Journal of Infectious Diseases & Therapy
ISSN: 2332-0877

Research Article Open Access

Salivary Rt-Pcr Can Be A Valid Frontline Non Invasive Diagnostic Test For Detection Of Covid-19.

Rashmi[1], Vikas Deswal[2], Teena Wadhwa[1], Pooja Sharma[3], Manish K Singh[3], Smita Sarma[3], Sushila Kataria[1,2*]

[1]*Department of Microbiology, Medanta-The Medicity, Gurugram, India*
[2]*Division of Internal Medicine, Medanta-The Medicity, Gurugram, India*
[3]*Medanta Institute of Education and Research, Medanta-The Medicity, Gurugram, India*

Abstract

Background: Cases of pneumonia of unknown aetiology were reported in Wuhan, China in December 2019. The disease was identified to be caused by Severe Acute Respiratory Syndrome Corona Virus-2 (SARS-CoV-2) and was named Corona Virus Disease 2019 (COVID-19).

Methods: The samples (salivary and NPS) were randomly collected from 60 adults, who were clinically suspected of COVID-19 infection and reported to the hospital. Both samples were collected on day 0 and repeat sampling was done between day 7 – day 14 for all the participants. All the samples were run for rRT-PCR. A Visual Analog Score form was used to evaluate patient comfort with the procedure.

Results: Sixty participants were enrolled in this study which included 35 males and 25 females. The median age was 32 years (IQR: 25-51 years). A positive test by either sample route (saliva/NPS) tested by rRT-PCR was considered as true positive and considered as reference standard. The 19 participants whose NPS tested negative for COVID-19, also tested negative with saliva (True negatives). Out of 41 positive cases (True positives), 40(97.5%) were accurately diagnosed as SARS Co-V-2 positive with saliva and 37(90.2%) with NPS. With saliva samples the sensitivity was 97.56%, specificity was 100%, positive predictive value was 100%, negative predictive value was 95% and accuracy was 98.33%. Whereas with NPS the sensitivity was 90.24%, specificity was 100%, positive predictive value was 100%, negative predictive value was 82.61% and accuracy was 93.33%. The Ct values of both the sampling methods were comparable. Saliva was also found to be more consistent. 70% of the participants found saliva as the preferred method of sampling.

Conclusion: Saliva is a viable, more sensitive, consistent and non-invasive method of sampling for COVID-19. It can be further evaluated as a valid frontline non-invasive diagnostic test for detection of COVID-19.

Keywords: Saliva; COVID-19; Diagnostic test

Abbreviations: WHO: World Health Organization; SARS-CoV-2 – Severe Acute Respiratory Syndrome Corona Virus 2; COVID-19: Corona Virus Disease 2019; CoVs: Corona viruses; ACE 2: Angiotensin-Converting Enzyme 2; SARS CoV: Severe Acute Respiratory Syndrome Corona Virus; rRT-PCR: real time Reverse Transcriptase PCR; NPS: Nasopharyngeal Swab; OPS: Oropharyngeal Swab; PPE: Personal Protective Equipment; OPD: Out Patient Department; IPD: In Patient Department; NABL: National Accreditation Board of Laboratories; BSL-2: Biosafety Level 2; Ct: Cycle Threshold; ICMR: Indian Council of Medical Research; CDC: Centre for Disease Control and Prevention; SOP: Standard Operating Procedures; VTM: Viral Transport Medium; TNA: Total Nucleic Acid; PCR: Polymerase Chain Reaction; SARI: Severe Acute Respiratory Infection; ICU: Intensive Care Unit; IQR: Inter Quartile Range; HTN: Hypertension; DM: Diabetes Mellitus; Sn: Sensitivity; Sp: Specificity; NPV: Negative Predictive Value; PPV: Positive Predictive Value; US-FDA: United States Food and Drug Administration

Introduction

The World Health Organization (WHO) reported cases of pneumonia of unknown aetiology in Wuhan city, China in December 2019. The disease was identified to be caused by a novel corona virus which was later named as Severe Acute Respiratory Syndrome Corona Virus-2 (SARS-CoV-2) and the disease was named Corona Virus Disease 2019 (COVID-19). It has been declared as a pandemic by the WHO [1].

