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.

Indian Journal of Medical Microbiology 45 (2023) 100384

Contents lists available at ScienceDirect

Indian Journal of Medical Microbiology

journal homepage: www.journals.elsevier.com/indian-journal-of-medical-microbiology

Original Research Article

Validating saliva as a biological sample for cost-effective, rapid and routine screening for SARS-CoV-2

B.R. Ansil [a,*], Carolin Elizabeth George [b], Sindhulina Chandrasingh [c], Ashwin Viswanathan [d], Mukund Thattai [a], Padinjat Raghu [a], Santhosha Devadiga [e], Arun Geetha Harikumar [e], Pulleri Kandi Harsha [e], Indu Nair [f], Uma Ramakrishnan [a,*], Satyajit Mayor [a]

[a] National Centre for Biological Sciences, Tata Institute of Fundamental Research, Bangalore, Karnataka, 560065, India
[b] Community Health and Research Division, Bangalore Baptist Hospital, Bangalore, Karnataka, 560024, India
[c] Department of Microbiology, Bangalore Baptist Hospital, Bangalore, Karnataka, 560024, India
[d] Nature Conservation Foundation, Mysore, Karnataka, 570017, India
[e] COVID-19 Testing Laboratory, Institute for Stem Cell Science and Regenerative Medicine, Bangalore Life Science Cluster, Bangalore, Karnataka, 560065, India
[f] Department of Medicine and Infectious Diseases, Bangalore Baptist Hospital, Bangalore, Karnataka, 560024, India

ARTICLE INFO

Keywords:
SalivaDirect
Nasopharyngeal swab
SARS-CoV-2
Respiratory virus
Pandemic
Surveillance

ABSTRACT

Purpose: Compared to nasopharyngeal/oropharyngeal swabs (N/OPS-VTM), non-invasive saliva samples have enormous potential for scalability and routine population screening of SARS-CoV-2. In this study, we investigate the efficacy of saliva samples relative to N/OPS-VTM for use as a direct source for RT-PCR based SARS-CoV-2 detection.

Methods: We collected paired nasopharyngeal/oropharyngeal swabs and saliva samples from suspected positive SARS-CoV-2 patients and tested using RT-PCR. We used generalized linear models to investigate factors that explain result agreement. Further, we used simulations to evaluate the effectiveness of saliva-based screening in restricting the spread of infection in a large campus such as an educational institution.

Results: We observed a 75.4% agreement between saliva and N/OPS-VTM, that increased drastically to 83% in samples stored for less than three days. Such samples processed within two days of collection showed 74.5% test sensitivity. Our simulations suggest that a test with 75% sensitivity, but high daily capacity can be very effective in limiting the size of infection clusters in a workspace. Guided by these results, we successfully implemented a saliva-based screening in the Bangalore Life Sciences Cluster (BLiSC) campus.

Conclusion: These results suggest that saliva may be a viable alternate source for SARS-CoV-2 surveillance if samples are processed immediately. Although saliva shows slightly lower sensitivity levels when compared to N/OPS-VTM, saliva collection is logistically advantageous. We strongly recommend the implementation of saliva-based screening strategies for large workplaces and in schools, as well as for population-level screening and routine surveillance as we learn to live with the SARS-CoV-2 virus.

Introduction

The COVID-19 pandemic spread rapidly in India, infecting more than 30 million people in two years [1]. Given this magnitude and speed, COVID-19 presents various diagnostic challenges to a country like India in the context of massive population density and limited diagnostic and health infrastructure capabilities. Viral diagnosis has progressed tremendously, and of the various modalities for SARS-CoV-2 diagnosis, the most reliable test is the reverse transcription-polymerase chain reaction (RT-PCR) on Nasopharyngeal/Oropharyngeal swabs collected in Viral Transport Medium (N/OPS-VTM) [2,3]. This requires skilled technical staff and involves procedural complexities such as viral inactivation and RNA extraction. Besides, the sample collection protocol causes significant discomfort to the patient [4] and demands strict

