Check for updates

Received: 12 March 2021 | Revised: 6 July 2021 | Accepted: 7 July 2021

DOI: 10.1111/ipd.12885

SHORT COMMUNICATION

INTERNATIONAL JOURNAL OF PAEDIATRIC DENTISTRY WILEY

Saliva as a reliable sample for COVID-19 diagnosis in paediatric patients

Alvina C. Felix[1] | Anderson V. de Paula[1] | Andreia C. Ribeiro[2] | Francini C. da Silva[2] | Marta Inemami[2] | Angela A. Costa[2] | Cibele O. D. Leal[1] | Walter M. Figueiredo[2] | Dmitry J. S. Sarmento[3] | Tatiana A. Sassaki[4] | Claudio S. Pannuti[1] | Paulo H. Braz-Silva[1,4] | Camila Malta Romano[1,5]

[1]Instituto de Medicina Tropical de São Paulo, Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil

[2]Serviço Especial de Saúde de Araraquara, Universidade de São Paulo, Araquara, Brazil

[3]Departamento de Medicina Oral, Universidade Estadual da Paraíba, Araruna, Brazil

[4]Departamento de Estomatologia, Faculdade de Odontologia da Universidade de São Paulo, São Paulo, Brazil

[5]Laboratório de Virologia (LIM-52), Hospital das Clinicas da Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil

Correspondence
Paulo Henrique Braz-Silva, Instituto de Medicina Tropical de São Paulo, Faculdade de Medicina da Universidade de São Paulo – IMTSP/FMUSP, Av. Dr. Enéas Carvalho de Aguiar, 470 - Jardim América, São Paulo – SP, Brazil.
Email: pbraz@usp.br

Funding information
Fundação de Amparo à Pesquisa do Estado de São Paulo, Grant/Award Number: 2019/03859-9

Population-based testing is one of the most effective strategies for preventing COVID-19 transmission by allowing early identification of cases and decision-making based on the pandemic's behaviour.[1] Nasopharyngeal swab (NPS) is the standard sample for molecular test, which causes much discomfort and possible cross-contamination during the collection.[1,2] Therefore, a trained healthcare professional is required for performing this procedure.[1,3] Saliva has been shown to be a reliable, safe diagnostic fluid for the detection of SARS-CoV-2, with a sensitivity ranging from 80%–100% compared with NPS in adult population.[1,3,4] In paediatrics, the use of saliva has clear advantages because the collection of this fluid is less invasive, thus reducing the discomfort and allowing self-collection.[2,5] Few studies have been conducted for SARS-CoV-2 molecular detection by using saliva in children. A recent published study has shown sensitivity higher than 80% compared with NPS.[6] The objective of this study was to compare the performance of saliva to that of NPS in the detection of SARS-CoV-2 in paediatric patients with mild symptoms.

This study evaluated saliva samples from children with suspected COVID-19 who attended public healthcare services of Araraquara, which is a medium-sized city located in the State of São Paulo, with a population of 238 339 in 2020.

As part of the COVID-19 contingency plan, the city of Araraquara offers molecular tests for the detection of SARS-COV-2 by using NPS in all symptomatic patients seeking healthcare service.

Parents and their children were invited to participate in this study at the time of NPS collection, in which the children were asked to spit into a sterile container for a collection of about 1 ml of saliva. The saliva samples were immediately stored at 4°C until being taken to the laboratory (<48 h). Symptoms and delays between their onset and sample collection (days) were also recorded. Total RNA was extracted by using the viral RNA mini kit (Qiagen, GE). SARS-COV-2 detection was made by using the Altona RealStar® SARS-CoV-2 RT-PCR Kit 1.0 (Altona Diagnostics GmbH), which employs a B-COV-specific probe directed to the E gene and a SARS-COV-2-specific probe directed to the S gene. Results were considered positive when one or both genes were amplified with a cycle threshold (Ct) <40. The positivity in saliva

 © 2021 BSPD, IAPD and John Wiley & Sons Ltd

was later compared with the results of NPS obtained by the Araraquara health surveillance. Unfortunately, no information on viral load in the NPS test was available for further comparisons.

This study was approved by the Research Ethics Committee of the University of São Paulo School of Medicine under protocol number 4235245.

The sample consisted of 50 patients, in which 27 were girls (54%) and 23 were boys (46%). Ten were positive for SARS-CoV-2 in at least one sample collected (saliva or NPS). The mean age was 10.24 ± 3.52 years old, and saliva was collected after 4.76 ± 1.31 days from the symptoms. Of the 50 patients evaluated, symptoms were reported by 46 during the saliva collection and the main ones were the following: coryza (60.9%), cough (56.5%), sore throat (45.7%), headache (39.1%) and fever (30.4%). None of these symptoms was statistically associated with the diagnosis of COVID-19 (Table 1).

