# To examine the RT-PCR patterns of the SARS-CoV-2 genetic material in individuals diagnosed with COVID-19

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**Abstract**
In the fourth year of the pandemic, we are still learning about viral dynamics, infectivity, and RT-PCR positivity. For this, the retrospective, observational study was conducted at MMG district hospital in Ghaziabad. COVID-19-positive patients were included in the current study. Age, gender, symptomatic and asymptomatic details, and SARS-CoV-2 RT-PCR results from nasooropharyngeal swabs obtained at various times were extracted from hospital lab records. Most of the 50,000 patients were male (55.1%). Furthermore, COVID-19 cases ranged from 15 to 63 years old, with a median age of 35. No symptoms were reported by 78.99% of patients. A total of 86,500 RT-PCR assays were performed on nasooropharyngeal tissues from 50,000 patients. These patients yielded 41598 virus-positive cases. The nasooropharyngeal RT-PCR test had a sensitivity of 90.57% compared to the gold standard for SARS-CoV-2 molecular diagnosis. Our study found that a small number of SARS-CoV-2 patients may meet the clinical case definition yet have negative RT-PCR results. By the time severe illness patients seek medical assistance, the virus may be quite low in the nasooropharyngeal tract. Thus, rigorous serological testing on suspected infected patients will help determine RT-PCR testing sensitivity**.**

**Keywords:** Asymptomatic, Nasooropharyngeal, RT-PCR, SARS-CoV-2

## 1. Introduction

After the initial case on January 30, 2020, Indian COVID-19 cases climbed gradually. March 24 began a 70-day national lockdown. After the shutdown, mid-September saw 98,000 new cases[1]. After a decline, cases peaked in mid-February 2021. New, extremely contagious variations may initiate wave two. Two large ICMR serosurveys revealed early Covid-19 infection in India. Adult Indians may have contracted the virus by early May 2020 at 0.73% (95% confidence interval: 0.34—1.13). The August 2020 prediction was 7.1% (95% CI: 6.2—8.2). This and other studies suggested uneven COVID-19 dissemination in India[2]. These studies show dispersion, especially in metropolitan regions where 30% of Indians dwell. Despite their vast populations, Uttar Pradesh (UP) and Bihar had few cases in the initial wave[3].

Before immunisations, thorough infection testing stopped the outbreak. Urgency questioned test method optimisation. Testing usually accomplishes two goals[4]. We diagnose symptoms. Identifying disease in symptomatic and asymptomatic people helps epidemiologists measure population spread. India screened 1-1.5 million people every day, 0.1% of its 1.34 billion people, during the first pandemic wave[5]. The main RAT was SD Biosensor Q COVID-19 in 49% of investigations. Initial ICMR certification. The rest was 51% RT-PCR. TrueNat and CBNAAT, which use tuberculosis-specific PCR, contributed less than 5%. Due to Indian state and national government efforts, RT-PCR testing costs have fallen but remain higher than RATs[6]. Community-acquired SARS persists. Since SARS-CoV-2 is ubiquitous, COVID-19-specific locations are needed. Determine SARS-CoV-2 RT-PCR sensitivity[7].

Overestimation of sensitivity by clinical staff, and a lack of use of testing results in combination with clinical features of their presentation, may lead to patients with disease being incorrectly diagnosed, and placed in non-COVID-19 areas with the subsequent risk of infection to others; underestimation of the sensitivity by clinical staff may lead to patients who are SARS-CoV-2 negative being erroneously placed in COVID-19 areas[8]. The reverse-transcriptase polymerase-chain-reaction (RT-PCR) test has close to 100% sensitivity and specificity in a laboratory setting, but delays in returning results, as well as increased costs[9]. some Indian states, including the large states of Uttar Pradesh (pop. 227.9 million) and Bihar (pop. 121.3 million) use a much higher proportion of such tests[10].

Various meta-analysis estimates the sensitivity of reverse transcription polymerase chain reaction (RT-PCR) testing of upper respiratory tract samples as 89%, but this meta-analysis and a subsequent one highlights several limitations in the literature[11]. These include small sample size (<100 patients with COVID-19), reliance on RT-PCR itself as the gold standard for diagnosis, use of computed tomography (CT) scans rather than clinical criteria as a gold standard for the diagnosis of COVID-19, and absence of comprehensive RT-PCR testing for all included patients[12]. Finally, only a single study to our knowledge has examined the cumulative sensitivity of repeat testing for SARS-CoV-

**2.** Here we examine in a large, comprehensive dataset the sensitivity of RT-PCR testing of nasooropharyngeal specimens for COVID-19[13].

