

Assessment of the use and quick preparation of saliva for rapid microbiological diagnosis of COVID-19

Evaluación del uso y preparación rápida de saliva para el diagnóstico microbiológico rápido de COVID-19

Mariana Fernández-Pittol¹, Juan Carlos Hurtado^{1,2}, Estela Moreno-García³, Elisa Rubio^{1,2}, Mireia Navarro^{1,2}, Marta Valiente¹, Aida Peiró², Alicia Capón¹, Nuria Seijas⁴, Miguel J. Martínez^{1,2,5}, Climent Casals-Pascual^{1,2,5}, Jordi Vila^{1,2,5 a}

¹ Department of Clinical Microbiology, Hospital Clinic, University of Barcelona, Barcelona, Spain.

² Institute of Global Health of Barcelona (ISGlobal), Barcelona, Spain.

³ Department of Infectious Diseases, Hospital Clinic, University of Barcelona, Barcelona, Spain.

⁴ Hospital at Home, Medical and Nurse Direction, Hospital Clinic, Barcelona, Spain

⁵ CIBER Enfermedades Infecciosas (CIBERINFEC), Instituto de Salud Carlos III, Madrid, Spain



Citar como: Fernández-Pittol M, Hurtado JC, Moreno-García E, Rubio E, Navarro M, Valiente M, *et al.* South Health; 2024;1:e003. doi: 10-21142/SH-01-2024-e003.

Artículo recibido: 19/11/2023

Artículo aceptado: 20/02/2024

Revisado por pares.



© Los autores, 2024. Publicado por la Universidad Científica del Sur (Lima, Perú)

^a Corresponding author:

Address: Department of Clinical Microbiology, Hospital Clinic, Villarroel, 170;

08036 Barcelona, Spain.

Phone: +34932275522

E-mail: jvila@clinic.cat

COVID-19 is a very devastating pandemic infection caused by SARS-CoV-2, which originated in China and has currently spread all over the world (Chen *et al.*, 2020). Rapid diagnosis of COVID-19 is essential for the management of patients mainly in the emergency department (Loeffelholz and Tang, 2020). Although some assays based on antigen-antibody reaction have been commercialized to detect either antigens or antibodies (IgA, IgM, or IgG), the most sensitive tool to detect SARS-CoV-2 continues to be reverse-transcription real time polymerase chain reaction (RT-PCR) which specifically amplifies different genes encoded in the viral RNA from nasopharyngeal and/oropharyngeal swabs.

Shortages in swabs for collecting nasopharyngeal and oropharyngeal samples as well as insufficient RNA extraction kits can lead to a critical situation during a pandemic in which a huge number of samples must be processed. Therefore, the main objective of this study was to evaluate the use of saliva as an alternative and easier-to-collect clinical sample to detect COVID-19. The diagnostic performance of nasopharyngeal and oropharyngeal swabs was compared to the performance of direct heated saliva to RNA extracted samples.

Consecutive patients attending the Emergency Department of the Hospital Clinic of Barcelona, Spain, with laboratory or clinical-radiologic findings compatible with a diagnosis of COVID-19 were included in the study. Patients presenting infections or lesions in the oropharyngeal area were excluded. All patients included in the study had a primary diagnosis of COVID-19 by a RT-PCR from oropharyngeal and nasopharyngeal swabs samples. Oropharyngeal and nasopharyngeal swabs together with saliva samples were obtained from 51 patients clinically diagnosed as potentially having COVID-19 in the period from May to June 2020. The study was approved by the local ethical committee (HCB/2020/1001). A written informed consent to participate in this study was provided by the patients.

The gold standard used was the detection of SARS-CoV-2 genes in the platform Cobas 6800[®] (Roche molecular system, Branchburg, NJ). This platform allows qualitative detection of SARS-CoV-2 through two target regions: *ORF1 a/b* (specific for SAR-CoV-2) and the detection of pan-Sarbecovirus (through a conserved region in the structural protein of the envelope gene E) for the Sarbecovirus subgenus that includes SARS-CoV-2. Nasopharyngeal and oropharyngeal swabs were deposited in a tube with 2 mL lysis buffer (guanidine thiocyanate, 2M; sodium citrate pH 7.0, 30 mM; dithiothreitol, 2 mM and triton X-100, 1%). During all the process a protocol to avoid cross-contamination was followed. First all the samples were processed in a laminar flow cabin (bio security cabin level 2). A previous step of inactivation was used for COBAS 6800[®] and, in all the cases before transfer the samples out of the cabin (were inactivated or not) all the tubes were decontaminated individually with bleach at 0.5 %.

