



Clinical Validation of Standard Q COVID-19 Antigen and IgM/IgG Combo Kit Assay at a Tertiary Care Center in Northern India

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Abstract

Background Expansion of the testing capacities for severe acute respiratory syndrome-coronavirus-2 is an important issue in the face of ever-increasing case load. So, there is need of point-of-care diagnostic tests in the existing laboratory capacities for early treatment, isolation, and clinical decision making, especially in resource limited settings.

Materials and Methods This prospective cohort study was conducted at Jai Prakash Narayan Apex Trauma Center, All India Institute of Medical Sciences, New Delhi. Nasopharyngeal samples and blood samples were collected for antigen and antibody testing. Rapid antigen test was performed as per the kit's instructions. The performance of the kit was compared with the gold standard reverse transcription polymerase chain reaction (RT-PCR) testing.

Results Eighty-eight out of 110 patients tested positive by RT-PCR for coronavirus disease 2019 in last 48 to 72 hours were included in the study. Overall, the sensitivity of combined antibody test was 52%, antigen test 26%, and combined sensitivity of both antigen and antibody was 72.7%, respectively.

Conclusion The combo kit needs to be used with caution in low prevalence settings, where cases may be missed.

Keywords

- ▶ antigen test
- ▶ antibody test
- ▶ point-of-care test

Introduction

Coronavirus disease-2019 (COVID-19) has infected more than 176 million people worldwide resulting in approximately 3.8 million deaths.¹ With the rapid surge of patients/contacts overwhelming existing laboratory capacities, there is a need for point-of-care rapid diagnostic tests (RDT) for early diagnosis of COVID-19. It would allow early

treatment and isolation of cases thus reducing the spread of COVID-19, especially in resource-constrained settings.

Laboratory diagnosis and management of COVID-19 have been helpful in combating the spread of severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2). At present, the gold standard for COVID-19 diagnosis is reverse-transcription-quantitative PCR (RT-qPCR) that uses nasopharyngeal swabs, throat swabs, or saliva samples.² RT-qPCR kits

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that do not require viral RNA extraction and high-throughput RT-qPCR systems have also been developed. Although such tests are widely utilized in tertiary care centers and large well-equipped hospitals, they are rarely available in the local clinics that are more approachable for the patients who are under suspicion.

Studies done on rapid antigen tests have shown sensitivity of 61.70% and specificity of 98.26% for the diagnosis of COVID-19.³ A cross-sectional, single-blinded study by Gupta et al showed overall sensitivity and specificity 81.8 and 99.6%, respectively. The sensitivity of RDT was higher (85.9%) in participants with a duration of illness up to 5 days.⁴

To aid in the proper management and timely diagnosis of COVID-19, validated and accurate laboratory testing is crucial. This will help the clinicians and infection control practitioners to combat infection at the health-care level and detect clinical cases timely. Therefore, it would further help in appropriate treatment, prompt isolation, and consequently deceleration of the cases.

Methodology

This is prospective cohort study conducted at the Department of Microbiology at Jai Prakash Narayan Apex Trauma Center, All India Institute of Medical Sciences, New Delhi. In this study, a total of 110 subjects were included. Samples were collected from the hospital inpatients who were laboratory confirmed cases of COVID-19.

RT-PCR for COVID-19 was done for these patients in last 48 to 72 hours to ensure that the desired number of true positives was being enrolled.

We used STANDARD Q COVID-19 antigen and immunoglobulin M/immunoglobulin G (IgM/IgG) combo kit (SD Biosensor, Inc., Gurugram, Haryana, India) that is a rapid immunochromatography test designed for the qualitative detection of specific antigens in human nasopharynx and IgM/IgG in humoral fluid (► Fig. 1).

For antigen-based tests, nasopharyngeal swab was collected (as per manufacturer's instructions). The samples were immediately placed in the buffer (provided with the

test kit). Test was performed as per manufacturer's instructions. For antibody-based test, blood/plasma/serum was collected as per manufacturer's instructions. All necessary reagents are provided with the kit and no equipment is required. The rapid antigen tests were performed as per the test kit instructions.

The detection time for antibodies is 10 to 15 minutes and the specimen types are whole blood (20 µL) and serum/plasma (10 µL) suitable for detection. The antigen test, with nasopharyngeal sample, gives results in 30 minutes. The reading for the rapid antigen test should be taken as per time mentioned in the manufacturer's instructions. Pictures of the rapid test strips results were taken for the documentation.