* Corresponding authors.
E-mail addresses: ansilbr@ncbs.res.in (B.R. Ansil), carolinelizabethj@gmail.com (C.E. George), sindhulinabbh@gmail.com (S. Chandrasingh), ashwinv2005@gmail.com (A. Viswanathan), thattai@ncbs.res.in (M. Thattai), praghu@ncbs.res.in (P. Raghu), santhosh.bailur@fathermuller.in (S. Devadiga), arungh1993@gmail.com (A.G. Harikumar), harshabkrishnan@gmail.com (P.K. Harsha), indudeep@yahoo.com (I. Nair), uramakri@ncbs.res.in (U. Ramakrishnan), mayor@ncbs.res.in (S. Mayor).

https://doi.org/10.1016/j.ijmmb.2023.100384
Received 30 January 2022; Received in revised form 12 November 2022; Accepted 11 May 2023
Available online xxxx

protocols for the prevention of infection to healthcare workers. These procedural complexities increase cost and turnaround time.

The SalivaDirect protocol is an alternative RT-PCR-based method that does not require RNA extraction, and is cost-effective with a short turnaround time and less dependence on the supply chain [5]. Using saliva as a source sample has several advantages, including: (1) samples can be collected by patients without any help of trained personnel; (2) stringent personal protective equipment (PPE) is not required; (3) non-invasive and routine testing is possible; (4) swabs or VTM are not involved, and so this method is more flexible; (5) RNA extraction is eliminated, reducing test cost, widening its applicability. Because of these advantages, several studies are now exploring the potential use of saliva for cost-effective SARS-CoV-2 testing [6–8].

Unfortunately, very few studies from India explore the validity of saliva in SARS-CoV-2 diagnosis [9]. In this study, we assessed the performance of saliva relative to N/OPS-VTM for use as a direct source (without RNA extraction) for the RT-PCR based SARS-CoV-2 detection. We also investigated possible reasons for discordance between N/OPS-VTM and saliva sample pairs, and used simulations to evaluate the effectiveness of the SalivaDirect protocol in a real-world scenario. Finally, we present a case study on implementing such a strategy in an educational institution. Through this study, we provide evidence for a low cost, easy, fast, and accurate test that had a considerable advantage in a country like India, especially when learning to 'live with the virus'.

Materials and methods

Ethical statement

The study was approved by the Institutional Review Board of Bangalore Baptist Hospital (BBB/IRB/2020/010) and Institutional Human Ethics Committee (NCBS/IEC-22/01, NCBS/IEC-26/03) and Institutional Biosafety Committee (TFR: NCBS:34IBSC/UR1) of National Centre for Biological Sciences.

Sample collection, processing and testing for SARS-CoV-2

Clinical samples were obtained from patients of Bangalore Baptist Hospital between December 2020 and May 2021. We collected an N/OPS-VTM and up to 5 ml of saliva from each individual. All samples were transported to the COVID-19 testing laboratory at Institute for Stem Cell Science and Regenerative Medicine (inStem), Bangalore Life Science Cluster (BLiSC). On arrival, samples were stored in a 4 °C refrigerator in the biosafety laboratory. Storage time before processing the samples varied from 0 to 15 days with a mean of 4 days. In case of storage beyond two days, the samples were moved to a −20 °C freezer within the biosafety laboratory.

RNA was extracted from inactivated N/OPS-VTM using a magnetic bead-based automated viral RNA extraction protocol (Beckman Coulter Life Sciences). Saliva were processed following the SalivaDirect protocol [5] and tested for RdRP, E and N genes of SARS-CoV-2 and human RNase P gene using NeoDx-CoviDx™ mPlex-4R SARS-CoV-2 RT-PCR Detection kits. Additional details of sample collection and testing strategies are provided in the Supplementary Material.