## 2. Methodology

### 2.1. Materials

#### 2.1.1. Data sources and mode of ascertainment

Data of all SARS-CoV-2 (COVID-19) registered under the OPD and IPD in the MMG district hospital, Ghaziabad area was collected. Data was collected for two years between JUNE 2020 to JUNE 2022. Data was collected from SARS-CoV-2 (COVID-19) registers and lab reports from a major SARS-CoV-2 (COVID-19) Unit in the MMG district hospital, Ghaziabad. Data obtained from the lab reports was cross-checked by verification of cases from SARS-CoV-2 (COVID-19) registers for the respective years.

#### 2.1.2. Data entry and variables

Data was entered into a Microsoft Excel worksheet (version 2019). Data of two period (JUNE 2020 to JUNE 2022 was collected for the following:

a. Total number of chest symptomatic registered in the MMG district hospital, Ghaziabad between JUNE 2020 to JUNE 2022.

b. Total number of SARS-CoV-2 (COVID-19) cases diagnosed in the MMG district hospital, Ghaziabad based on Nasoorpharyngeal swab Rapid Antigen Detection (RAD) test JUNE 2020 to JUNE 2022.

#### 2.1.3 Analysis of data

The diagnostic and screening efficiency of current routinely used diagnostics was determined through this analysis. Screening efficiency was defined as the number of SARS-CoV-2 (COVID-19) cases diagnosed using a given diagnostic from among all symptomatic. Diagnostic efficiency was defined as the number of SARS-CoV-2 (COVID-19) cases diagnosed based on positive smear results or other diagnostics in the diagnostic paradigm amongst all SARS-CoV-2 (COVID-19) cases.

### 2.2. Methods

#### 2.2.1. Ethical considerations

Before testing of clinical samples brainstorming sessions with Chief Medical Officer (SARS-CoV-2 (COVID-19)), Chief Pathologist and Radiologists of the SARS-CoV-2 (COVID-19) Unit were organized. Necessary permission from the MMG district hospital, Ghaziabad authorities was taken to test the sample after it had been processed for all investigations at the SARS-CoV-2 (COVID-19) Unit. Diagnostic decisions were made by site physicians according to the COVID-19 guidelines.

#### 2.2.2 Inclusion Criteria

15-47years (Youth group) and 48-63years (Middle age group) were included in the present study.

#### 2.2.3 Exclusion Criteria

The paediatric group (0-14 years), old age group (>64 years), pregnant women, and patients suffering from other life-threatening diseases were not included in the present study.

#### 2.2.2 Laboratory Analysis

##### 2.2.2.1 Specimen collection

The Nasooropharyngeal swab was placed in the sterile viral transport media (total volume 3 mL) and sealed securely. Sample (n=50,000) was stored at -4-to -20°C for 24 hours and -80°C for long time. Virus concentrations in samples were estimated from cycle threshold (Ct) value. All the specimens were processed in biosafety cabinet level 2 (BSL 2 Advanced) following all infection control practices[14].

##### 2.2.2.2. Molecular diagnosis

Samples were processed through the following steps:

**2.2.2.2.1 Viral Nucleic Acid Inactivation:** Total nucleic acid was manually isolated from nasopharyngeal swabs using the Q-line RNA extraction kit. Briefly, we added 200 mL viral transport media, 600ul lysis buffer,5uL Carrier RNA, 8ul magnetic beads for 1 reaction[15].

**2.2.2.2.2 Viral Nucleic Acid Extraction**

600 µL of wash buffer was added to an Eppendorf tube on a magnetic stand. Nucleic acids were eluted with 50 µL of elution solution per reaction[16]. The total nucleic acid was immediately subjected to an RT-qPCR test, and residual samples were stored at -80°C.

