conical centrifuge tube where 2-mL of DMEM were added. d) Pool imbibed with the medium and vortexed, ready to RNA extraction.

Finally, the approach of testing pooled samples is a cost-effective strategy useful to decrease the costs of laboratory analyses, scaling and speeding up the monitoring and epidemiological activities of local or national health authorities or enabling alternative control measures. For instance, the University of South Carolina, in the attempt of assessing the risk of repeated SARS-CoV-2 infection in a population of young people, has disposed a mandatory weekly saliva tests during the spring 2021 semester (Rennert and McMahan, 2021). Similar monitoring plans could be easily carried out with our sampling strategy. Also, the reduced costs in terms of disposable material for sampling and the possibility of collecting samples without the need of trained personnel are particularly desirable for laboratories in low-income countries, where limited economical resources can hamper the activation of surveillance plans in the population (Takeuchi et al., 2020).

5. Conclusions

In conclusion, the following *Salivary sampling proposal* is advised:

A sterile single test tube and one bibula strip should be provided for each subject (Fig. 1a), who will be requested to self-collect saliva specimens by placing the bibula strip for few seconds (3–5s) inside the mouth (Fig. 1b). This should correspond to about 30μL of saliva

absorbed by the strip. Each individual strip should be inserted into the provided test tube, closed and transported to the laboratory at +4 °C. After delivery in the laboratory, all the bibula strips of the monitored population group (i.e. classroom, office, or day-care center) will be collected in a single 50mL sterile conical centrifuge tube added with 2mL of DMEM to form a pool of up to 30 strips (Fig. 1c). Each sample pool will be treated as described above (Fig. 1d). If the sample pool tests negative, no further testing is required. If the sample pool tests positive, re-screening of all individuals of the population group is required to individuate the infected persons.

CRediT authorship contribution statement

Annamaria Pratelli: Conceptualization, Investigation, Writing – original draft, Writing – review & editing, Project administration. **Maria Stella Lucente:** Methodology, Investigation. **Viviana Mari:** Methodology, Investigation. **Marco Cordisco:** Methodology. **Alessio Sposato:** Methodology. **Paolo Capozza:** Software, Data curation. **Gianvito Lanave:** Formal analysis, Data curation. **Vito Martella:** Validation, Supervision, Writing – review & editing. **Alessio Buonavoglia:** Conceptualization, Visualization, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The Authors are grateful to Carlo Armenise for his excellent assistance and sample collection at the slaughterhouse.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

Azzi, L., Carcano, G., Gianfagna, F., Grossi, P., Gasperina, D.D., Genoni, A., et al., 2020. Saliva is a reliable tool to detect SARS-CoV-2. J. Infect. 81e, 45–50. https://doi.org/10.1016/j.jinf.2020.04.005.

Callahan, C.J., Lee, R., Zulauf, K.E., Tamburello, L., Smith, K.P., Previtera, J., et al., 2020. Open development and clinical validation of multiple 3D-printed sample-collection swabs: rapid resolution of a critical COVID-19 testing bottleneck. J. Clin. Microbiol. 58, e00876–e00920. https://doi.org/10.1128/JCM.00876-20.

Czumbel, L.M., Kiss, S., Farkas, N., Mandel, I., Hegyi, A., Nagy, Á., et al., 2020. Saliva as a candidate for COVID-19 diagnostic testing: a Meta-Analysis. Front. Med. 7, 465. https://doi.org/10.3389/fmed.2020.00465.

Decaro, N., Martella, V., Ricci, D., Elia, G., Desario, C., Campolo, M., et al., 2005. Genotype-specific fluorogenic RT-PCR assays for the detection and quantification of canine coronavirus type I and type II RNA in faecal samples of dogs. J. Virol. Methods 130, 72–78. https://doi.org/10.1016/j.jviromet.2005.06.005.

Decaro, N., Elia, G., Campolo, M., Desario, C., Mari, V., Radogna, A., et al., 2008. Detection of bovine coronavirus using a TaqMan-based real-time RT-PCR assay. J. Virol. Methods 151, 167–171. https://doi.org/10.1016/j.jviromet.2008.05.016.

Falzone, L., Gattuso, G., Tsatsakis, A., Spandidos, D.A., Libra, M., 2021. Current and innovative methods for the diagnosis of COVID19 infection. Int. J. Mol. Med. 47, 100. https://doi.org/10.3892/ijmm.2021.4933.

Herrera, L.A., Hidalgo-Miranda, A., Reynoso-Noverón, N., Meneses-Garcí, A.A., Mendoza-Vargas, A., Reyes-Grajeda, J.P., et al., 2021. Saliva is a reliable and accessible source for the detection of SARS-CoV-2. Int. J. Infect. Dis. 105, 83–90. https://doi.org/10.1016/j.ijid.2021.02.009.