Analysis

We determined the number of individuals that were (a) positive for SARS-CoV-2 in both N/OPS-VTM and saliva, (b) positive only in N/OPS-VTM, (c) positive only in saliva, and (d) negative in both N/OPS-VTM and saliva. We used these data to compute the sensitivity of the test on each sample type with the help of a reference diagnosis. Since nasopharyngeal swabs have been shown to produce false negatives by RT-PCR [10], we did not use N/OPS-VTM results as the reference but instead considered any individual with a positive result on either sample as true positive [6]. We therefore defined sensitivities for the saliva and the N/OPS-VTM as (a + c)/(a + b + c) and (a+b)/(a+b + c) respectively [6].

We compared positive outcomes from the saliva results with true positives and used a Generalized Linear Model (GLM) with four variables (age, sex, severity of disease and duration of sample storage) to understand the factors that explain result agreement (or disagreement). We modelled result agreement/disagreement (success/failure) as a function of the sex of the patient, age, severity of the symptoms, and storage duration (number of days between sample collection and testing), assuming a binomial error distribution. The severity of symptoms for each patient was clinically diagnosed and reported in two categories: asymptomatic and symptomatic. The storage duration of samples was grouped into two bins: two days and less (0–2), and 3–15 days. We did not include individuals who showed inconclusive results (samples with no amplification of viral genes and internal control) in this analysis. We also assessed the Ct-value distribution of all test genes for samples that showed result agreement and compared them using a Wilcoxon test [11].

Modelling infection spread on a network

We used a Monte Carlo simulation of infection transmission in a network of 1600 individuals to understand transmission risk under scenarios derived from the results of this study. We focused on three key factors of the testing protocol: (a) test sensitivity, (b) daily testing capacity and (c) delay in reporting results.

In the simulation, a subset of individuals were tested each day, and test results were reported after some delay. Positive individuals were isolated, and their contacts were subsequently tested and isolated if positive. We started with a single positive case and ran the simulation until no infected individuals remained. The model was stochastic, so each run of the simulation produced a different result. The total number of infections at the end of the simulation defined the size of the cluster. An important goal of mitigation was to limit the size of a cluster seeded by a single infection. Details of the model used for simulation are provided in the Supplementary Material.

Note that this model was not meant to replicate the transmission dynamics of an actual workforce but as a proof of principle to identify key factors that influence screening success.

Real-world implementation

We explored the effectiveness of saliva-based RT-PCR by implementing a screening program in the large Bangalore Life Sciences Cluster (BLiSC) campus of ca. 1400 adults. Participants donated saliva once every seven days, and we collected personal information using a mobile phone application. A video played at the collection centre with no verbal instruction informed individuals about the sample collection protocol. Samples were tested on the same day, the maximum delay between sample collection and testing being ca. 8 hrs. In six months, we tested ca. 20,000 samples of saliva for SARS-CoV-2 using the protocols described here. An average of 160 samples were tested each day, with a maximum of 300 samples on a single day. Samples were collected between 10 a.m. and 2 p.m. Monday to Friday, processed in about 6 h, and final results were delivered soon after (the same evening).

Results

SARS-CoV-2 detection in paired N/OPS-VTM and saliva samples

We observed a 75.4% agreement between N/OPS-VTM and saliva sample pairs, 30.3% as positives and 45.1% as negatives (Table 1). Of the respective totals, 1.1% and 5.7% of N/OPS-VTM and saliva samples were inconclusive, while 3.4% of saliva samples were positive when the corresponding N/OPS-VTM samples were negative. We calculated the overall sensitivity of saliva and N/OPS-VTM as 70.2% and 92.9% respectively. We found that the storage time of samples impacted the effectiveness of the SalivaDirect (Supplementary Table 1). Among

Table 1
A matrix showing the alignment of results from the two methods - N/OPS-VTM and Saliva - for all samples (N = 175). The asterisk indicate result agreements.

