# Diagnostic Accuracy of Saliva for Molecular Detection of SARS CoV-2 for Diagnosis of COVID 19: a Comparative Study

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**Abstract:** Throat swab, Nasal swab, Nasopharyngeal swab, Bronchoalveolar lavage fluid and Endotracheal aspirate are the recommended samples by Indian Council of Medical Research for molecular detection of SARS-CoV-2. Saliva specimen is less invasive, can be collected by oneself thus minimizing the risk to the healthcare workers to SARS-CoV-2 exposure. The present study was aimed to evaluate better possible sample collection method among nasal swab, Throat swab and saliva samples of patients for precise COVID-19 diagnosis.

A prospective study conducted for diagnosis of COVID-19 samples. three type of samples Nasal and Throat swab and saliva collected for COVID-19 diagnosis. If sample was found positive then Throat and nasal swabs were collected every alternative day while saliva collected every day for COVID-19 diagnosis by RT-PCR.

172 samples of patients were found to be positive for COVID-19. 103 (59.88 %) males and 69 (40.11 %) were females. Throat and Nasal swabs were collected every alternative day. Nasal swab was the best method of sample collection and gave most effective results followed by throat swab in comparison with the saliva sample.

Saliva specimen showed positivity only for upto 3 days, while TS and NS specimens showed higher positivity in comparison with saliva specimens for upto 7 days. Further research on usefulness of less invasive saliva specimen is required.

Keywords: Saliva specimen, RT-PCR, COVID-19.

## 1. INTRODUCTION

An outbreak of pneumonia with an unknown cause emerged in Wuhan, Hubei (China) in a cluster of patients with community acquired pneumonia in early December 2019. This outbreak was later confirmed to be caused by a new coronavirus infection and International committee on Taxonomy of Viruses (ICTV) on 10 January 2020, named this virus as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [2]. Molecular detection by Real time Polymerase chain reaction (RT-PCR) based nucleic acid detection are the most effective method for the diagnosis of suspected COVID-19 patients. Throat swab (TS), Nasal swab (NS), Nasopharyngeal swab, Bronchoalveolar lavage fluid (BAL) and Endotracheal aspirate are the recommended samples by Indian Council of Medical Research (ICMR) for molecular detection of SARS-CoV-2 [8].

The collection of these specimens requires close contact between healthcare workers and patients thus increasing the biosatety risk to healthcare workers through creation of droplets. Moreover, the collection of above mentioned

specimens requires some degree of skills by healthcare workers [9]. Viral transport medium (VTM) is required which can be additional burden in resource poor settings or remote areas in vast countries like India or china. Also the above mentioned specimens may cause discomfort to patients as well complications like bleeding in targeted tissues can be a problem especially in thrombocytopenic individuals [9]. Saliva specimen on the other hand is less invasive, can be collected by oneself thus minimizing the risk to the healthcare workers to SARS-CoV-2 exposure [4]. Since, SARS-CoV-2 infection is transmitted by salivary droplets, testing of viral RNA using salivary specimen can be used an alternative to NS or TS specimens which are considered as the gold standard specimens [4,5].

Since, viral pneumonia typically do not yield production of purulent sputum hence, the present study was conducted to assess the diagnostic accuracy of saliva in comparison with nasopharyngeal swab, nasal swab, throat consumables like swab sticks, swab for molecular detection of SARS-CoV-2.

### Aims and Objectives

The present study was aimed to evaluate better possible sample collection method among nasal swab (NS), Throat swab (TS) and saliva samples (SS) of patients for precise COVID-19 diagnosis.

### Objectives

1. To study the positivity rate in saliva samples for detection of SARS CoV-2 for diagnosis of COVID -19.
2. To compare the Ct value between positive RT-PCR test for saliva sample in comparison with nasopharyngeal swab, NS and TS for detection of SARS CoV-2 for diagnosis of COVID-19.
3. To study clinical features of COVID 19 patients tested positive by RT-PCR test.