Statistical Analysis

Stata 14.0 Statistical Software (Stata Corp LLC, Texas, United States) was used for data analysis. Diagnostic characteristics such as sensitivity and specificity of the test with RT-PCR as reference were calculated. The RDT was also evaluated considering days since infection. For each of the summary measures, frequency with percentage and 95% confidence interval (CI) was also computed.

Result

This is a prospective cohort study in which we have evaluated 110 number of patients for the study. All patients were laboratory confirmed cases of COVID-19. Out of 110 patients, only 88 patients met the inclusion criteria. The gender distribution of the present population was male 62 (70.4%) and females 26 (29.6%), respectively. The median duration of illness at the time of testing among symptomatic patients was 1 day (range: 1–10). The most common symptoms among the participants were fever (71.5%), cough (25.4%), and fatigue/malaise (12.8%).

The study found that 26.7% (95% CI: 17.2–36.3) of total patients tested positive for antigen test (► Fig. 2A). The sensitivity for IgM was found to be 48.6% (95% CI: 37.2–60.1), whereas 51.3% (95% CI: 39.8–62.81) of patients show presence of IgG antibody (► Table 1A–D). The sensitivity of

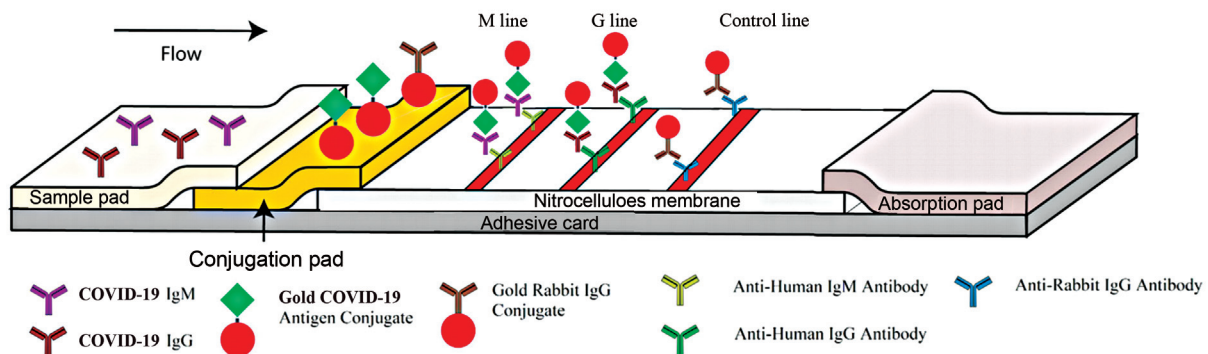


Fig. 1 Schematic diagram of immunoglobulin M/immunoglobulin G (IgM/IgG) severe acute respiratory syndrome-coronavirus-2 antibody tests (<https://doi.org/10.1002/jmv.25727>). COVID-19, coronavirus disease 2019.