		N/OPS-VTM (N = 175)			
		Positive	Negative	Inconclusive	Total
Saliva (N = 175)	Positive	30.3% (53)*	3.4% (6)	0.6% (1)	34.3% (60)
	Negative	14.3% (25)	45.1% (79)*	0.6% (1)	60.0% (105)
	Inconclusive	5.7% (10)	0.0% (0)	0.0% (0)	5.7% (10)
	Total	50.3% (88)	48.6% (85)	1.1% (2)	**175**

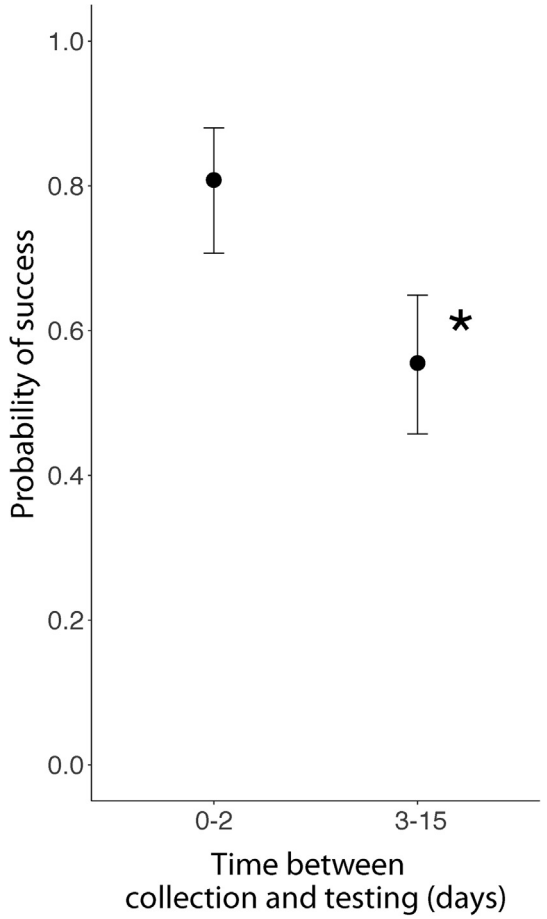

Fig. 1. Probability of result agreement in positive samples (0–2 days: N = 49; 3–15 days: N = 46). Samples stored for more than two days showed high result disagreement between paired samples. Error bars are standard errors and the asterisk denotes a significant difference.

positive patients, we observed a clear drop in result agreement (Fig. 1) from 80.8% (SE 70.7–88) to 55.5% (SE 45.7–64.9) when samples were stored for more than two days (p = 0.025). Concordance (83%) and the sensitivity (74.5%) of the saliva test improved when we only considered samples tested within two days of collection. This also resulted in a significant improvement (decrease) in inconclusive test results (1.8% in saliva and 0.9% in N/OPS-VTM) (Table 2). We also estimated saliva sensitivity separately after considering N/OPS as standard method and found marginally lower values 68% and 72% for all samples and samples processed within two days of collection respectively. We also found that viral loads were statistically indistinguishable in positive sample pairs for all three viral genes (Wilcoxon p > 0.05, Fig. 2) among positive concordant samples.

Simulations reveal that saliva-based screening can limit infection spread

We tracked the probability that a single starting infection leads to a large cluster (size 25 or more) as the testing parameters vary (Fig. 3). In the absence of testing and isolation, we found that the probability of a large cluster is 20% under the assumed parameter values. By using a test with 75% sensitivity at 200 tests per day with a one-day delay for results, however, we found that the probability of a large cluster reduces to 2% (ten-fold). We also show that this protocol works better than a test with 100% sensitivity, but with half the capacity or twice the delay.