Hung, K.F., Sun, Y.C., Chen, B.H., Lo, J.F., Cheng, C.M., Chen, C.Y., et al., 2020. New COVID-19 saliva-based test: how good is it compared with the current nasopharyngeal or throat swab test? J. Clin. Med. Assoc. 83, 891–894. https://doi.org/10.1097/JCMA.0000000000000396.

Ji, T., Liu, Z., Wang, G., Guo, X., Akbar Khan, S., Lai, C., et al., 2020. Detection of COVID-19: a review of the current literature and future perspectives. Biosens. Bioelectron. 166, 112455 https://doi.org/10.1016/j.bios.2020.112455.

Lohse, S., Pfuhl, T., Berkó-Göttel, B., Rissland, J., Geißler, T., Gärtner, B., et al., 2020. Pooling of samples for testing for SARS-CoV-2 in asymptomatic people. Lancet Infect. Dis. 20, 1231–1232. https://doi.org/10.1016/S1473-3099(20)30362-5.

McMahan, C.S., Tebbs, J.M., Bilder, C.R., 2012. Informative Dorfman screening. Biometrics 68, 287–296. https://doi.org/10.1111/j.1541-0420.2011.01644.x.

Nagura-Ikeda, M., Imai, K., Tabata, S., Miyoshi, K., Murahara, N., Mizuno, T., et al., 2020. Clinical Evaluation of Self-Collected Saliva by Quantitative Reverse Transcription-PCR (RT-qPCR), Direct RT-qPCR, Reverse Transcription-Loop-Mediated Isothermal Amplification, and a Rapid Antigen Test To Diagnose COVID-19. J. Clin. Microbiol. 24 (58), e01438–e01520. https://doi.org/10.1128/JCM.01438-20.

Pasomsub, E., Watcharananan, S.P., Watthanachockchai, T., Rakmanee, K., Tassaneetrithep, B., Kiertiburanakul, S., et al., 2021. Saliva sample pooling for the detection of SARS-CoV-2. J. Med. Virol. 93, 1506–1511. https://doi.org/10.1002/jmv.26460.

Rajendrakumar, A.L., Nair, A.T.N., Nangia, C., Chourasia, P.K., Chourasia, M.K., Syed, M. G., et al., 2021. Epidemic landscape and forecasting SARS-CoV-2 in India. J. Epidemiol. Glob. Health 11, 55–59. https://doi.org/10.2991/jegh.k.200823.001.

Rennert, L., McMahan, C., 2021. Risk of SARS-CoV-2 reinfection in a university student population. Clin. Infect. Dis. https://doi.org/10.1093/cid/ciab454 in press.

Savela, E.S., Winnett, A., Romano, A.E., Porter, M.K., Shelby, N., Akana, R., et al. 2021. SARS-CoV-2 is detectable using sensitive RNA saliva testing days before viral load reaches detection range of low-sensitivity nasal swab tests. MedRxiv Apr 7 2021. 10.1101/2021.04.02.21254771; in press.

Takeuchi, Y., Furuchi, M., Kamimoto, A., Honda, K., Matsumura, H., Kobayashi, R., 2020. Saliva-based PCR tests for SARS-CoV-2 detection. J. Oral. Sci. 62, 350–351. https://doi.org/10.2334/josnusd.20-0267.

To, K.K.W., Tsang, O.T.Y., Leung, W.S., Tam, A.R., Wu, T.C., Lung, D.C., et al., 2020a. Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study. Lancet Infect. Dis. 20, 565–574. https://doi.org/10.1016/S1473-3099(20)30196-1.

To, K.K.W., Tsang, O.T.Y., Yip, C.C.Y., Chan, K.H., Wu, T.C., Chan, J.M.C., et al., 2020b. Consistent detection of 2019 novel coronavirus in saliva. Clin. Infect. Dis. https://doi.org/10.1093/cid/ciaa149. Published online 2020 Feb 12.

Watkins, A.E., Fenichel, E.P., Weinberger, D.M., Vogels, C.B.F., Brackney, D.E., Casanovas-Massana, A., et al., 2021. Increased SARS-CoV-2 testing capacity with pooled saliva samples. Emerg. Infect. Dis. 27, 1184–1187. https://doi.org/10.3201/eid2704.204200.

Wong, S.C.Y., Tse, H., Siu, H.K., Kwong, T.S., Chu, M.Y., Yau, F.Y.S., et al., 2020. Posterior oropharyngeal saliva for the detection of SARS-CoV-2. Clin. Infect. Dis. 71, 2939–2946. https://doi.org/10.1093/cid/ciaa797.

Wyllie, A.L., Fournier, J., Casanovas-Massana, A., Campbell, M., Tokuyama, M., Vijayakumar, P., et al., 2020. Saliva or nasopharyngeal swab specimens for detection of SARS-CoV-2. N. Engl. J. Med. 383, 1283–1286. https://doi.org/10.1056/NEJMc2016359.