In addition, all patients were asked to provide a saliva sample from the posterior oropharynx before tooth brushing and meal intake. Patients were instructed and supervised by a medical care team. Saliva samples with a volume less than 500µL were not included in the study. The samples were transported to the Clinical Microbiology Department within less than 2 hours after collection. All saliva samples were stored at -80oC and processed together.

Two different methods were compared. In the first method, 350 µL of saliva were mixed with 350µL of lysis buffer (MagNA Pure Compact RNA Isolation Kit, Roche). RNA was extracted from 500 µl of sample using a MagNA Pure Compact Instrument (Roche, Basel, Switzerland) with an elution volume of 50µl. In the second method, 700µL of saliva were heat-inactivated at 96 oC for 15 minutes, and 5 µl were subjected directly to RT-PCR. One step real time RT-PCR was performed using the RNA Process Control Kit (Roche, Basel, Switzerland) with 5 µl of extracted RNA or directly from 5 µl of heated sample. RT-PCR was performed targeting the SARS-CoV-2 envelope (E) gene region (Corman *et al.*, 2020). Diagnostic performance was assessed using the results of the RT-PCR from nasopharyngeal and oropharyngeal swabs as the gold standard.

Statistical analyses were conducted using Stata 16.0 and positive and negative-predictive (post-test) values were calculated for a disease prevalence of 10%.

A total of 51 patients with COVID-19-like symptoms were included in the study (Supplementary data 1), 37 patients gave positive by RT-PCR using oro- and naso-pharyngeal swabs, whereas 14 were negative. The overall sensitivity, specificity, positive and negative predictive values were 81.08%, 92.86%, 55.78% and 97.79%, respectively when RNA extraction was included in the protocol with saliva, whereas sensitivity, specificity, positive and negative predictive values were 83.78%, 92.86%, 56.58% and 98.10%, respectively, for the heat-inactivation protocol (Table 1). However, when the analysis was performed exclusively on saliva samples (heated only) with a limited time from the onset of symptoms (<9 days, N=28), these values were 90%, 87.5%, 44.44% and 98.75% for the heat-inactivation protocol and 85%, 87.5%, 62.9% and 95.8%, respectively, for the RNA extracted protocol (Table 2).

Table 1. Sensitivity and specificity of RT-PCR in saliva sample using RNA extraction protocol or heat-inactivation protocol.

Saliva (N=51)	RNA extraction	Heat inactivation
	% (95% CI)	% (95% CI)
Sensitivity	81.08 (64.8-92.0)	83.7 (67.9-93.8)
Specificity	92.8 (66.1-99.8)	92.8 (66.1-99.8)
Positive likelihood ratio	11.3 (1.71-75.5)	11.73 (1.76-77.9)
Negative likelihood ratio	0.20 (0.10-0.40)	0.17 (0.08-0.37)
Positive predictive value	55.78 (15.94-89.35)	56.58 (16.4-89.65)
Negative predictive value	97.79 (95.71-98.87)	98.10 (96.07-99.09)
Accuracy	91.6 (80.4-97.5)	91.9 (80.8-97.7)

CI: confidence interval.

Table 2. Sensitivity and specificity of RT-PCR in saliva sample using RNA extraction protocol or heat-inactivation protocol.

Saliva (N=28)	RNA extraction	Heat inactivation
	% (95% CI)	% (95% CI)
Sensitivity	85.0 (69.35-100)	90.0 (68.3-98.7)
Specificity	87.5 (69.35-100)	87.5 (47.35-99.68)
Positive likelihood ratio	6.8 (5.08-9.10)	7.20 (1.14-45.30)
Negative likelihood ratio	0.71 (0.14-0.20)	0.11 (0.03-0.44)
Positive predictive value	62.96 (26.44-88.94)	64.29 (27.28-89.58)
Negative predictive value	95.89 (79-99.31)	97.22 (80.96-99.65)
Accuracy	87.00 (70.0-95.04)	88.0 (71.23-95.60)

CI: confidence interval.