Implementation of a saliva screening program

Out of the 20,000 samples collected during the testing of this method in the BLiSC campus, nine saliva samples tested positive. Of these, eight individuals were completely asymptomatic, and one had very mild generic symptoms. Among the nine individuals, three tested positive on N/OPS-VTM on the same day, one tested negative, and the remaining 5 declined further testing and preferred to isolate as per public health guidelines. We noted only 10 instances where amplification of the

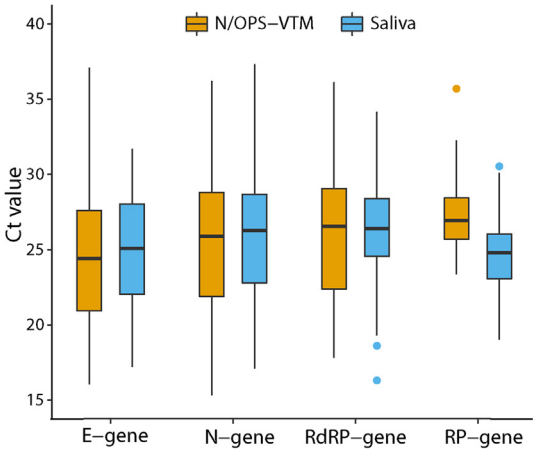

Fig. 2. Boxplot showing Ct value distribution for three SARS-CoV-2 genes and human RNAse P gene. There is no significant difference in mean Ct values for viral genes between N/OPS-VTM and saliva samples.

Table 2
A matrix showing the alignment of results from the two methods - N/OPS-VTM and Saliva - for samples tested within two days of collection (N = 112). The asterisk indicate result agreements.

		N/OPS-VTM (N = 112)			
		Positive	Negative	Inconclusive	Total
Saliva (N = 112)	Positive	27.7% (31)*	3.6% (4)	0.9% (1)	32.1% (36)
	Negative	10.7% (12)	55.4% (62)*	0.9% (1)	67.0% (75)
	Inconclusive	0.9% (1)	0.0% (0)	0.0% (0)	0.9% (1)
	Total	39.3% (44)	59.0% (66)	1.8% (2)	**112**

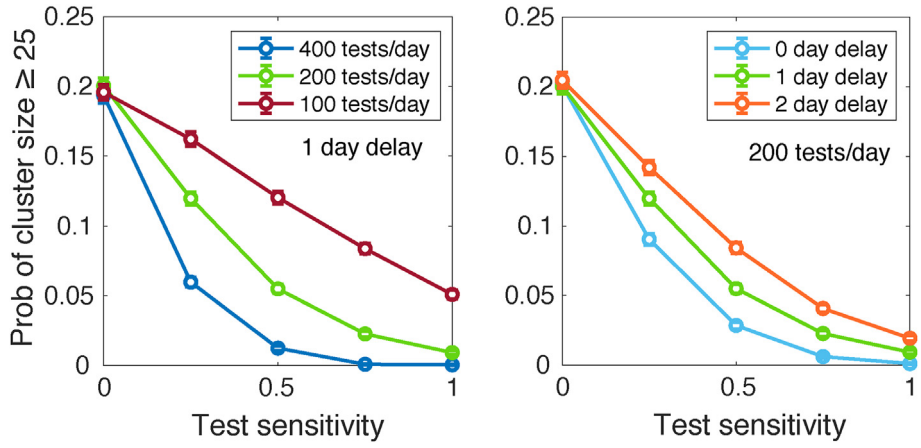

Fig. 3. Results of a Monte Carlo simulation of infection transmission on a network of 1600 individuals. We track the probability that a single starting infection seeds a cluster of 25 or more infections. We compare a baseline protocol that has a capacity of 200 tests per day and a delay in reporting of one day (green), with variations having higher or lower capacities or delays (see legend). Error bars represent SEM values over 5000 replicate simulations.

internal control (RNase P gene) did not occur, presumably due to inhibitory factors in the saliva sample.