The Severe Acute Respiratory Syndrome Corona Virus (SARS-CoV), responsible for the 2003 epidemic and SARS CoV-2 share 80% sequence homology, but SARS CoV-2 is much more contagious [2]. The novel corona virus also employs the host cell angiotensin-converting enzyme-2 (ACE-2) as the main host receptor for cellular entry, like SARS- CoV [3]. Studies have shown higher levels of ACE 2 expression in salivary glands compared with that in the lungs [4]. SARS-CoV was also detected in saliva samples [5]. This suggested that the salivary glands could be a potential target for SAR-CoV-2 infection, and hence saliva could be a potential sample for SARS-CoV-2 detection [6].

Accurate and rapid diagnostic testing is a key factor to control the spread of pandemic caused by the novel corona virus [7]. Real Time Reverse Transcriptase Polymerase Chain Reaction (rRT-PCR) on respiratory specimens like Oropharyngeal Swab (OPS), Nasopharyngeal Swab (NPS), bronchoalveolar lavage and tracheal aspirates, is the current gold standard test for detection of SARS Co-V-2. The most commonly used sample type is NPS which has been found to be inconsistent during serial testing and has relatively poor sensitivity in early infection [7].

The collection of NPS has various disadvantages like it requires close contact between the healthcare worker performing the collection and the patient. This exposes the healthcare workers to aerosols generated due to sneezing and coughing by the patient during sample collection, putting them at high risk. The other disadvantages are due to invasiveness of the procedure, which include the discomfort caused and risk of bleeding in thrombocytopenic patients. Also in this state of

***Corresponding author:** Sushila Kataria, Division of Internal Medicine, Medanta-The Medicity, Gurugram, Haryana, India, Tel: +91- 9953069025; E-mail: Sushila.kataria@medanta.org

Received: September 25, 2020; **Accepted date:** October 09, 2020; **Published date:** October 16, 2020

Citation: Rashmi, Deswal V, Wadhwa T, Sharma P, Singh M K, et al. (2020) Salivary Rt-Pcr Can Be A Valid Frontline Non Invasive Diagnostic Test For Detection Of Covid-19. J Infect Dis Ther S2:007.

health emergency, where we are facing challenges with the supply of swabs and Personal Protective Equipment (PPE) for healthcare workers this method further limits us to perform large scale testing [7]. Thus, keeping in view all these points and overburdened testing centres there is a need to explore other sample types for detecting the virus.

With this prospective observational study we aimed to compare the sensitivity and specificity of paired salivary and NPS, rRT-PCR with total rRT-PCR as the reference standard for diagnosis of COVID-19 infection.

The study was approved by the Institutional Review Board and Ethics Committee of Medanta-The Medicity. All authors vouch for correctness of data.

Materials and Methods

It is a prospective observational study conducted at a tertiary care 1500 bedded hospital, in the National Capital Region, Delhi over a period of 40 days (21.05.2020-30.06.2020). The samples (salivary and NPS) were randomly collected from 60 adults, who reported at the Out Patient Department (OPD) and In Patient Department (IPD) of the hospital and were clinically suspected of COVID-19 infection. A written informed consent was taken from all participants. The patients who were on drugs affecting saliva production like anticholinergics, had xerostomia or any active oral disease or injury and mechanically ventilated or critically ill patients were excluded from the study. The samples were collected at the baseline (day 0) and positives were repeated between 7-14 days.

All the samples were run for rRt-PCR for SARS CoV-2 at the same Indian Council of Medical Research (ICMR) approved laboratory and their Cycle Threshold (Ct) values were recorded. Ct value is defined as the number of cycles when the sample fluorescence exceeds a chosen threshold above the calculated background fluorescence [8].

A Visual Analog Score form (scale 1-5, with 1 as very uncomfortable and 5 as comfortable) was used to evaluate patient comfort with the sample collection procedure (both NPS and saliva).

Sample collection and processing

The samples were collected with all aseptic precautions in accordance with the current institutional Standard Operating Procedures (SOP). NPS was collected by a trained health care professional using a flexible, flocked swab which was passed through the patient's nostril until the posterior nasopharynx was reached. It was left for 10 seconds in place to absorb secretions and removed slowly while rotating. The swab was put in 3 ml sterile Viral Transport Medium (VTM) and sealed securely.