**2.2.2.2.3 One-step RT-qPCR**

According to the protocol developed by Q-line, we performed one-step RT-qPCR to detect SARS-CoV-2. The primer/probe set amplified the nucleocapsid (N) gene and ORF1ab gene of SARS-CoV-2. The reaction mixture (9ul COVID-19 enzyme mix,1ul COVID-19 Prime Probe and 10ul template) was added in each well and mixed it properly. For the internal positive control, the human ribonuclease P gene was used. Positive control and negative control were in two well in 96 well plate. The RT-qPCR assays were conducted in Quant studio 5, applied biosystem (Thermo Fisher Scientific) with the following cycling conditions: $50^0$C for 15 min for reverse transcription, $95^0$C for 3 min for cDNA initial denaturation, and 45 cycles of $95^0$C for 15s for denaturation and 55 $^0$C for 40s for annealing, extension and fluorescence measurement[17]. The threshold was set to 0.2. A threshold cycle (Ct) value was assigned to each PCR reaction, and the amplification curve was visually assessed. Following the protocol, we deemed a sample to be positive when a visible amplification plot was observed, but negative when no amplification was observed. The absolute copy number of the viral load was determined using the Ct value of the Q-line

molecular. The targeted genes were E, N, ORF1ab/ Rdrp.

**2.2.2.2 Classification of test results**

**Table 1: Classification of test results**

| SNO. | Description | Classification |
|---|---|---|
| 1. | Single negative test | True negative |
| 2. | An initial positive test, with or without subsequent testing. | True positive |
| 3. | More than one negative test, no positive test result at any point | Clinical records were reviewed to identify whether should be classified as true negative, or potential false negative |
| 4. | A series of one or more negative tests followed by a positive test, with or without subsequent testing. | Clinical records were reviewed to identify whether a single or multiple clinical presentation. If two distinct clinical presentations with independent testing, are treated as discrete episodes, and test was classified as a true positive. If a single episode, the test was classified as a false negative. |

Patients who were tested negative for COVID-19 were considered to be truly negative if they had only one negative RT-PCR test. However, those who tested negative multiple times and later tested positive were investigated to determine if they had two different presentations of the virus. The results of individual positive and negative tests were analyzed, and COVID-19 was diagnosed based on clinical criteria, discharge diagnosis, or death certificate verification[18]. The reports of clinical, radiological, and hematological tests were evaluated according to WHO criteria. If there was any disagreement between the clinical team and radiology team, the diagnosis was made based on the case records. SARS-CoV-2 RTq-PCR, was used to retest the first respiratory samples of potential false negatives. Positive results from early testing indicated the need for clinical intervention. After two negative RT-PCR tests, a positive result did not indicate COVID-19. Clinically inappropriate sample IDs were matched to the COVID-19 Genomics Sequencing Consortium NCDC (National Centre for Disease Control). NCDC positive samples passed.

**2.2.2.4 Statistical analysis**

The sensitivity was computed by dividing the proportion of true positives found on first testing and re-testing of suspected false negatives by the number of true positives added to convincing false negatives, as estimated by respiratory and serology tests[19]. Based on recurrent RT-PCR retesting, specificity was estimated by dividing true negatives by the number of true negatives added to false positives. Divide the number of true positives by the sum of true and false positives to get the positive predictive value. Divide the number of true negatives by the sum of true and false negatives to calculate the negative predictive value. These estimations' confidence intervals were obtained using two-sided exact binomial test with a 0.95 confidence level (by R).

## 3. Results and Discussion

Out of the total samples tested from suspected COVID-19 cases (n=50,000) by real-time RT-PCR assay. The median age for COVID-19 cases was 35 years (range 15-63 years) (Table 2). Out of the total infected cases, 27550 were males (55.1%) and 22,450 were females (44.9%) (Table 3). (Figure 1)

**Table 2: Age distribution among patients (n=50000)**

| Variable (Age) | % patients |
|---|---|
| 15-47 | 38522 (77.04%) |
| 48-63 | 27550 (55.1%) |

**Table 3: Gender distribution among patients (n=50000)**

| Variable (Gender) | % patients |
|---|---|
| Male | 27550 (55.1%) |
| Female | 22450 (44.9%) |

**Table 4: Case distribution among patients (n=50000)**

| Variable (Age) | % patients |
|---|---|
| Symptomatic | 10502 (21.04%) |
| Asymptomatic | 39498 (78.99%) |