Discussion

Saliva is emerging as an effective alternative sample type for RT-PCR based SARS-CoV-2 testing, with very high sensitivity and specificity [5,12]. Within this context, we examined the efficacy of saliva for direct RT-PCR and potential use for large-scale testing. Our results suggest that saliva is an excellent alternative to conventional N/OPS-VTM with a reasonable concordance of 75.4%, that increased to 83% in samples processed within two days from collection. Since saliva were collected without any stabilization media, RNA stability might have been compromised during the storage and freeze-thaw, resulting in lower positivity [13,14]. We found that the sensitivity of these quick-processed samples (74.5%) was comparable to those reported in several recent studies [6,8].

We found that saliva was less sensitive than N/OPS-VTM. We believe that this difference in sensitivity may have resulted from differences in the persistence of SARS-CoV-2 RNA in nasopharyngeal/oropharyngeal swabs compared to saliva after patient recovery [15,16]. Samples for this study were collected from patients after a week of hospitalisation (see sample collection strategy in Supplementary material), perhaps sufficient time for this difference to emerge, which may have led to more false-positive results in N/OPS (but true-negative results in saliva) and skewed sensitivity estimations. Recent studies have found a higher percentage of viral positivity in saliva when collected within ten days of COVID-19 diagnosis [17,18]. Importantly, we found that both positive N/OPS-VTM and saliva had a similar viral load (similar Ct value profiles for three viral genes), suggesting that saliva is a valuable alternative sample type for SARS-CoV-2 detection [19].

A saliva-based protocol has slightly lower sensitivity than N/OPS-VTM but provides several logistical advantages, therefore meeting all criteria for effective workplace screening [20]. It is non-invasive, simple and self-collected without any PPE and can be used directly for RT-PCR [21]. The SalivaDirect protocol does not require RNA extraction, a significant bottleneck in the testing workflow [5]. These unique features of saliva screening significantly reduce testing costs and complexity compared to N/OPS based testing [8], although we have not performed a formal cost analysis. Adopting saliva-based screening could yield higher testing capacity under resource constraints and result in shorter delays in reporting. In case of a high test burden, saliva pooling can also be employed to further reduce overall turnaround time [22,23].

In choosing a testing protocol, one confronts a trade-off between test sensitivity on the one hand, and testing capacity and testing delay on the other. Rapid Antigen Tests, for example, produce immediate results with low sensitivity, while the gold-standard N/OPS RT-PCR achieves high sensitivity but with limited capacity and delayed results. An N/OPS RT-PCR test, even if 100% sensitive, cannot prevent spread since only a fraction of individuals are sampled each day, and individuals in early stages of the infection may not test positive. We explored these trade-offs using a simulation of infection transmission. We found that increased testing capacity and shorter delay more than offset decreased test sensitivity in SalivaDirect, therefore preventing the emergence of large infection clusters [20]. We found that a higher testing capacity was particularly important because it enabled a more rapid cycle for testing an entire workforce, increasing the chance that an infection missed in one cycle would be picked up in the next. Our results suggest that this approach can be scaled up for routine surveillance efforts and implemented in schools, offices, academic institutions, and residential apartments. Repeatedly testing and identifying asymptomatic individuals in a workforce is a proactive approach that can help isolate sources of SARS-CoV-2 infection.

Given these benefits and the observed concordance between N/OPS-VTM and saliva, we piloted saliva-based COVID-19 screening in the BLiSC academic campus. We found that this screening and surveillance effort was successful, enabling the campus to remain functional during the ongoing COVID-19 pandemic. Regular screening, which depends heavily on participant compliance, allowed the resumption of regular workplace activity with enhanced safety with respect to COVID-19 infection.

Conclusion

We present a comprehensive study where we validate a saliva-based screening protocol in India, adding to global evidence supporting its use as a source sample for SARS-CoV-2 detection. We emphasise that saliva is an excellent cost-effective alternative sample type for SARS-CoV-2 screening, and recommend sample processing without significant delay. We hope this study can serve as an example and provide guidelines to set up saliva-based testing protocols. Setting up such rapid and efficient approaches in large establishments, including schools and industries, will facilitate their safe functioning during public health emergencies from any respiratory pathogens.