## 2. MATERIALS AND METHODS

This was a prospective study conducted for diagnosis of COVID-19 samples received at Virology Laboratory, Chhindwara Institute of Medical Sciences (CIMS), Chhindwara, Madhya Pradesh, India. The present study was conducted during third wave of COVID-19 from January 2022 to March 2022. All the diagnosis of collected samples was done at Virology Laboratory, CIMS. This study was reviewed and approved by the Institutional Ethical committee, Chhindwara Institute of Medical Sciences (CIMS), Chhindwara, Madhya Pradesh (IEC no. CIMS/Ethics Committee/2021/1166 dated 21/02/2022).

Before sample collection a consent form filed by the patient was taken and all the three type of samples NS, TS, and SS were collected for COVID-19 diagnosis from suspected patient. After first diagnosis of COVID-19 using TS, NS and SS of the patient if sample was found positive then respective confirmed COVID-19 positive patients included for further diagnosis of 7 day. TS and NS swabs were collected every alternative day while SS collected every day from the patient and diagnosed for COVID-19 by RT-PCR. Virology Laboratory each day tested ~2300 sample collected from the entire Chhindwara district of Madhya Pradesh including main city Chhindwara (rural and urban area). Total 21066 samples were collected from Chhindwara district and tested during the study period. Among them, the patient whose sample first time diagnosed as positive was included for further study with their convenience and consent.

### Diagnosis of COVID-19 virus

For viral RNA extraction, MagMAX Viral/Pathogen Nucleic Acid Isolation Kit (MVP I) (Thermo-fisher, USA) was used. All the collected samples were diagnosed using RT-PCR machine (Quant studio 5, Thermofisher). Genes2me multiplex RT-PCR kit (India) was used for diagnosis purpose as protocol provided by the manufacturer. All the other reagents used were molecular grade and purchased from Hi-media, India.

### Statistical Analysis

Individual study parameters were recorded in the Microsoft Office Excel sheet. Data presented as percentages, mean, standard deviations and chi square test. *p*-values with a significance level of <0.05 were recorded as statistically significant. Descriptive statistics were analyzed by Sigma plots (10.0 versions) and Origin 7.0 software.

## 3. RESULTS

Period, from 01 January 2022 to 31 March 2022 RT-PCR lab CIMS, Chhindwara tested total 21066 samples collected from only Chhindwara district (rural and urban Chhindwara area). Total 172 samples of patients were found to be positive for COVID-19 and they were from different age groups **(Table 1)**. Out of these 172 patients, 103 (59.88 %) males and 69 (40.11 %) were females. The youngest patient was 7 year old and the oldest patient was 85 year old. All the COVID-19 confirmed positive patients included in the study were in home isolation. The mean age of the participants was 39.62 ± 7.1 SD years. All the patients involved in this study were fully vaccinated with 2 dose of COVID-19 vaccine except age group from 1-18.

**Table 1:** Demographic characteristics of COVID-19 confirmed patients

| Characteristics | Variables | N (%) |
|---|---|---|
| Age group | 0-10 | 01 (0.58) |
| | 11-20 | 17 (9.88) |
| | 21-30 | 47 (27.32) |
| | 31-40 | 28 (16.27) |
| | 41-50 | 33 (19.18) |
| | 51-60 | 18 (10.46) |
| | 61-70 | 20 (11.62) |
| | 71-80 | 07 (4.06) |
| | 81-90 | 01 (0.58) |
| Mean age | 39.62 ± 7.1 | |
| Gender | Male | 103 (59.88) |
| | Female | 69 (40.11) |
| Admitted facility | Home isolation | 172 (100) |
| Vaccination | Male | 99 (57.55) |
| | Female | 65 (37.79) |

The purpose of this study was to find the better way that overcomes the irritating sample collection procedure of COVID-19 *via*, NS and TS swabs. All the three type of samples NS, TS, and SS collected for precise COVID-19 diagnosis each day from confirmed COVID-19 positive patient. TS and NS swabs were collected every alternative day to less hurt the patient while SS collected every day from the patient and diagnosed for COVID-19 by RT-PCR. **Table 2** showed that NS was the best method of sample collection because it gave most effective results than SS and TS showed second most precise results of COVID-19 diagnosis. However, SS showed the least precise or false negative results of COVID-19 diagnosis. In case of patients belongs to younger age 0-20 and from 21-40 age group SS sample showed negative results of COVID-19 diagnosis after 3 and 4 days, respectively. While, NS and TS swabs samples showed positive results of same patient after the diagnosis.