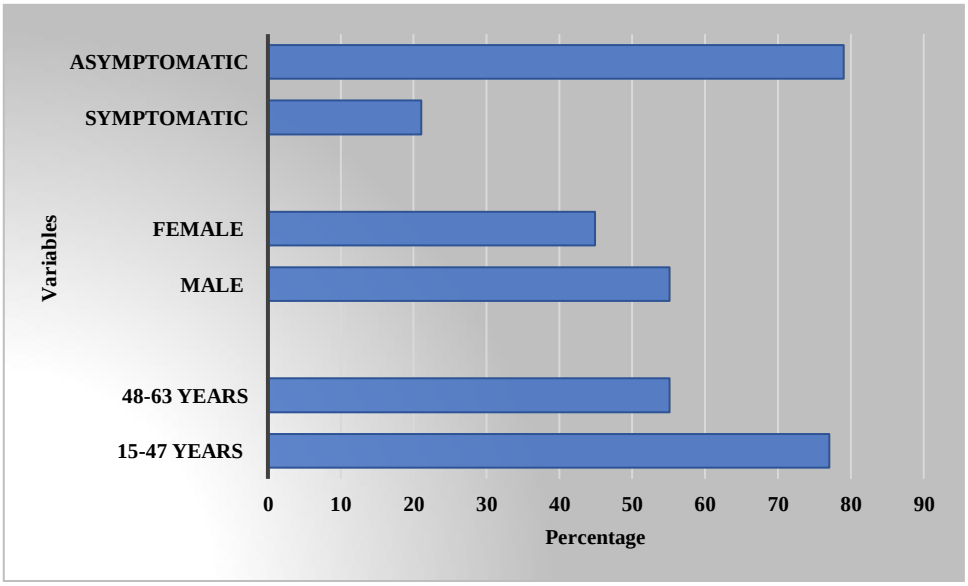


**Figure 1: Representation of Different variables among patients (n=50000)**

Between December 2021 and December 2023, MMG district hospital in Ghaziabad conducted a total of 86500 RT-PCR tests on nasooropharyngeal specimens of 50000 patients to detect SARS-CoV-2. Out of these patients, 41598 tested positive for the virus (as shown in Table 5 and Figure 1). The nasooropharyngeal RT-PCR test for the whole group had an overall sensitivity of 90.57%, calculated based on the molecular diagnosis of SARS-CoV-2 on nasooropharyngeal RT-PCR as the gold standard.

**Table 5: Real-time RT-qPCR**

| SNO. | Testing pattern | Number | Percent of all patients | Percent of positive patients |
|---|---|---|---|---|
| **A.** | **Single negative test** | 6337 | 12.674 | NA |
| **B.** | **More than 1 negative test** | 2065 | 4.13 | NA |
| **C.** | **Single positive test** | 37678 | 75.356 | 90.57 |
| **D.** | **Initial negative test followed by positive test** | 3765 | 7.53 | 9.05 |
| **E.** | **Positive test after two or more negative tests** | 155 | 0.31 | 0.37 |
| | **Total positive** | (C+D+E=41598) | | |