ROC curve analysis was performed in order to assess sensitivity and specificity of RT-PCR between saliva and NPS, with the positive cases considered a gold standard. The results showed a statistically significant curve for both samples, saliva and NPS, with same results (AUC = 0.900, SE = 0.076; p <.001; 95% CI = 0.750–1.00). With these results, we can state that saliva can be safely used for the diagnosis of COVID-19 in paediatric patients. We also tested the concordance between saliva and NPS by using Kappa concordance test (k = 0.702; p <.001), with 96% of the samples being concordant (Table 2). Additionally, the concordance between these fluids was assessed individually, in which positive cases (10/50 patients) were considered as true infections. It was observed that the saliva and NPS showed the same values for Kappa concordance test (k = 0.865, p <.001).

Consistent scientific evidence has pointed to the effectiveness of the use of saliva as a diagnostic fluid for COVID-19 in adult population.[1,3,4] The advantages of a less invasive and painless sample collection have been shown to be more evident in paediatric populations, which may include self-collection and multiple collection possibilities.[2] There are, however, a few studies of paediatric patients and different ways to sampling for SARS-CoV-2 detection, and their results are conflicting.[6-9] A recent study from Dubai has shown similar rates of SARS-CoV-2 detection in saliva and NPS by using RT-PCR. Sensitivity and specificity of saliva were 87.7% and 98.5% respectively.[6] Our results showed that saliva had the same diagnostic performance as that of NPS for COVID-19, showing that this fluid is a good alternative for SARS-CoV-2 detection.

The use of less invasive strategies for COVID-19 surveillance has a crucial importance for children not only in the understanding of SARS-CoV-2 behaviour in this population, but also in the re-opening of schools based on constant

Why this paper is important to paediatric dentists?

- The COVID-19 has changed the approach of clinical activities in paediatric dentistry.
- To stablish the COVID-19 vigilance in paediatric population, it is important to include molecular tests for SARS-CoV-2 detection.
- Saliva is a less invasive and easy to handle sample for COVID-19 diagnosis. Our results have showed the same diagnostic performance of saliva compared to nasopharyngeal swabs in SARS-CoV-2 molecular detection in paediatric population.

TABLE 1 List of symptoms reported

Symptoms		n	%	p[a]
Fever	Yes	14	30.4	.242
	No	32	69.6	
Cough	Yes	26	56.5	.150
	No	20	43.5	
Shortness of air	Yes	9	19.6	.384
	No	37	80.4	
Coryza	Yes	28	60.9	.717
	No	18	39.1	
Headache	Yes	18	39.1	.999
	No	28	60.9	
Myalgia	Yes	7	15.2	.163
	No	39	84.8	
Fatigue	Yes	8	17.4	.664
	No	38	82.6	
Nausea	Yes	4	8.7	.201
	No	42	91.3	
Vomit	Yes	4	8.7	.999
	No	42	91.3	
Diarrhoea	Yes	6	13.0	.315
	No	40	87.0	
Abdominal pain	Yes	8	17.4	.055
	No	36	82.6	
Loss of smell (anosmia)	Yes	0	0	–
	No	46	100	
Loss of taste (ageusia)	Yes	2	4.3	.391
	No	44	95.7	
Sore throat	Yes	21	45.7	.306
	No	25	54.3	

[a]Association of the symptom with diagnosis of COVID-19. Fisher's exact test.

1365263x, 2022, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/ipd.12885 by Univ of Sao Paulo - Brazil, Wiley Online Library on [30/04/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

TABLE 2 Description of positive and negative cases of COVID-19 for saliva and NPS by using RT-PCR

		COVID-19		
RT-PCR		Yes N(%)	No N(%)	Total
Saliva	Positive	8 (16)	0 (0)	8 (16)
	Negative	2 (4)	40 (80)	42 (84)
Total		10 (20)	40 (80)	50 (100)
NPS	Positive	8 (16)	0 (0)	8 (16)
	Negative	2 (4)	40 (80)	42 (84)
Total		10 (20)	40 (80)	50 (100)

Note: Sensitivity–80%; Specificity–100% for saliva and NPS; 95% CI (0.75–1.00).

