





Saliva Sampling and Its Direct Lysis, an Excellent Option To Increase the Number of SARS-CoV-2 Diagnostic Tests in Settings with Supply Shortages

Joaquín Moreno-Contreras,^a Marco A. Espinoza,^a Carlos Sandoval-Jaime,^a Marco A. Cantú-Cuevas,^b Héctor Barón-Olivares,^c Oscar D. Ortiz-Orozco,^c Asunción V. Muñoz-Rangel,^c Manuel Hernández-de la Cruz,^c César M. Eroza-Osorio,^c  Carlos F. Arias,^a  Susana López^a

^aDepartamento de Genética del Desarrollo y Fisiología Molecular, Instituto de Biotecnología Universidad Nacional Autónoma de México, Cuernavaca, Morelos, Mexico

^bSecretaría de Salud del Estado de Morelos, Cuernavaca, Morelos, Mexico

^cServicios de Salud del Estado de Morelos, Cuernavaca, Morelos, Mexico

ABSTRACT As part of any plan to lift or ease the confinement restrictions that are in place in many different countries, there is an urgent need to increase the capacity of laboratory testing for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Detection of the viral genome through reverse transcription-quantitative PCR (RT-qPCR) is the gold standard for this virus; however, the high demand of the materials and reagents needed to sample individuals, purify the viral RNA, and perform the RT-qPCR has resulted in a worldwide shortage of several of these supplies. Here, we show that directly lysed saliva samples can serve as a suitable source for viral RNA detection that is less expensive and can be as efficient as the classical protocol, which involves column purification of the viral RNA. In addition, it bypasses the need for swab sampling, decreases the risk of the health care personnel involved in the testing process, and accelerates the diagnostic procedure.

KEYWORDS COVID-19, diagnostic tools, RT-qPCR, SARS-CoV-2

With the worldwide COVID-19 health emergency, there is an urgent need for rapid and reliable methods of diagnosis for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The accepted gold standard for detection of this virus is the amplification of regions of the viral genome by reverse transcription-quantitative PCR (RT-qPCR) in nasopharyngeal and oropharyngeal swabs (1, 2). Unfortunately, given the enormous demand for the reagents needed to collect the biological samples and to purify the viral RNA, there have been shortages of many of the reagents needed for the diagnostic tests. Swabs, viral transport media, and kits for viral RNA extraction are among the most common consumables that have become scarce, compromising the number of tests that can be done in many parts of the world.

Recently, several reports have demonstrated the possibility of using saliva instead of oral and nasal swabs to detect the genome of SARS-COV-2 (3–5). Saliva collection also has many collateral benefits, including self-collection, which decreases the risk to health care workers in charge of taking the swabs and does not require the use of personal protective equipment (PPE), which has also become a scarce item in this pandemic (6, 7). In addition, the methods to extract the RNA from biological samples require the use of purification kits, whose availability has also become limited due to the heavy worldwide demand.

In this study, we compared the RT-qPCR results from 253 paired samples obtained from saliva and swabs of ambulatory patients; the RNA in the swab samples was

Citation Moreno-Contreras J, Espinoza MA, Sandoval-Jaime C, Cantú-Cuevas MA, Barón-Olivares H, Ortiz-Orozco OD, Muñoz-Rangel AV, Hernández-de la Cruz M, Eroza-Osorio CM, Arias CF, López S. 2020. Saliva sampling and its direct lysis, an excellent option to increase the number of SARS-CoV-2 diagnostic tests in settings with supply shortages. *J Clin Microbiol* 58:e01659-20. <https://doi.org/10.1128/JCM.01659-20>.

Editor Angela M. Caliendo, Rhode Island Hospital

Copyright © 2020 American Society for Microbiology. All Rights Reserved.

Address correspondence to Susana López, susana@ibt.unam.mx.

Received 28 June 2020

Returned for modification 17 July 2020

Accepted 21 July 2020

Accepted manuscript posted online 23 July 2020

Published 22 September 2020

extracted using a commercial RNA purification kit, and the saliva samples were directly mixed with a lysis buffer, boiled, and used for the RT-qPCR protocol. We found a very good correlation of results between the two types of samples, and we propose that saliva sampling and direct lysis, which together simplify the sampling of patients and accelerate the preparation of RNA for RT-qPCR, represent an excellent alternative that will facilitate sampling and diagnosis of a larger number of persons at a reduced cost.

