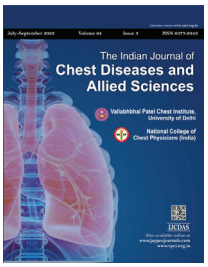
ORIGINAL ARTICLE

# Viral Load-based Evaluation of the Sensitivity of Antigen-based Rapid Detection Assay for SARS-CoV-2

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## Abstract

**Background:** Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has become a global public health problem. The real-time reverse transcription-polymerase chain reaction (RT-PCR) is the gold standard test for the detection of SARS-CoV-2. However, the assay requires hours to get the final results. Therefore, antigen-based rapid assays are being used extensively to reduce the time. We have evaluated the performance of the antigen-based rapid test for the detection of SARS-CoV-2 virus in comparison with RT-PCR.

**Materials and methods:** Nasopharyngeal and throat swabs were collected from 366 suspected patients of COVID-19 visiting our institute and subjected to qualitative RT-PCR and antigen-based rapid assays to detect the presence of SARS-CoV-2 virus. The sensitivity and specificity of the antigen-based assay were calculated in comparison with RT-PCR.

**Results:** Compared with RT-PCR, sensitivity and specificity of the antigen-based rapid assay were observed to be 70.5% and 98.6%, respectively, in comparison with RT-PCR. However, the sensitivity of antigen-based rapid assay varied significantly with decreasing viral load. The sensitivity of the rapid antigen assay was equivalent to RT-PCR (23/23, 100%) at a higher viral load (Ct value 15–20). In contrast, the antigen assay could only detect 3/21 (14.28%) samples with Ct value >30.

**Conclusion:** The antigen-based assay could assist in the rapid screening of a large population. However, the rapid antigen assay might not detect early stages of infection represented by low viral load. Therefore, the antigen-based assay could not replace RT-PCR testing. The study reiterates that all antigen-based negative tests should be confirmed by RT-PCR.

**Keywords:** Assay sensitivity, COVID-19 pandemic, Rapid antigen test, SARS-CoV-2, Viral load, Viral surveillance.

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## Abbreviations used in this Article

SARS-CoV-2 = Severe acute respiratory syndrome coronavirus-2; RT-PCR = Reverse transcription-polymerase chain reaction; WHO = World Health Organization; IC = Internal control; POC = point-of-care.

## Introduction

The coronavirus disease 2019 (COVID-19) is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The city of Wuhan in China, in December 2019, recorded the initial cases of SARS-CoV-2 infection.[1] The localized infection in China soon turned into a pandemic, which imprisoned the whole world within a noticeably short period. As per the World Health Organization (WHO) data, there were more than 170.4 million confirmed cases of COVID-19 by June 2021, with 3.55 million deaths.[2] The infection statistic is increasing at a distressing rate. Since there was no promising treatment or vaccine available for the SARS-CoV-2 till the last quarter of 2020, various preventive measures such as social distancing, isolation, and temporary lockdowns were in place to minimize virus spread.[3] The worldwide public health regulators faced a number of problems in preventing the further spreading of the virus. The long incubation period and asymptomatic carriers of the SARS-CoV-2 facilitate the virus's unnoticed and fast spread. Therefore, the testing of the maximum suspected individuals for SARS-CoV-2 infection is the primary strategy adopted worldwide against the virus, as endorsed by WHO to control viral spread.

There are various indicative approaches for viral diagnostics. The real-time RT-PCR-based detection of the viral genome is considered a highly sensitive and effective approach for viral

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diagnostics.[4] However, the whole process of RT-PCR-based testing takes 3–4 hours in the generation of the results. Further, the PCR-based detection needs specialized laboratory equipment, skilled technical staff, and nuclease- and contamination-free laboratory setup. Therefore, it is challenging to screen the larger population with RT-PCR tests, especially when the patients need urgent medical attention. The RT-PCR approach is also difficult to implement in remote areas where the required setup is not available.

Therefore, viral antigen-based rapid detection kits are considered a viable alternative to RT-PCR. It enables the detection of viral infection in patients within 15–30 minutes without the requirement of a dedicated diagnostic facility or expensive equipment. Further, a person with minimum technical skill can perform this test. However, in comparison to RT-PCR-based tests, the sensitivity of the assay is compromised in antigen-based tests.

In the current study, we have compared the sensitivity and specificity data of RT-PCR and antigen-based rapid immunoassay on suspected COVID-19 samples. The data may help health authorities and medical processors to make informed decisions in COVID-19 management.