**Table 2:** Day wise diagnosis of Covid-19 positive patient's nasal swab (NS), throat swab (TS), and saliva sample (SS) samples

| Age Group | Patient number | Diagnosis of Covid-19 sample | | | | | | | | | | | | | | | *P* value |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Day 1 | | | Day 2 | | Day 3 | | Day 4 | | Day 5 | | Day 6 | | Day 7 | | |
| | | TS | NS | SS | SS | TS | NS | SS | SS | TS | NS | SS | SS | TS | NS | SS | |
| 0-10 | 01 | +++ | +++ | +++ | ++ | +++ | +++ | - | - | ++ | ++ | - | - | + | + | - | - |
| 11-20 | 17 | +++ | +++ | +++ | ++ | +++ | +++ | - | - | ++ | ++ | - | - | + | + | - | 0.023 |
| 21-30 | 47 | +++ | +++ | ++ | ++ | ++ | +++ | + | - | + | ++ | - | - | - | + | - | 0.022 |
| 31-40 | 28 | +++ | +++ | ++ | ++ | +++ | +++ | + | - | ++ | ++ | - | - | - | + | - | 0.026 |

| Age Group | Patient number | Diagnosis of Covid-19 sample | | | | | | | | | | | | | | | *P* value |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Day 1 | | | Day 2 | | Day 3 | | Day 4 | | Day 5 | | Day 6 | | Day 7 | | |
| | | TS | NS | SS | SS | TS | NS | SS | SS | TS | NS | SS | SS | TS | NS | SS | |
| 41-50 | 33 | +++ | +++ | +++ | ++ | +++ | +++ | ++ | + | ++ | +++ | - | - | + | + | - | 0.047 |
| 51-60 | 18 | +++ | +++ | +++ | ++ | +++ | +++ | ++ | + | ++ | +++ | - | - | + | +++ | - | 0.051 |
| 61-70 | 20 | +++ | +++ | +++ | +++ | +++ | +++ | +++ | ++ | +++ | +++ | ++ | + | ++ | +++ | + | 0.082 |
| 71-80 | 07 | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | ++ | + | ++ | +++ | + | 0.083 |
| 81-90 | 01 | +++ | +++ | +++ | +++ | +++ | +++ | +++ | ++ | +++ | +++ | ++ | + | + | ++ | - | - |

$^{+++}$ Ct value 18-22, $^{++}$ Ct value 23-28, $^{+}$ Ct value 29-34 and (-) negative. All the patients are asymptomatic and vaccinated except age group 0-10 and 11-18

## 4. DISCUSSION

COVID-19 diagnosis relies on molecular laboratory methods such as RT-PCR along with clinical and radiological findings of the suspected cases. RT-PCR is considered as a gold standard method for SARS CoV-2 viral antigen detection and is also a highly sensitive method for detection of nucleic acid sequence of SARS CoV-2. In India, various samples such as nasopharyngeal swabs (NPSs), oropharyngeal swabs (OPSs), bronchoalveolar lavage, tracheal aspirate, sputum are the recommended specimens as per ICMR for detection of SARS-CoV-2 virus 2 [8]. NPS and OPS were the standard specimens recommended for the diagnosis of COVID-19. However, these specimens have increased risk of exposure to healthcare worker, difficulty in self collection and extra burden of supply of personal protective equipments (PPE) in resource limited settings. On the other hand, saliva specimen can be collected easily and posing minimal risk to healthcare workers but less sensitive for diagnosis of COVID-19.