MATERIALS AND METHODS

Sample collection. A total of 253 paired samples from oropharyngeal and nasopharyngeal swabs (OPSs and NPSs, respectively) and saliva were collected during a span of 30 days (from 2 May to 31 May) by health care workers from the epidemiology department of the health ministry of the state of Morelos, Mexico (Secretaría de Salud Morelos [SSM]). All but 3 samples were from ambulatory patients; the 3 exceptions were collected from hospitalized patients.

Swab sampling. Oropharyngeal and nasopharyngeal swabs were taken from 71 patients, while a single oropharyngeal swab was taken from each of 182 patients. After their collection, swabs were placed in 2.5 ml of viral transport medium.

Saliva collection. Saliva was self-collected by patients that were asked to spit on several occasions into sterile urine cup containers until completing roughly 2 to 3 ml of saliva. No viral transport media, or stabilizing agents, were added to the saliva samples.

After collection, both swab and saliva samples were stored at 4°C until transported to the Institute of Biotechnology/UNAM for their analysis, which was within 24 to 36 h after sample collection.

Nucleic acid extraction and SARS-CoV-2 detection by RT-qPCR. Total RNA was extracted from swab samples using the QIAamp viral RNA minikit (Qiagen) following the manufacturer's protocol, using 140 μ l of viral transport medium from each swab, and the purified RNA was eluted in 60 μ l of elution buffer.

Saliva samples were treated with Quick Extract DNA extraction solution (QE; Lucigen) by mixing 50 μ l of saliva with 50 μ l of the QE reagent and heating for 5 min at 95°C; the mixture was then cooled on ice and kept at 4°C until use (within 1 h of QE treatment). For saliva samples that had high viscosity, 1 volume of sterile phosphate-buffered saline (PBS) was added and mixed by repeated pipetting, and the diluted saliva sample was extracted as mentioned above.

SARS-CoV-2 detection was performed using the Berlin protocol, using the reported oligonucleotides and probes for viral gene E and for human RNase P (8). The RT-qPCRs were performed using the StarQ one-step RT-qPCR (Genes 2 Life) kit, using 5 μ l of the column-extracted total RNA in 20 μ l of reaction mixture or 2.5 μ l of the QE-treated saliva in 22.5 μ l of reaction mixture. Samples were analyzed in an ABI Prism 7500 sequence detector system (Applied Biosystems) with the following thermal protocol: 50°C for 15 min, 95°C for 2 min, and then 45 cycles of 95°C for 15 s and 60°C for 30 s. All samples with a threshold cycle (C_T) value equal to or less than 38 were classified as positive.

Determination of viral copy number. To determine the viral copy number in a sample, a standard curve was generated using a 10-fold serial dilution of an *in vitro* T7 RNA transcript that carries the sequence recognized by oligonucleotides and probe for gene E. Briefly, the logarithm of concentration of each dilution was plotted against the C_T and the viral copy number from unknown samples was determined by extrapolating the C_T value onto the corresponding standard curve.

Statistical analysis. Statistical analysis was performed using GraphPad Prism 6.0 (GraphPad Software Inc.) as described in Results.

RESULTS

Detection of SARS-CoV-2 in paired swab and saliva samples. To evaluate if saliva is a good source of viral RNA for the RT-qPCR, we determined the presence of the SARS-CoV-2 genome in paired saliva and swab samples from 253 ambulatory patients. All patients had two or more symptoms related to COVID-19 (8, 9); 116 (45.4%) were male and 137 (54.1%) female, with a median age of 41 (\pm 14.4) years. Samples were taken from ambulatory patients in the respiratory triage of the Tlaltenango health center in Cuernavaca, Morelos, Mexico. The RT-qPCR Berlin protocol was used to detect SARS-CoV-2, using only the primers and probe for gene E, since previous studies have shown a weak detection of viral RNA when the RNA-dependent RNA polymerase (RdRp) gene is probed (9, 10). As an internal control of RNA content in the samples, the RNase P gene was detected. Total RNA was purified from swabs using the QIAamp viral RNA minikit; the RNA in saliva was directly obtained using the QE lysis buffer (Lucigen) and boiling for 5 min, as reported previously (11).

During the course of the study, and due to the shortage of swabs, the health center shifted temporarily from collecting two swabs per person (nasopharyngeal swab [NPS] plus oropharyngeal swab [OPS]) to only one swab (OPS) per individual. For the 253 patients included in this study, two swabs were used in 71 (28%) of the cases, while a

TABLE 1 Summary of results obtained from parallel testing of swab and saliva samples from patients suspected of having COVID-19

Result for saliva	No. of patients with indicated result for single swab (OPS)			No. of patients with indicated result for double swabs (OPS + NPS)		
	Positive	Negative	Total	Positive	Negative	Total
Positive	41	28	69	19	6	25
Negative	11	102	113	9	37	46
Total	52	130	182	28	43	71

single OPS was taken from the other 182 (72%) irrespective of the number of swabs collected, saliva samples were taken from all patients.