CRediT author statement

B.R. Ansil: Conceptualization, Data curation, Analysis, Writing - Original Draft, Writing - Review and Editing. **Carolin Elizabeth George:** Conceptualization, Data curation, Analysis, Writing - Original Draft, Writing - Review and Editing. **Sindhulina Chandrasingh:**

Conceptualization, Data curation, Writing - Original Draft, Writing - Review and Editing. **Ashwin Viswanathan:** Conceptualization, Analysis, Writing - Original Draft, Writing - Review and Editing. **Mukund Thattai:** Conceptualization, Methodology, Analysis, Writing - Original Draft, Writing - Review and Editing. **Padinjat Raghu:** Conceptualization, Methodology, Writing - Original Draft, Writing - Review and Editing. **Santhosha Devadiga:** Investigation, Data curation. **Arun Geetha Harikumar:** Investigation, Data curation, Writing - Review and Editing. **Pulleri Kandi Harsha:** Investigation, Data curation. **Indu Nair:** Conceptualization, Data curation. **Uma Ramakrishnan:** Conceptualization, Writing - Original Draft, Writing - Review and Editing, Supervision. **Satyajit Mayor:** Conceptualization, Writing - Review and Editing and Supervision.

Conflict of Interest

The authors declare that they have no conflict of interest.

Acknowledgements

This work was primarily supported by NCBS/TIFR core fund, and we acknowledge the support of the Department of Atomic Energy (DAE), Government of India, under Project Identification No. RTI 4006; and philanthropic support from the Azim Premji Foundation. We acknowledge the CCAMP-InDx program for facilitating the saliva collection receptacle and transport medium from Kriyamed (https://www.kriyamed.com/) for campus screening. We are grateful to COVID-19 testing laboratory staff and InStem for helping with sample processing and RT-PCR testing; Mr. Prem Chandra Gautam, technical and research services teams at NCBS for their assistance in implementing saliva testing. We thank Prof. Apurva Sarin and Prof. Colin Jamora for facilitating the project. ABR is supported by the SPM fellowship by CSIR-HRDG. UR is a Senior fellow DBT-Wellcome Trust India Alliance (IA/S/16/2/502714). SM is a JC Bose Fellow of the Department of Science and Technology, Government of India, and a Margadarshi Fellow of the DBT-Wellcome Trust India Alliance (IA/M/15/1/502018).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijmmb.2023.100384.