The youngest patient was 7 years old and the oldest patient was 85 years old and all are in home isolation **(Figure 1)**. The mean age of the participants was 39.62 ± 7.1 SD years. All the patients involved in this study were fully vaccinated with 2 dose of COVID-19 vaccine except age group from 1-18. SS showed least precise or false negative results of COVID-19 diagnosis and may bewilder or confuse the diagnosing lab and doctors **(Table 2)**. In case of patients belongs to younger age 0-20 and from 21-40 age group SS sample showed negative results of COVID-19 diagnosis after 3 and 4 days, respectively. While, NS and TS swabs samples showed positive results of same patient after COVID-19 diagnosis. These false negative results of COVID-19 diagnosis by using SS samples may lead to create critical condition for patient or may more spread the COVID-19 virus. In all the age groups the Ct value on all the consecutive days decreased for saliva specimen and from day 5 onwards to day 7 saliva specimen became negative in comparison to the TS and NS specimen which were still positive on day 7 but had decreased Ct value. The *p* value for all the specimens in all the age groups was not significant. The viral RNA concentration was highest during the early phase of illness gradually decreasing over time. Lai et al [6] and Jamal et al [3] have reported lower sensitivity of 68.7 % in deep-throat saliva specimens and 72 % in saliva samples in comparison with NPS specimens, respectively. Lower sensitivity of deep-throat saliva specimens and saliva samples 30 % to 50 % was demonstrated as compared with NPS specimens in the community setting [1]. Our findings were comparable with the previous studies. To et al [10, 11] have reported sensitivity of saliva specimen as 86.9 % and 91.7 % in comparison with NPS specimens. Liu et al [7] have reported that epithelial cells lining salivary gland ducts in the upper respiratory tracts of rhesus macaques were early target cells of SARS-CoV infection. Similarly, the present study also confirmed the diagnosis using TS and NS samples which were collected from upper respiratory tracts of patients. In patients with COVID-19, infection of salivary gland was suggested possibly by the presence of COVID-19 in saliva specimen though the saliva can be secreted either from major or minor salivary glands or these secretions can come down from nasopharynx or from lungs *via* cilia lining the airway [11]. However, more research is required to decipher the source of COVID-19 in saliva.

Limitations of the study included that specimens of severe and critically ill patients could not be processed. This study included the specimens of suspected COVID-19 disease only from the population of rural and urban Chhindwara. The sample size in our study was small and larger sample size would be necessary to get more insight about saliva as a specimen for COVID-19.

## 5. CONCLUSION

To conclude, saliva specimen showed positivity only for upto 3 days, while TS and NS specimens showed higher positivity in comparison with saliva specimens for upto 7 days. TS and NS can be used as standard specimens and NPS as the gold standard specimen. Further research on usefulness of less invasive alternative specimen combinations such as saliva specimen is still required.

### Data availability

ICMR Specimen Referral Form for COVID-19 (SARS-CoV2) and patient's medical record were used for data collection.

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### Author's Contributions

PCT has made substantial contribution to conception, design, acquisition, analysis, interpretation, drafting the manuscript. RKS has contributed in design, acquisition, analysis and drafting the manuscript. RU has contributed in analysis, interpretation and drafting the manuscript. HS has contributed in acquisition and drafting. All authors read and approved the final manuscript.

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### Declarations

**Ethical Approval:** This study was reviewed and approved by the Institutional Ethical committee, Chhindwara Institute of Medical Sciences (CIMS), Chhindwara, Madhya Pradesh (IEC no. CIMS/Ethics Committee/2022/623).

**Consent for publication –** Not applicable

**Conflicts of interest –** The author(s) declare no potential conflicts of interest.

**Competing Interest -** The author(s) declare that they have no competing interest.

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### Abbreviations

ICTV - International committee on Taxonomy of Viruses

SARS-CoV-2 - Severe acute respiratory syndrome coronavirus 2

RT-PCR – Real time Polymerase chain reaction

COVID-19 - Coronavirus disease 2019

TS - Throat swab

NS - Nasal swab

BAL - Bronchoalveolar lavage fluid

ICMR - Indian Council of Medical Research

CIMS – Chhindwara Institute of Medical Sciences

NPS – Nasopharyngeal swabs

OPS - Oropharyngeal swabs

PPE – Personal protective equipments

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