The participant was requested not to eat, drink, smoke or chew gum for at least 30 minutes before giving saliva sample. Prior to spitting, participant was asked to relax and rub cheeks gently for 30 seconds to make saliva. The sample of 2-3 ml saliva (minus any bubbles that form on the top of the vial) took around 5 minutes to collect. Participants self-collected the saliva sample using the drooling technique, in a sterile vial containing 3ml of VTM, which was sealed and shared with the laboratory personnel. No special saliva collection devices were used. Both sample types were processed within 4 hours of collection.

Processing of samples

Total nucleic acid was extracted from 200 µl of inoculated VTM from the NP swab or saliva samples using the Maxwell Total Nucleic Acid (TNA) extraction kit on Promega automated extraction system following the manufacturer's protocol. From each sample processed, 5 µl of elute was used for reaction set up using TaqPath COVID-19 combo kit. One negative control, 1 positive control and 1 internal control (MS2 phage as an extraction control) was processed with each run. After Polymerase Chain Reaction (PCR) reaction is set up, the reaction plate is run in Thermofisher Quantstudio-5X for analysis of results. The kit provided 3 genes for detection-ORF1ab, N and S.

Samples were classified as positive for SARS-CoV-2 when all or any 2 of the 3 (ORF1ab, N and S) genes detected with<40 Ct value.

Statistical methods

The analysis included profiling of patients on different demographic, clinical and laboratory parameter. Distribution of quantitative parameters were assessed using the Kolmogorov–Smirnov test. Descriptive analysis of quantitative parameters were expressed as median and Inter Quartile Range (IQR). Categorical data were expressed as absolute number and percentage. Wilcoxon Signed Ranks Test was used for testing of median. The diagnostic accuracy of NPS and saliva were evaluated in terms of sensitivity, specificity, negative predictive value, positive predictive value and accuracy with respect to the reference standard total rRT-PCR. Agreement between NPS rRT-PCR and salivary rRT-PCR with total rRT-PCR was assessed by Cohen's kappa statistics. P-value<0.05 was considered statistically significant. All analysis was done using SPSS software, version 24.0.

Sample size

Assuming the agreement between paired NPS rRT-PCR and salivary rRT-PCR with total rRT-PCR test is about 90%. Under these assumptions the minimum sample size calculated was 60 with 99% confidence level and 10% precision.

Results

A total of 60 participants were included in this study. In all, 210 samples were collected from 60 participants including the follow-up sampling done between day 7-day 14 for positives. (Table 1) depicts the characteristics of participants enrolled in the study. The median age of the participants was 32 years (IQR: 25-51 years) with 35(58.3%) males and 25(41.7%) females.

Characteristics	Number, (n=60)	Percentage (%)
Age (years), median (IQR)	32(25-51)	
Gender		
Male, n	35	58.30%
Female, n	25	41.70%
Onset of symptoms before the test (days), median (IQR)	2(1-2)	
Symptoms at presentation		
Dysguesia	3	5%
Anosmia	4	6.67%
Fever	37	61.67%
Cough	16	26.67%
Running Nose	8	13.33%
Bodyache	10	16.67%
Sore throat	12	20%
Shortness of breath	6	10%
Headache	3	5%

Table 1: Characteristics of participants enrolled in the study.

The median number of days for onset of symptoms before the first test was 2 days (IQR:1-2 days). The most common symptom being fever seen in 37(61.67%) followed by cough-16 (26.67%), sore

throat – 12(20%), bodyache-10(16.67%), running nose – 8(13.33%), breathlessness-6 (10%), anosmia-4(6.67%, dysguesia – 3(5%) and headache -3(5%).

Co-morbidities were seen in 14(23.3%) cases. The most common co-morbidities were hypertension and Diabetes Mellitus seen in 7(11.7%) cases.

A positive test by either sample route (saliva/NPS) tested by rRT-PCR was considered as true positive and the numerical sum of positive results with both sampling methods was used as the reference standard. The 19 patients whose NPS tested negative for COVID-19 also tested negative with saliva. These were considered as true negatives. Out of 41 positive cases (true positives), 40(97.5%) were accurately diagnosed as COVID-19 positive with saliva and 37 (90.2%) with NPS. Thus, 4 COVID-19 cases were missed with NPS while only 1 was missed with saliva.