Of the 182 patients with a single swab collected, 80 (43.9%) were positive for SARS-CoV-2 as determined by either the swab or saliva samples. Of these, 41 (51.2%) were positive as determined by both types of samples, while 28 (35%) were positive only by saliva and not by the swab sample and 11 (13.7%) were positive only by the OPS. In total, out of the 80 individuals found to be positive for the virus, 69 (86.2%) were correctly identified using saliva, while only 52 (65%) were identified with the OPS (Table 1 and Fig. 1).

On the other hand, 34 (47.8%) of the 71 patients with two swabs collected were found to be positive for SARS-CoV-2 by either the swabs or the saliva samples. Of these, 19 (55.8%) were positive by both swabs and saliva, while 6 (17.6%) were positive only by saliva and 9 (26.4%) were positive only by the two swab samples. In total, in this group of patients, of the 34 individuals identified as positive for the virus, 25 (73.5%) were identified by testing saliva, while 28 (82.3%) were positive by the swabs (Table 1 and Fig. 1).

Quantitation of viral RNA. When the numbers of viral genome copies in the single OPS and saliva samples were compared, a significant difference in the geometric mean was detected, with saliva samples having a titer 1.9 log₁₀ higher than that observed in the swabs (*P* < 0.0024 [Fig. 2A]). This can be better appreciated when the viral copy numbers in paired swabs and saliva from the same patient are plotted and represented

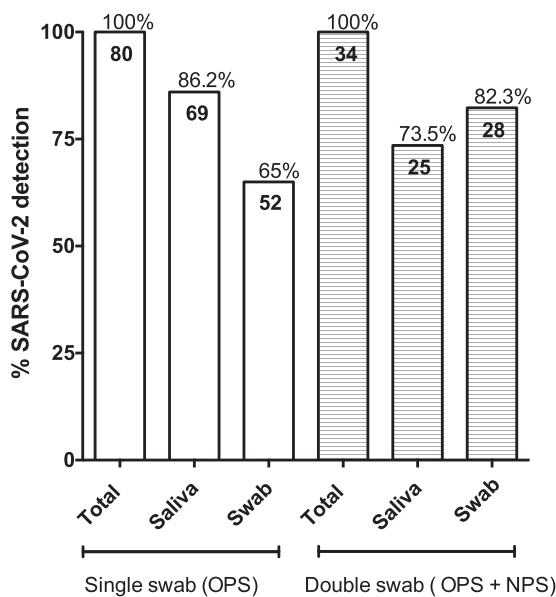


FIG 1 Detection of SARS-CoV-2 in paired swab and saliva samples. Percentages and numbers of positive samples detected among single OPSs and saliva, or double swabs (OPS plus NPS) and saliva, as indicated, are shown. Data are extracted from Table 1.