## Materials and Methods

### Clinical Specimen's Collection

The nasopharyngeal and oropharyngeal swabs were collected from the suspected COVID-19 patients visiting Vallabhbhai Patel Chest Institute, University of Delhi, from May 2020 to November 2020. The sample was collected in 3 mL viral transport media (Labsystems diagnostics). The sample was transported from the hospital wing to the diagnostic laboratory at 2–8°C. All the samples were processed in a biosafety level-2-enhanced (BSL-2+) facility by trained technical staff as per the WHO guidelines.

Extraction of viral RNA and detection of SARS-CoV-2 genome using real-time RT-PCR Viral RNA was extracted using the QIAamp Viral RNA isolation kit (QIAGEN GmbH – Germany) as per the manufacturer's instructions. Viral RNA was eluted in 60-µL elution buffer, which was subsequently used for RT-PCR.

The PCR-based diagnostic kit from LabGun (Korea) was used for COVID-19 detection, which targets envelope gene (E) of the Sarbecovirus and RNA-dependent RNA polymerase (RdRp) gene of Wuhan-specific strain of SARS-CoV-2. The RT-PCR reaction was performed as per the manufacturer's instructions. Briefly, the individual reaction for each gene was prepared by mixing 10 µL reaction buffer, 4 µL primer-probe mix, 1 µL enzyme mix, and 1 µL internal control to the 5 µL of extracted sample RNA. The CFX-96 real-time thermal cycler (Bio-Rad Laboratories, Inc., Hercules, CA, USA) was used for the RT-PCR. The temperature conditions for RT-PCR were as follows: single cycle for 30 minutes at 50°C, 15 minutes at 95°C for activation of hot-start enzyme followed by 45 cycles of 15 seconds at 95°C, 60°C for 30 seconds. The probe of RdRp was labeled with FAM, the E gene was labeled with Cy5, and the internal control (IC) was labeled with HEX. The data were acquired by CFX manager3.1 software (Bio-Rad laboratories). The sample with a cycle threshold value (Ct-value) <40 for all three target genes was considered positive. The sensitivity of the antigen-based assay was also evaluated with viral load (Ct-value) since there is always some difference in the Ct value of two genes. Therefore, to maintain data consistency, the Ct value of the E gene was considered while comparing the antigen data. The E gene is among the most widely evaluated gene for SARS-CoV-2 and is recommended by WHO.

### Rapid Antigen Detection Assay for SARS-CoV-2

A lateral flow-based rapid chromatographic immunoassay, Standard Q COVID-19 Ag test (SD Biosensor®, Republic of Korea), was used to detect SARS-CoV-2. The rapid test device comprises a window with two precoated lines; the test (T-line) and control (C-line). The nasopharyngeal sample from suspected COVID-19 patients was processed as per the manufacturer's instructions. Briefly, the sample swab was inserted into the extraction buffer while squeezing and stirring the tube. The swab was removed, and the nozzle was fitted on extraction buffer tubes. Three drops of extraction buffer were added to the well of the testing device, and the result was read in 15–30 minutes. The appearance of two lines, T- and C-lines, in the window of the device, was read as a positive sample for SARS-CoV-2, while the appearance of a single line, C-line, was recorded as a negative specimen for SARS-CoV-2.

### Statistical Analysis

The continuous data are presented in the average range. The categorical data are presented in numbers, 95% confidence interval, and percentage. The online tool, GraphPad, was used for statistical analysis.

## Results

### Sensitivity and Specificity of Antigen Kit

A total of 366 clinical samples were processed with RT-PCR and rapid antigen kits to detect the SARS-CoV-2 virus infection. Out of these, 78 samples were detected positive for SARS-CoV-2 virus by RT-PCR. The rapid antigen kit detected only 55 samples as positive for SARS-CoV-2. Therefore, the overall sensitivity of the antigen-based rapid test was observed to be 70.51% as compared to RT-PCR.

As shown in Table 1, a total of 288 samples were detected as negative by RT-PCR. Out of a total of 288 samples, the rapid antigen detection kit detected 284 samples as negative, while four samples were detected as false positive by the kit. These four samples developed faint bands which were interpreted as a positive test as per the manufacturer's instructions (SD Biosensor®, Republic of Korea). Therefore, the overall specificity of the rapid antigen kit detected was observed to be 98.60%.

### Performance of Rapid Kit in Association with Thresholds Cycle

To evaluate the distribution of sensitivity data in relation to thresholds cycle (Ct value), the detection rate of the antigen kit was also analyzed with the cycle threshold (Ct) value.