Thus, salivary samples identified 3(7.3%) additional COVID positive cases as compared to NP samples (Tables 2 and 3).

Saliva rRT-PCR	Reference standard (Total rRT-PCR)		
	Test Positive	Test Negative	Total
Test Positive	40	0	40(66.7%)
Test Negative	1	19	20(33.3%)
Total	41(68.3%)	19(31.7%)	60(100.0%)

Table 2: Detection of SARS Co-V-2 using saliva rrt-PCR.Out of the 41

NPS rRT-PCR	Reference standard (Total rRT-PCR)		
	Test Positive	Test Negative	Total
Test Positive	37	0	37(61.7%)
Test Negative	4	19	23(38.3%)
Total	41(68.3%)	19(31.7%)	60(100.0%)

Table 3: Detection of SARS Co-V-2 using NPS rrt-PCR.

positive cases, 40 had mild/moderate disease while one patient, 72 years old male developed Severe Acute Respiratory Infection (SARI) and required Intensive Care Unit (ICU) admission during the course of disease. None of the study participants required mechanical ventilation and no death was reported.

To determine the diagnostic performance of saliva and NPS tests, the cumulative positive rRT-PCR results of both samples were used as the reference standard and the sensitivity and specificity of saliva and NPS, rRT-PCR were calculated separately. Tables 2 and 3 depict the diagnostic performance of salivary rRT-PCR and NPS rRT-PCR.

The sensitivity of salivary rRT-PCR samples was 97.56% (95%CI: 87.14% to 99.94%), specificity was 100.00% (95%CI: 82.35% to 100.00%), positive predictive value was 100.00%, negative predictive value was 95.00% (95%CI: 73.27% to 99.25%) and accuracy was 98.33%(95%CI: 91.06% to 99.96%). The overall agreement for virus detection between the two methods salivary rRT PCR and total rRT PCR was 96.2%(95%CI: 88.8-100.0).

Whereas the sensitivity of NPS rRT-PCR was 90.24%(95%CI: 76.87% to 97.28%), specificity was 100.00%(95%CI: 82.35% to 100.00%), positive predictive value was 100.00%, negative predictive value was 82.61%(95%CI: 65.18% to 92.34%) and accuracy was 93.33%(95%CI: 83.80% to 98.15%). The overall agreement for the virus detection between the two methods NPS rRT PCR and total rRt-PCR was 85.4%(95%CI: 71.8-99.1) (Table 4).

Statistical parameter	Saliva rRT-PCR		NPS rRT-PCR	
	Value	95% CI	Value	95% CI
Sensitivity	0.9756	87.14% to 99.94%	0.9024	76.87% to 97.28%
Specificity	1	82.35% to 100.00%	1	82.35% to 100.00%
Positive Predictive Value	1		1	
Negative Predictive Value	0.95	73.27% to 99.25%	0.8261	65.18% to 92.34%
Accuracy	0.9833	91.06% to 99.96%	0.9333	83.80% to 98.15%
kappa coefficient	0.962	0.888 to 1.000	0.854	0.718 to 0.991

Table 4: Diagnostic Test evaluation parameters.

Thus, the sensitivity, negative predictive value and accuracy of saliva samples were higher than NPS. Hence, we can say that salivary rRT-PCR is more sensitive and accurate as compared to NPS. Salivary method also showed better agreement with total rRT-PCR than NPS.

With NPS, the median (IQR) Ct values of the N gene was 22.8(IQR: 19.8-24.6), ORF1ab gene was 21.6(IQR: 17.6-25.6) and S gene was 21.7(IQR: 18.8-24.7 at the time of diagnosis while in saliva specimens the median (IQR) Ct values of the N gene was 20.6(IQR: 18.4 – 25.2), of ORF1ab gene was 22.5(IQR: 18.4-24.2) and of S gene was 21.2(IQR: 17.6-25.9). At follow-up testing, with NPS the median (IQR) Ct values of the N gene was 29.6(26.9-30.8), ORF 1ab gene was 30.6(28.6-31.7) and S gene was 30.4(27.3-32.0) while in saliva specimens the median (IQR) Ct values of the N gene was 30.4(24.6-32.4), ORF 1ab gene was 30.3(26.8-32.3) and S gene was 31.2(26.0-32.4). No statistically significant difference was seen in the median Ct values of N, ORF1ab and S genes at the time of diagnosis and follow up testing for both NPS and saliva, rRT-PCR (Tables 5 and 6).