References

[1] Ministry of Health and Family Welfare, Government of India. https://www.mohfw.gov.in. [Accessed 10 December 2021].
[2] Sethuraman N, Jeremiah SS, Ryo A. Interpreting diagnostic tests for SARS-CoV-2. JAMA 2020;323:2249. https://doi.org/10.1001/jama.2020.8259.
[3] Lee RA, Herigon JC, Benedetti A, et al. Performance of saliva, oropharyngeal swabs, and nasal swabs for SARS-CoV-2 molecular detection: a systematic review and meta-analysis. J Clin Microbiol 2021;59:17.
[4] Wang H, Liu Q, Hu J, et al. Nasopharyngeal swabs are more sensitive than oropharyngeal swabs for COVID-19 diagnosis and monitoring the SARS-CoV-2 load. Front Med 2020;7:334. https://doi.org/10.3389/fmed.2020.00334.
[5] Vogels CBF, Watkins AE, Harden CA, et al. SalivaDirect: a simplified and flexible platform to enhance SARS-CoV-2 testing capacity. Medicine 2021;2:263–280.e6. https://doi.org/10.1016/j.medj.2020.12.010.
[6] Ibrahimi N, Delaunay-Moisan A, Hill C, et al. Screening for SARS-CoV-2 by RT-PCR: saliva or nasopharyngeal swab? Rapid review and meta-analysis. PLoS One 2021;16:e0253007. https://doi.org/10.1371/journal.pone.0253007.
[7] Azmi I, Faizan MI, Kumar R, et al. A saliva-based RNA extraction-free workflow integrated with Cas13a for SARS-CoV-2 detection. Front Cell Infect Microbiol 2021;11:632646. https://doi.org/10.3389/fcimb.2021.632646.
[8] Bastos ML, Perlman-Arrow S, Menzies D, Campbell JR. The sensitivity and costs of testing for SARS-CoV-2 infection with saliva versus nasopharyngeal swabs: a systematic review and meta-analysis. Ann Intern Med 2021;174:501–10. https://doi.org/10.7326/M20-6569.
[9] Bhattacharya D, Parai D, Rout UK, et al. Saliva for diagnosis of SARS-CoV-2: first report from India. J Med Virol 2021;93:2529–33. https://doi.org/10.1002/jmv.26719.
[10] Woloshin S, Patel N, Kesselheim AS. False negative tests for SARS-CoV-2 infection — challenges and implications. N Engl J Med 2020;383:e38. https://doi.org/10.1056/NEJMp2015897.
[11] Wilcoxon F. Individual comparisons by ranking methods. Biomed Bull 1945;1:80. https://doi.org/10.2307/3001968.
[12] Butler-Laporte G, Lawandi A, Schiller I, et al. 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. https://doi.org/10.1001/jamainternmed.2020.8876.
[13] Matic N, Stefanovic A, Leung V, et al. Practical challenges to the clinical implementation of saliva for SARS-CoV-2 detection. Eur J Clin Microbiol Infect Dis 2021;40:447–50. https://doi.org/10.1007/s10096-020-04090-5.
[14] Pasomsub E, Watcharananan SP, Watthanachockchai T, et al. Saliva sample pooling for the detection of SARS-CoV-2. J Med Virol 2021;93:1506–11. https://doi.org/10.1002/jmv.26460.
[15] Wu X, Wang Z, He Z, et al. A follow-up study shows that recovered patients with repositive PCR test in Wuhan may not be infectious. BMC Med 2021;19:77. https://doi.org/10.1186/s12916-021-01954-1.
[16] Wong J, Koh WC, Momin RN, et al Probable causes and risk factors for positive SARS-CoV-2 test in recovered patients: evidence from Brunei Darussalam. 5.
[17] Wyllie AL, Fournier J, Casanovas-Massana A, et al. Saliva or nasopharyngeal swab specimens for detection of SARS-CoV-2. N Engl J Med 2020;383:1283–6. https://doi.org/10.1056/NEJMc2016359.
[18] Uwamino Y, Nagata M, Aoki W, et al. Accuracy and stability of saliva as a sample for reverse transcription PCR detection of SARS-CoV-2. J Clin Pathol 2021;74:67–8. https://doi.org/10.1136/jclinpath-2020-206972.
[19] Yokota I, Hattori T, Shane PY, et al. Equivalent SARS-CoV-2 viral loads by PCR between nasopharyngeal swab and saliva in symptomatic patients. Sci Rep 2021;11:4500. https://doi.org/10.1038/s41598-021-84059-2.
[20] Larremore DB, Wilder B, Lester E, et al. Test sensitivity is secondary to frequency and turnaround time for COVID-19 screening. Sci Adv 2021;7:eabd5393. https://doi.org/10.1126/sciadv.abd5393.
[21] Medeiros da Silva RC, Nogueira Marinho LC, de Araújo Silva DN, et al. Saliva as a possible tool for the SARS-CoV-2 detection: a review. Trav Med Infect Dis 2020;38:101920. https://doi.org/10.1016/j.tmaid.2020.101920.
[22] Perchetti GA, Sullivan K-W, Pepper G, et al. Pooling of SARS-CoV-2 samples to increase molecular testing throughput. J Clin Virol 2020;131:104570. https://doi.org/10.1016/j.jcv.2020.104570.
[23] Watkins AE, Fenichel EP, Weinberger DM, et al. Pooling saliva to increase SARS-CoV-2 testing capacity. In: Infectious diseases (except HIV/AIDS); 2020.