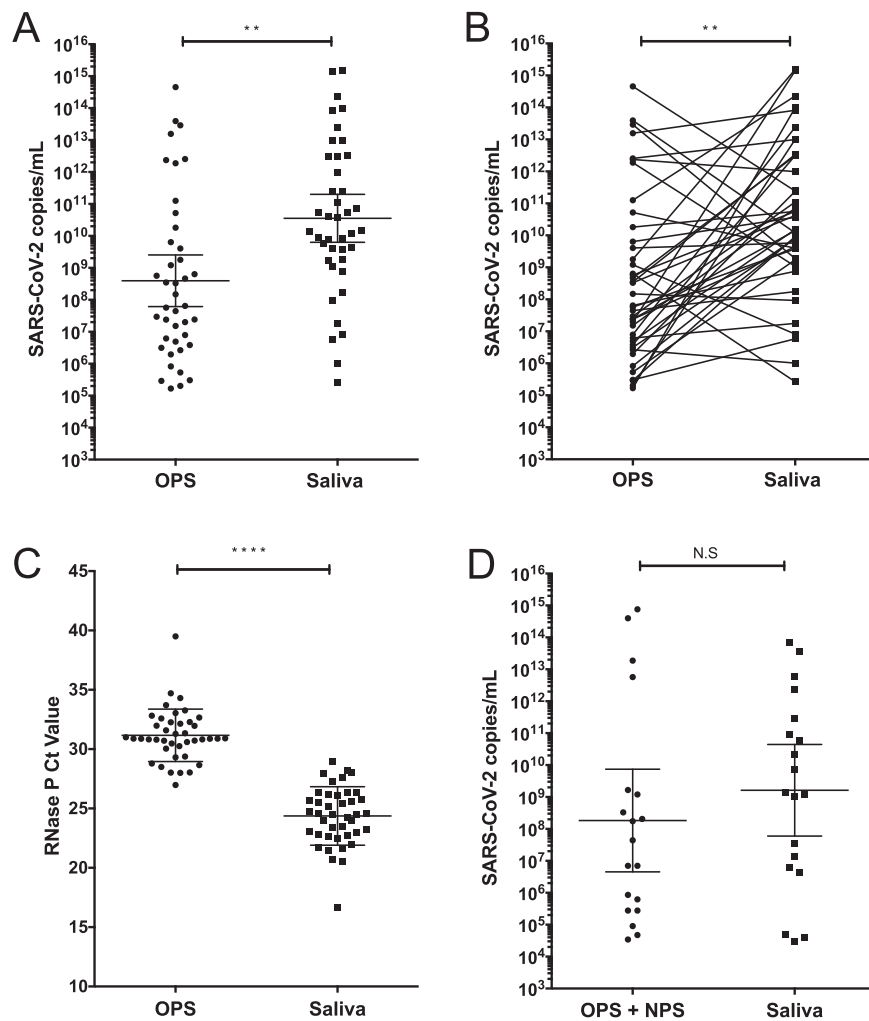


FIG 2 A high SARS-CoV-2 genome copy number is detected in saliva samples. (A) Viral titer detected in paired OPS and saliva samples. (B) Viral titer detected in paired OPS and saliva samples are represented by lines connecting both samples. Data were compared by a Wilcoxon test (**, $P < 0.0024$). (C) RT-PCR cycle C_T values for RNase P detected in OPS and saliva samples. Data were compared by Wilcoxon test (****, $P < 0.0001$). (D) Viral titer detected in paired double (NPS plus OPS) and saliva samples. Data were compared by Wilcoxon test; no statistical significance (N.S) was found ($P < 0.6226$). Bars represent the geometric medians and 95% confidence intervals.

as connecting lines (Fig. 2B); for 31 of the paired samples, the number of viral copies was higher in saliva samples than in swabs. Human RNase P was used as an internal control of sampling quality; of interest, the comparison between the mean of C_T values obtained from OPS and saliva samples showed a difference of at least 6.8 C_T units between both types of samples (Fig. 2C), indicating that there is more cellular material in saliva, as reported in other studies (12). The viral genome copy numbers in the double-swab and saliva samples were not statistically different, although a larger set of data would be needed to confirm these results (Fig. 2D).

DISCUSSION

In this study, we analyzed 253 paired samples from either a single OPS compared to saliva or a double OPS and NPS and saliva. RNA purified from swabs using commercial column kits was compared with saliva samples directly lysed with QE buffer (surpassing the RNA extraction protocol) as a source for the RT-qPCR assay. Although the coincidence rate between the single OPS and saliva samples was relatively low (51.2%), the saliva samples were clearly more efficient in detecting the virus than single OPS samples (86.2% versus 65%). On the other hand, the efficiency of detection of the virus

in saliva compared to the double OPS and NPS was slightly lower (73.5% versus 82.3%), with a coincidence rate of 55.8%.

Taken together, these results suggest that saliva is a good source for SARS-CoV-2 detection, especially compared with a single OPS. Furthermore, it can be implemented for diagnostic tests using a simple QE buffer-based sample preparation in place of the column-based RNA purification method that is currently employed for swab analyses.

The reason for the low coincidence in the positive results obtained with swab and saliva samples is not clear. The failure of identification of SARS-CoV-2 in swabs when the saliva samples were positive for the virus could be due to bad swab sampling, what can be corroborated by the higher C_T values of RNase P detected in these samples (Fig. 2C), with the consequent low viral copy number. This is a major concern, since the medical personnel in charge of taking the samples frequently do not do it correctly for the risk associated with this process. It has been reported that oropharyngeal swabs have a lower viral titer than nasopharyngeal swabs (1); thus, this could contribute to the discrepancies observed. Furthermore, it has also been previously reported that nasopharyngeal swabs have a lower viral titer than saliva samples (12), which could also contribute to explain our findings. On the other hand, the false negatives in saliva could be due either to the absence or undetectable levels of virus in the saliva samples or to unknown problems during the collection, transport, and/or storage of the sample before its arrival to the laboratory.