Table 5 and 6 depict the median (IQR) Ct values of positive cases for NPS and saliva, rRT-PCR for N, ORF1ab and S genes at the time of diagnosis and at follow-up testing.

Out of 36 positive cases with both NPS and salivary samples on day 0, 8 tested positive on follow up testing with both NPS and saliva samples and 27 tested negative. One patient tested positive with saliva on the same day of follow up testing, when his NPS showed conversion to negative.

Type of sample and gene	Ct value NPS (n=36) Median (IQR)	Ct value saliva (n=39) Median (IQR)	Type of sample and gene
N gene	22.8(19.8-24.6)	20.6(18.4-25.2)	0.118
ORF1ab gene	21.6(17.6-25.6)	22.5(18.4-24.2)	0.975
S gene	21.7(18.8-24.7)	21.2(17.6-25.9)	0.307
*p-value<0.05, statistically significant			

Table 5: Mean Ct values of cases for NPS and saliva, rrt-PCR for N, ORF1ab and S genes at the time of diagnosis.

Type of sample and gene	Ct value NPS (n=8) Median (IQR)	Ct value saliva (n=8) Median (IQR)	p-value Value
N gene	29.6(26.9-30.8)	30.4(24.6-32.4)	0.401
ORF1ab gene	30.6(28.6-31.7)	30.3(26.8-32.3)	0.889
S gene	30.4(27.3-32.0)	31.2(26.0-32.4)	0.917
*p-value<0.05, statistically significant			

Table 6: Mean Ct values for NPS and saliva, rrt-PCR for N, ORF1ab and S genes at follow up testing.

Discussion

This is a prospective observational study which compared paired NPS and salivary rRT-PCR with total rRT-PCR as the reference standard for detecting SARS CoV-2 in 60 participants.

In the present study, salivary samples had a higher sensitivity and accuracy as compared to NPS for detecting SARS CoV-2. The sensitivity of salivary rRT-PCR was 97.56% as compared to 90.24% for NPS rRT-PCR while the accuracy of saliva samples was 98.33% and only 93.33% for NPS.

In a pionner study from Hong Kong by To et al., SARS-CoV-2 was detected in 11 out 12 laboratory confirmed COVID-19 patients' saliva, and the 33 participants who were negative with NPS were also negative with salivary samples [9]. Similar, findings were also seen in our study where the virus was identified in all but 1 participant who was positive only with NPS. The patient tested negative with saliva even on repeat testing 2 days later. Also there were 4 instances where saliva could detect the SARS Co-V-2 virus and the NPS could not. The researchers at Yale University, USA also detected SARS Co-V-2 virus from saliva and not in NPS from 8 participants. Pasomsub et al. and Azzi et al. also demonstrated similar results in their study [2,6,7].

Of the 4 patients who tested positive only with saliva in this study, 2 patients tested negative with NPS on day 0 of testing while positive with saliva on the same day. Both patients were again tested on day 2 and the results were consistent. The Ct values of salivary samples were also similar on both occasions. Interestingly, in other 2 patients on repeat sampling 2 days later NPS also turned positive. This proves NPS to be a less consistent sample type in comparison to saliva due to differing quality of NPS collection. The Yale researchers also found NPS to be inconsistent for detection of the virus [7]. All 4 participants were treated as COVID-19 patients according to the standard hospital protocol. All of them recovered and were discharged in stable condition. It is important for a sample type to be consistent for correct and early diagnosis of COVID-19 to contain the pandemic.

Moreover, one case was still positive with saliva on day 12 of testing when his NPS showed conversion to negative. Azzi et al. also reported a similar scenario in their study [2].