Abbreviation: NPS, Nasopharyngeal swabs.

tracking of asymptomatic cases.[2,6] Additionally, with the emergence of the variants of concern and vaccination campaigns that were initially not aimed at this age group, saliva could help on the detection of them in paediatric patients. The use of saliva makes COVID-19 surveillance viable, since this strategy is more largely accepted by individuals for being painless and for requiring no professional sample collection, avoiding possible technical problems on performing NPS, especially in paediatric population.[2-6] Depending on the age group, the individuals themselves can be remotely instructed (eg, videos) to perform the sample collection.[2,3]

The limitations of this preliminary work are in the fact that larger samples and inclusion of asymptomatic children should be considered in further studies. Our data allow us to conclude that saliva is a viable alternative fluid for the molecular diagnosis of COVID-19 in children.

ACKNOWLEDGMENTS

The authors would like to thank José Tadeu Sales for the language correction of the manuscript. This study was supported by the São Paulo Research Foundation (FAPESP) according to grant # 2019/03859-9.

AUTHOR CONTRIBUTIONS

Alvina C. Felix, Walter M. Figueiredo and Dmitry J. S. Sarmento conceptualized and designed the study, drafted the initial manuscript and reviewed and revised the manuscript. Anderson V. de Paula, Andreia C. Ribeiro, Francini C. da Silva, Marta Inemami, Angela A. Costa, Cibele O. D. Leal and Tatiana A. Sassaki designed the data collection instruments, collected data, carried out the initial analyses and reviewed and revised the manuscript. Claudio S. Pannuti, Paulo H. Braz-Silva and Camila M. Romano conceptualized and designed the study, coordinated and supervised the data collection, draft the manuscript and critically reviewed the manuscript for important intellectual content. All authors approved the final manuscript as submitted and agreed to be accountable for all aspects of the work.

ORCID

Paulo H. Braz-Silva https://orcid.org/0000-0002-1842-9521

REFERENCES

1. Sapkota D, Søland TM, Galtung HK, et al. COVID-19 salivary signature: diagnostic and research opportunities. *J Clin Pathol*. 2021;74(6):344-349. doi:https://doi.org/10.1136/jclinpath-2020-206834
2. Santos CN, Rezende KM, Oliveira Neto NF, Okay TS, Braz-Silva PH, Bönecker M. Saliva: an important alternative for screening and monitoring of COVID-19 in children. *Braz Oral Res*. 2020;34:e0125. doi:https://doi.org/10.1590/1807-3107bor-2020.vol34.0125
3. Braz-Silva PH, Mamana AC, Romano CM, et al. Performance of at-home self-collected saliva and nasal-oropharyngeal swabs in the surveillance of COVID-19. *J Oral Microbiol*. 2020;13(1):1858002. doi:https://doi.org/10.1080/20002297.2020.1858002
4. To KK, Tsang OT, Yip CC, et al. Consistent Detection of 2019 Novel Coronavirus in Saliva. *Clin Infect Dis*. 2020;71(15):841-843. doi:https://doi.org/10.1093/cid/ciaa149
5. Khurshid Z, Asiri FYI, Al Wadaani H. Human saliva: non-invasive fluid for detecting novel coronavirus (2019-nCoV). *Int J Environ Res Public Health*. 2020;17(7):2225. doi:https://doi.org/10.3390/ijerph17072225
6. Al Suwaidi H, Senok A, Varghese R, et al. Saliva for molecular detection of SARS-CoV-2 in school-age children. *Clin Microbiol Infect*. 2021. doi:https://doi.org/10.1016/j.cmi.2021.02.009
7. Kam KQ, Yung CF, Maiwald M, et al. Clinical utility of buccal swabs for severe acute respiratory syndrome coronavirus 2 detection in coronavirus disease 2019-infected children. *J Pediatric Infect Dis Soc*. 2020;9(3):370-372. doi:https://doi.org/10.1093/jpids/piaa068
8. Chua GT, Wong JSC, To KKW, et al. Saliva viral load better correlates with clinical and immunological profiles in children with coronavirus disease 2019. *Emerg Microbes Infect*. 2021;10(1):235-241. doi:https://doi.org/10.1080/22221751.2021.1878937
9. Chan RWY, Chan KC, Chan KYY, et al. SARS-CoV-2 detection by nasal strips: A superior tool for surveillance of paediatric population. *J Infect*. 2021;82(4):84-123. doi: https://doi.org/10.1016/j.jinf.2020.11.009

How to cite this article: Felix AC, de Paula AV, Ribeiro AC, et al. Saliva as a reliable sample for COVID-19 diagnosis in paediatric patients. *Int J Paediatr Dent*. 2022;32:123–125. https://doi.org/10.1111/ipd.12885

1365263x, 2022, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/ipd.12885 by Univ of Sao Paulo - Brazil, Wiley Online Library on [30/04/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License