Figure 1 shows that the antigen kit's sensitivity was observed to be equivalent to the RT-PCR at higher viral load (Ct value 15–25), while it decreased to 20% at lower viral loads (Ct value 31–35). The data showed that the rapid antigen kit failed to detect the SARS-CoV-2 virus in a sample with a Ct value above 35.

## Discussion

The SARS-CoV-2 emerged globally at the end of 2019, and as of 2nd June 2021, the WHO reported 170,426,245 confirmed cases and 3,548,628 deaths worldwide.[2] A few days after the first report of COVID-19, an RT-PCR protocol for detection of SARS-CoV-2 was described.[5] The RT-PCR is the gold standard test for diagnosis of COVID-19 infection and the WHO and other health authorities released several PCR protocols to be followed during the pandemic.[6] However, the RT-PCR is a time-consuming assay that requires

**Table 1:** RT-PCR results in comparison to rapid antigen assay

| | *Rapid antigen assay* | | | | |
|---|---|---|---|---|---|
| *RT-PCR* | *Positive* | *Negative* | *Sensitivity* | *Specificity* | *Accuracy* |
| Positive | 55 | 23 | 70.51% | 98.60% | 92.62% |
| Negative | 4 | 284 | | | |

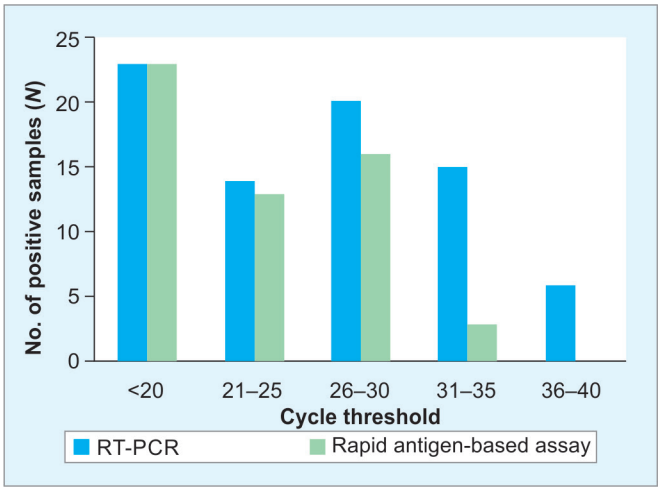


**Fig. 1:** The graph showing correlation between Ct value and sensitivity of rapid antigen kit. The "*N*" represents the number of SARS-CoV-2 virus-positive samples, as detected by RT-PCR

stringent protocols and well-trained laboratorians to perform the assays. The need for a point-of-care (POC) rapid assay that could be performed at the bedside led to the development of the antigen-based rapid test that used several targets such as the nucleocapsid, spike, or membrane proteins of the virus.[6] We evaluated an antigen-based rapid commercial assay with the gold standard RT-PCR. Our data showed that the performance of Standard Q COVID-19 antigen-based assay (SD Biosensor®, Republic of Korea) was inferior to the RT-PCR test. The sensitivity and specificity of the Standard Q COVID-19 antigen-based assay were shown to be 98.33% and 98.73%, respectively, in a study in Thailand,[7] whereas, a study in Uganda reported the sensitivity and specificity of the assay to be 70% and 92%, respectively.[8] The present data showed that the overall sensitivity of antigen-based assay is 70.51%, which is also in a similar range. Other crucial data generated from the study are that the sensitivity of antigen-based kits is not a static figure, instead, it is directly associated with viral load. At high viral load (low Ct), the observed sensitivity of the antigen-based assay was comparable to the RT-PCR, while at high Ct value, the antigen-based kit could not detect the virus. Higher Ct values, especially above 35, are usually represented by either onset or viral clearance phases of infection. These groups have a comparatively lower risk of the virus spreading than the person having a higher virus load. It has been established that viral load in COVID-19 patients varies depending on the time between disease onset and sample collection, and the quality of the sample.[6,9,10] Since the antigen assays are less sensitive than RT-PCR, they would not be reliable for diagnosis of COVID-19 patients with a low viral load.

Also, four of our samples were faintly positive by the antigen-based assay but negative by RT-PCR. Hence, faint-positive bands in the antigen-based kits should be confirmed by RT-PCR assays.

In conclusion, the antigen-based assay would not be a replacement for the RT-PCR test due to lower sensitivity at later stages of infection. However, it is a quick method to screen a large population. Therefore, fast spreading of the virus could be prevented by the first level of screening with the antigen-based assay. All rapid antigen-based negative tests should be followed up with an RT-PCR test.

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