SARS-CoV-2 has been detected in saliva at higher titers during the first days after the onset of symptoms, with the viral titer declining over time. It is not clear how long after symptom onset the viral RNA can be detected in saliva, although some reports suggest a short period of detection (~13 days) compared with that for nasopharyngeal swabs (~19 days) (13). However, other reports have recently demonstrated the detection of viral RNA in saliva for longer periods (~20 days or longer) (4, 14). The patients included in this study were ambulatory and according to their clinical interviews were between 1 and 7 days (median of 4 days) of the onset of symptoms. We did not find a significant difference between the onset of symptoms and the cases in which positivity was determined only with saliva versus those that were detected only with swabs.

Direct lysis of nasopharyngeal or oropharyngeal swab samples in viral transport medium using the QE buffer has been reported as a suitable method for direct RT-qPCR for SARS-CoV-2 detection, with rates similar to those of methods based on column purification (11, 15). However, we have found a great variability in the results obtained using the QE lysis protocol when applied to swab samples, most likely due to variations in the material of the swabs used and to variations in the preparation of the viral transport medium employed (data not shown). In this regard, it has recently been reported that the composition of viral transport media can affect the detection of RNA from SARS-CoV-2 and other viruses (16), and due to the scarcity of these media, several laboratories have started to prepare their own transport media, introducing an additional confusion factor. A similar situation occurs with swabs, since in view of the scarcity of suitable materials, other materials are being employed, despite the fact that some of them are known to inhibit RT-PCR (17).

The use of saliva samples offers the advantage that no additives or transport media need to be used for their preservation or analysis if stored in cold temperatures and analyzed up to 36 h after their collection. Our results indicate that a rapid processing of saliva using direct lysis with QE buffer offers an excellent alternative to the current swab analysis that uses RNA column purification, since it is a sensitive, fast, and inexpensive method that can be used for massive screening, in particular in those settings where common supplies needed for the classical methods are in shortage.

ACKNOWLEDGMENTS

We are grateful to the health care workers of Servicios Estatales de Salud de Morelos for their invaluable help in collecting the samples and to the personnel of the Laboratorio Estatal de Salud Pública del Estado de Morelos for their support in the

preparation and transport of the samples. The work of P. Gaytán, E. López, and J. Yañez from the DNA sequencing and synthesis unit is also acknowledged.

Some of the reagents used in this study were provided by the Instituto Nacional de Diagnóstico y Referencia Epidemiológica, supported by INSABI. This work was supported by grant 314343 from CONACyT. J.M.-C. was the recipient of a scholarship from CONACyTs.