Two studies have recently evaluated the use of saliva for diagnosing SARS-CoV-2 infection, showing that the saliva viral load was highest during the first week after symptom onset and subsequently declined over time, which suggests that saliva may be a good non-invasive sample for detecting the presence of SARS-CoV-2 during the first days after the onset of symptoms [To *et al.*, 2020a, 2020b). Using RT-PCR, Pasomsub *et al.* (2020) found a sensitivity and specificity for saliva samples of 84.2% [95% confidence interval (CI) 60.4%-96.6%], and 98.9% (95% CI 96.1%-99.9%), respectively. Analysis of the two specimens demonstrated 97.5% of agreement (kappa coefficient 0.851, 95% CI 0.723-0.979; $p < 0.001$). Moreover, it has also been suggested that saliva could be a more sensitive alternative to nasopharyngeal swabs (Wyllie *et al.*, 2020). Our results agree with the abovementioned studies, but in addition we show that RT-PCR can be performed using an RNA extraction-free protocol with 91.9% of concordance with oropharyngeal and nasopharyngeal swabs (considering only samples collected below 9 days of the onset of the symptoms) This protocol modification reduced the turnaround time by 40 minutes, taking into account the 10 minutes of pre-processing plus 30 minutes of RNA extraction. In addition, the cost is also decreased. Moreover, the combination of a test with a high negative predictive value, and the simplified logistics of sample collection (patients provide the saliva samples with no need for personal protective equipment) is especially helpful to rule out infection during times of low incidence and in low-resource settings. Larger studies are needed to prospectively validate these findings, and standardized saliva sample collection protocols are also necessary prior to implementation in the clinical setting.

Funding

We have economical support from the Spanish Ministry of Science and Innovation through the "Centro de Excelencia Severo Ochoa 2019-2023" Program (CEX2018-000806-S), and support from the Generalitat de Catalunya through the CERCA Program. Supported by Plan Nacional de I+D+i 2013-2016 and Instituto de Salud Carlos III, Subdirección General de Redes y Centros de Investigación Cooperativa, Ministerio de Economía, Industria y Competitividad, Spanish Network for Research in Infectious Diseases (REIPI RD16/0016/0010) - co-financed by European Development

Regional Fund “A way to achieve Europe”, Operative program Intelligent Growth 2014-2020. This work was also supported by award 2017 SGR 0809 from the Agència de Gestió d’Ajuts Universitaris de Recerca of the Generalitat de Catalunya.

Competing interests

The authors declare no conflict of interest.

Authors’ contributions

The work performed by each one of the authors were: Conceptualization and design, J.V., C.C. and M.M.; methodology, M.F., J.C.H.; E.M.G.; E.R.; M.N.; M.V.; A.P.; N.S. and A.C.; supervision and validation, M.F and J.C.H.; writing–original draft preparation, J.V and C.C.; writing–review and editing, M.M.; C.C. and J.V.; funding acquisition, J.V. All authors have read and agreed to the published version of the manuscript.

References

- Chen J, Lu H, Melino G, Boccia S, Piacentini M, Ricciardi W, *et al.* COVID-19 infection: the China and Italy perspectives. *Cell Death Dis.* 2020;11(6):438. doi: [10.1038/s41419-020-2603-0](https://doi.org/10.1038/s41419-020-2603-0).
- Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu DK, *et al.* Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. *Euro Surveill.* 2020;25(3):2000045. doi: [10.2807/1560-7917.ES.2020.25.3.2000045](https://doi.org/10.2807/1560-7917.ES.2020.25.3.2000045).
- Loeffelholz MJ, Tang YW. Laboratory diagnosis of emerging human coronavirus infections - the state of the art. *Emerg Microbes Infect.* 2020;9(1):747-756. doi: [10.1080/22221751.2020.1745095](https://doi.org/10.1080/22221751.2020.1745095).
- Pasomsub E, Watcharananan SP, Boonyawat K, Janchompoo P, Wongtabtim G, Suksuwan W, *et al.* Saliva sample as a non-invasive specimen for the diagnosis of coronavirus disease 2019: a cross-sectional study *Clin Microbiol Infect.* 2021;27(2):285.e1-285.e4. doi: [10.1016/j.cmi.2020.05.001](https://doi.org/10.1016/j.cmi.2020.05.001).
- To KK, Tsang OT, Leung WS, Tam AR, Wu TC, Lung DC, *et al.* 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.* 2020; 20(5):565-574. doi: [10.1016/S1473-3099\(20\)30196-1](https://doi.org/10.1016/S1473-3099(20)30196-1).
- To KK, Tsang OT, Yip CC-Y, Chan KH, Wu TC, Chan JM, *et al.* Consistent detection of 2019 novel coronavirus in saliva. *Clin Infect Dis.* 2020; 71(15):841-843. doi: [10.1093/cid/ciaa149](https://doi.org/10.1093/cid/ciaa149).
- Wyllie AL, Fournier J, Casanovas-Massana A, Campbell M, Tokuyama M, Vijayakumar P, *et al.* Saliva or nasopharyngeal swab specimens for detection of SARS-CoV-2. *N Engl J Med.* 2020;383(13):1283-1286. doi: [10.1056/NEJMc2016359](https://doi.org/10.1056/NEJMc2016359).