Mean Ct values in NP and salivary samples were comparable on both the occasions i.e. at day 0 and on follow up testing between day 7 day 14. Similar results have also been seen by Pasomsub et al. in their study, while the researchers at Yale University reported 5 times higher viral load in saliva [6,7]. In our study, Ct values were higher on follow up testing in all the cases which indicates that the viral load declined from baseline.

70%(42) of the study participants found salivary sampling method as a more comfortable, convenient and easy for compliance method of sampling as it is non-invasive and also they had a shorter waiting time at the collection centre.

Collection of saliva samples for testing has several advantages. Firstly, it is better accepted by the participant due to the simplicity of acquiring samples, secondly it can be self collected by the patient at the comfort of their home and hence reducing the waiting time and preventing overcrowding of testing centres. This is particularly important for busy clinical settings where a larger population is to be screened. Third and major advantage is it minimizes nosocomial spread of infection due to minimal contact between health care worker and individual. Finally, its cheaper since it alleviates the use of PPE and swabs [10]. Saliva as a valid sample type has been approved by the United States Food and Drug Administration (US-FDA) and an emergency use authorisation has been issued by them [11].

Conclusion

The present study proves saliva to be a viable, more sensitive, accurate, consistent and non-invasive method of sampling for diagnosing COVID-19. In resource limited setting like ours, where the cost of PPE and swabs are putting an extra burden on the health care system, we should consider alternate sample types like saliva which are also cost-effective, safer and more sensitive for detecting SARS Co-V-2 virus. We assert that saliva can be further evaluated as a valid frontline non-invasive diagnostic test for detection of COVID-19.

Limitation

Only symptomatic mild and moderate cases were included in the study. It needs further validation for severe and asymptomatic cases as well on a larger sample size.

Disclosures

No conflict of interest.

Funding

The research was funded by the Medanta Institute of Education and Research.

Acknowledgement

The authors gratefully acknowledge all the respected colleagues fighting the COVID-19 pandemic at each level.

References

1. Gupta N, Potdar V, Praharaj I, Giri S, Sapkal G, et al. (2020) Laboratory preparedness for SARS-cov-2 testing in India: Harnessing a network of Virus Research & Diagnostic Laboratories. Indian J Med Res 151:216-225.
2. Azzi L, Carcano F, Gianfagna F, Grossi P, Gasperina D, et al. (2020) Saliva as a reliable tool to detect SARS-cov-2. J Infect 81:e45-e50.
3. Hoffmann M, Kleine-Weber H, Schroeder S, Kruger N, Herrler T, et al. (2020) SARS-cov-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell 18:271-280.
4. Xu J, Li Y, Gan F, Du Y, Yao Y (2020) Salivary glands: Potential reservoirs for COVID-19 asymptomatic infection. J Dent Res 99:989.
5. Wang WK, Chen SY, Liu IJ, Chen YC, Chen HL, et al. (2004) Detection of SARS associated coronavirus in throat wash and saliva in early diagnosis. Emerg Infect Dis 10:1213-1219.
6. Pasomsub E, Watcharananan SP , Boonyawat K, Janchompoo P , Wongtabtim G, et al. (2020) Saliva sample as a non-invasive specimen for the diagnosis of coronavirus disease 2019: A cross sectional study. Clin Microbiol Infect.
7. Wyllie AL, Fournier J, Casanovas-Massana A, Campbell M, Tokuyama M, et al. (2020) Saliva is more sensitive for SARS-cov-2 detection in COVID-19 patients than nasopharyngeal swabs. Medrxiv.
8. Chang MC, Hur J, Park D (2020) Interpreting the COVID-19 test results. Am J Phys Med Rehabil 99:583-585.
9. KK, Tsang OT, Chik-Yan Yip C, Chan KH, Wu TC, et al. (2020) Consistent detection of 2019 novel coronavirus in saliva. Clin Infect Dis.
10. Vaz SN, Santana DS, Netto EM, Pedroso C, Wang WK, et al. (2020) Saliva is a reliable, non-invasive specimen for SARS-CoV-2 detection. Braz J Infect Dis.
11. Bajaj N, Granwehr BP, Hanna EY, Chambers MS (2020) Salivary detection of SARS-CoV-2 (COVID-19) and implications for oral health-care providers. Head Neck 42:1543-1547.