REFERENCES

- Pan Y, Zhang D, Yang P, Poon LLM, Wang Q. 2020. Viral load of SARS-CoV-2 in clinical samples. *Lancet Infect Dis* 20:411–412. [https://doi.org/10.1016/S1473-3099\(20\)30113-4](https://doi.org/10.1016/S1473-3099(20)30113-4).
- Wang Y, Kang H, Liu X, Tong Z. 2020. Combination of RT-qPCR testing and clinical features for diagnosis of COVID-19 facilitates management of SARS-CoV-2 outbreak. *J Med Virol* 92:538–539. <https://doi.org/10.1002/jmv.25721>.
- Kojima N, Turner F, Slepnev V, Bacelar A, Deming L, Kodeboyina S, Klausner JD. 2020. Self-collected oral fluid and nasal swabs demonstrate comparable sensitivity to clinician collected nasopharyngeal swabs for Covid-19 detection. medRxiv <https://doi.org/10.1101/2020.04.11.20062372>.
- To KK, Tsang OT, Chik-Yan Yip C, Chan KH, Wu TC, Chan JMC, Leung WS, Chik TS, Choi CY, Kandamby DH, Lung DC, Tam AR, Poon RW, Fung AY, Hung IF, Cheng VC, Chan JF, Yuen KY. 12 February 2020. Consistent detection of 2019 novel coronavirus in saliva. *Clin Infect Dis* <https://doi.org/10.1093/cid/ciaa149>.
- Williams E, Bond K, Zhang B, Putland M, Williamson DA. 23 July 2020. Saliva as a non-invasive specimen for detection of SARS-CoV-2. *J Clin Microbiol* <https://doi.org/10.1128/JCM.00776-20>.
- Khurshid Z, Asiri FYI, Al Wadaani H. 2020. Human saliva: non-invasive fluid for detecting novel coronavirus (2019-nCoV). *Int J Environ Res Public Health* 17:2225. <https://doi.org/10.3390/ijerph17072225>.
- Ng K, Poon BH, Kiat Puar TH, Shan Quah JL, Loh WJ, Wong YJ, Tan TY, Raghuram J. 2020. COVID-19 and the risk to health care workers: a case report. *Ann Intern Med* 172:766–767. <https://doi.org/10.7326/L20-0175>.
- Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu DK, Bleicker T, Brunink S, Schneider J, Schmidt ML, Mulders DG, Haagmans BL, van der Veer B, van den Brink S, Wijsman L, Goderski G, Romette JL, Ellis J, Zambon M, Peiris M, Goossens H, Reusken C, Koopmans MP, Drosten C. 2020. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. *Euro Surveill* 25:2000045. <https://doi.org/10.2807/1560-7917.ES.2020.25.3.2000045>.
- Nalla AK, Casto AM, Huang MW, Perchetti GA, Sampoleo R, Shrestha L, Wei Y, Zhu H, Jerome KR, Greninger AL. 2020. Comparative performance of SARS-CoV-2 detection assays using seven different primer-probe sets and one assay kit. *J Clin Microbiol* 58:e00557-20. <https://doi.org/10.1128/JCM.00557-20>.
- Ramirez JD, Munoz M, Hernandez C, Florez C, Gomez S, Rico A, Pardo L, Barros EC, Paniz-Mondolfi A. 2020. Genetic diversity among SARS-CoV2 strains in South America may impact performance of molecular detection. medRxiv <https://doi.org/10.1101/2020.06.18.20134759>.
- Ladha A, Joung J, Abudayyeh O, Gootenberg J, Zhang F. 2020. A 5-min RNA preparation method for COVID-19 detection with RT-qPCR. medRxiv <https://doi.org/10.1101/2020.05.07.20055947>.
- Wyllie AL, Fournier J, Casanovas-Massana A, Campbell M, Tokuyama M, Vijayakumar P, Geng B, Muenker MC, Moore AJ, Vogels CBF, Petrone ME, Ott IM, Lu P, Lu-Culligan A, Klein J, Venkataraman A, Earnest R, Simonov M, Datta R, Handoko R, Naushad N, Sewanan LR, Valdez J, White EB, Lapidus S, Kalinich CC, Jiang X, Kim DJ, Kudo E, Linehan M, Mao T, Moriyama M, Oh JE, Park A, Silva J, Song E, Takahashi T, Taura M, Weizman EO, Wong P, Yang Y, Bermejo S, Odio C, Omer SB, Dela Cruz CS, Farhadian S, Martinello RA, Iwasaki A, Grubaugh ND, Ko AI. 2020. Saliva is more sensitive for SARS-CoV-2 detection in COVID-19 patients than nasopharyngeal swabs. medRxiv <https://doi.org/10.1101/2020.04.16.20067835>.
- Iwasaki S, Fujisawa S, Nakakubo S, Kamada K, Yamashita Y, Fukumoto T, Sato K, Oguri S, Taki K, Senjo H, Sugita J, Hayasaka K, Konno S, Nishida M, Teshima T. 2020. Comparison of SARS-CoV-2 detection in nasopharyngeal swab and saliva. *J Infect* <https://doi.org/10.1016/j.jinf.2020.05.071>.
- To KK, Tsang OT, Leung WS, Tam AR, Wu TC, Lung DC, Yip CC, Cai JP, Chan JM, Chik TS, Lau DP, Choi CY, Chen LL, Chan WM, Chan KH, Ip JD, Ng AC, Poon RW, Luo CT, Cheng VC, Chan JF, Hung IF, Chen Z, Chen H, Yuen KY. 2020. 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](https://doi.org/10.1016/S1473-3099(20)30196-1).
- Sentmanat M, Kouranova E, Cui X. 2020. One-step RNA extraction for RT-qPCR detection of 2019-nCoV. bioRxiv <https://doi.org/10.1101/2020.04.02.022384>.
- Kirkland PD, Frost MJ. 2020. The impact of viral transport media on PCR assay results for the detection of nucleic acid from SARS-CoV-2 and other viruses. bioRxiv <https://doi.org/10.1101/2020.06.09.142323>.
- Hedman J, Radstrom P. 2013. Overcoming inhibition in real-time diagnostic PCR. *Methods Mol Biol* 943:17–48. https://doi.org/10.1007/978-1-60327-353-4_2.