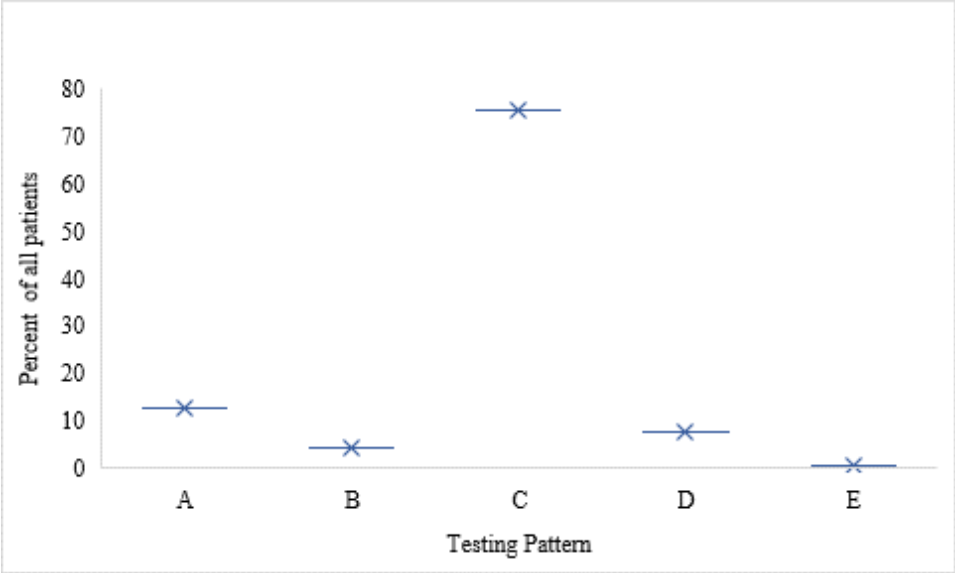


**Figure 1: Results of RTqPCR**

**A: Single Negative Test; B: More than one negative test; C: Single positive test; D: Initial negative test followed by positive test followed by; E: Positive test after two or more negative test**

Gaining knowledge on the development and spread patterns of SARS-CoV-2 is essential for effectively handling cases, halting transmission, and averting the future dissemination of the pandemic. Swift identification and segregation of cases are crucial to impede the dissemination of the virus. This study was undertaken during the lag phases of the pandemic in India, and it offers the most extensive dataset on the identification of SARS-CoV-2 RNA in samples taken from the nose and throat of individuals with symptoms or without symptoms of COVID-19. Due to limited feasibility and availability, viral isolation is not easily achievable. Therefore, the diagnosis of viral respiratory tract infections mostly depends on molecular testing, such as nucleic acid amplification and detection-based diagnostics. The duration of viral shedding can only be assessed by isolating the virus in respiratory samples that are suitable for testing. The results of SARS-CoV-2 RT-PCR tests have played a vital role in determining diagnostic, treatment, and discharge protocols in various nations. Furthermore, RT-PCR assays have been employed to ascertain the length of time that the virus remains detectable in respiratory tract samples. Respiratory samples from mildly ill people with SARS-CoV-2 have shown a median period of viral detection. Additionally, samples from severe cases exhibited greater viral loads compared to those from mild cases. The precise duration of infectivity for COVID-19 is unknown, but transmission from individuals without symptoms has been seen.

The study examined and analysed the relationship between age, sex, and symptomatology. There was no statistically significant disparity observed about gender. Xu et al. conducted a retrospective analysis to investigate patients who experienced extended viral shedding[20]. The study identified several risk variables related with this condition, including older age, male sex, corticosteroid medication, delayed hospital admission, and the need for mechanical ventilation. The heightened immunological dysfunction observed in older individuals has been proposed as a potential reason for the longer presence of SARS-CoV-2 in their respiratory tract.

A significant proportion of the cases in our study involved asymptomatic patients. Detecting asymptomatic carriers is crucial for implementing public health interventions and the high secondary attack rate of SARS-CoV-2 among households and close contacts. Asymptomatic cases are being given extra attention since the virus continues to be present in respiratory samples despite the absence of symptoms[21].

This study was a retrospective analysis of the RT-PCR results obtained from nasooropharyngeal specimens collected from individuals diagnosed with COVID-19. The study's primary advantages were its extensive sample size and diverse population. The sensitivity of RT-qPCR was found to be 90.57%. However, additional research is necessary to draw definitive conclusions about viral shedding and infectivity by comparing RT-PCR results with viral isolation.

**Conclusion**

A retrospective observational study was conducted at MMG district hospital in Ghaziabad and involved COVID-19-positive patients. Hospital lab records show that nasooropharyngeal swabs were utilized to collect age, gender, symptomatic and asymptomatic data, and SARS-CoV-2 RT-PCR results. Most of the patients were asymptomatic men with a median age of 35 years. The patients had 41598 virus-positive instances. Nasooropharyngeal RT-PCR was 90.57% sensitive

compared to the gold standard for SARS-CoV-2 molecular diagnosis. We also detected SARS-CoV-2 patients with clinical case criteria but negative RT-PCR data. The virus may have diminished in the nasooropharyngeal tract by the time severe disease patients seek medical assistance. Complete serological testing on suspected infected patients can evaluate RT-PCR sensitivity. Thus, from the perspective of management and containment, patients require unique concerns to be considered.

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