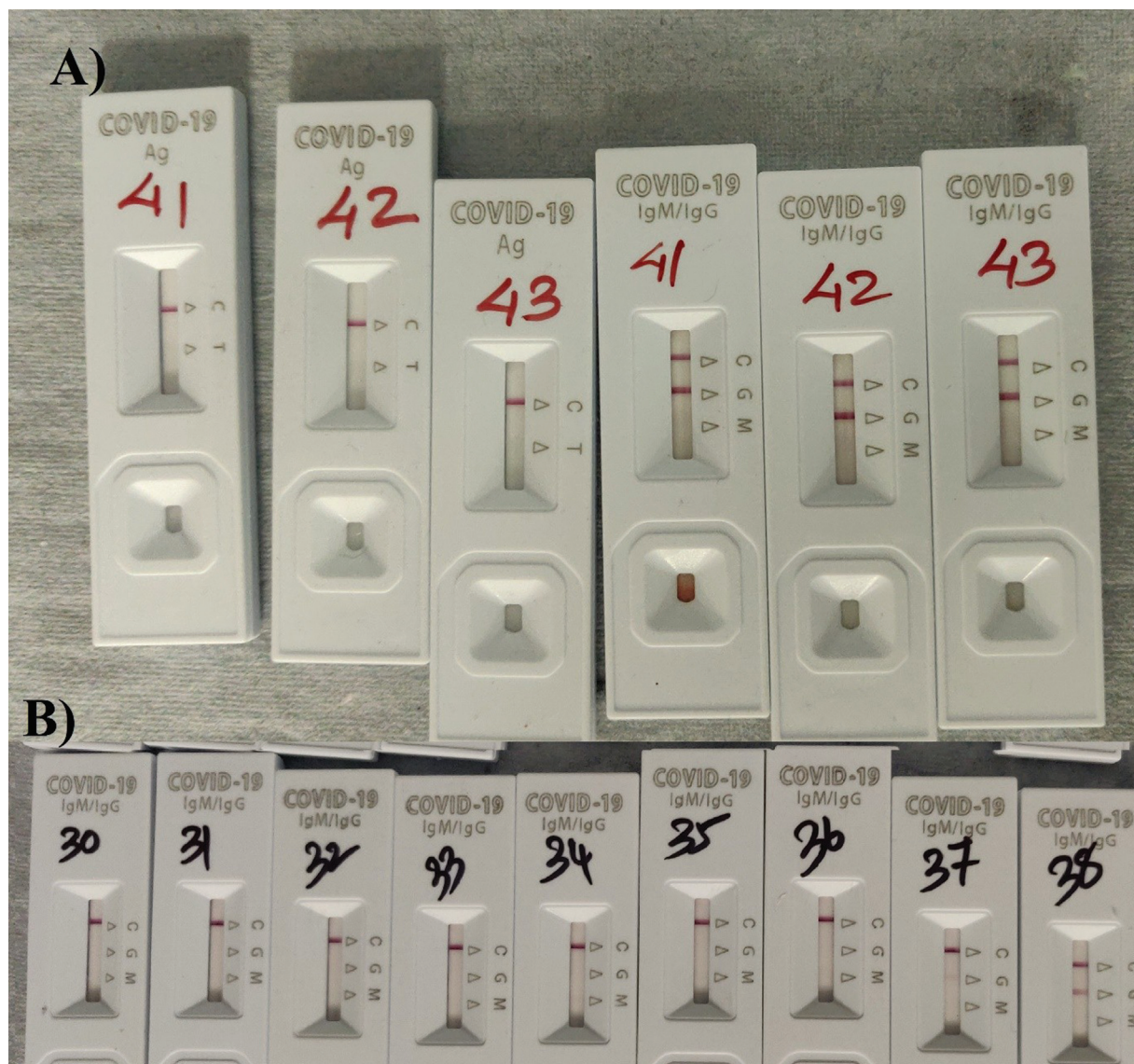


Fig. 2 Illustrations of (A) severe acute respiratory syndrome-coronavirus-2 antigen and (B) immunoglobulin M/immunoglobulin G antibody test.

combined antibody test (IgM and IgG both) was 52% (→ **Fig. 2B**). The sensitivity of combined testing, that is, both antigen and antibody test, was found to be 72.7%. There were 14.7% patients who were neither positive to antigen nor to antibody.

Discussion

RT-PCR is the gold standard test for SARS-CoV-2, but it is time-consuming and requires specialized laboratory and trained laboratory personnel. At the same time, RT-PCR is also very expensive. Due to higher contingency of SARS-CoV-2 and increasing number of patients, antigen and antibody tests may be utilized. Various tests detecting SARS-CoV-2 antigens and antibody have recently been developed and commercially available.^{5,6} Many studies have been done to evaluate the COVID-19 tests including antibody and antigen test.⁷⁻⁹ In the current study, median

time from onset of illness at the time of testing was 1 day (interquartile range [IQR]: 1–10 days), which is shorter than that in previous studies (6 days) (IQR: 3–13 days).¹⁰ IgM antibody was found in 48.6%, IgG antibody in 51.3%, and the sensitivity of the combined antibody test (IgM and IgG both) was calculated to be 52% from the specimens. However, in a study from Imai et al, IgM and IgG antibodies were detected in 43.2% and 14.4% and combined antibody test (IgM and IgG both) was 43.2%, respectively.¹⁰ The clinical effectiveness of serological tests for COVID-19 remains questionable due to interval between onset of symptoms and appearance of IgM and IgG antibodies in serum. This discrepancy seemingly reflects differences in timing of sampling because the health centers vary across countries. In this study, the antigen testing showed less sensitivity than antibody testing. In fact, the sensitivity to individual antibody test was more than the sensitivity of antigen test. The sensitivity of IgM and IgG individually is approximately

Table 1 (A) Sensitivity and 95% confidence interval of SD antigen–antibody combo kit, (B) comparison between antigen and IgM antibody, (C) comparison between antigen and IgG antibody, (D) comparison between IgM and IgG antibody

(A) SD antigen–antibody combo kit	Frequency	Sensitivity (%)	95% Confidence interval	
Antigen kit	23	26.74	17.2	36.29
Antibody kit (IgM)	37	48.68	37.19	60.18
Antibody kit (IgG)	39	51.32	39.82	62.81
(B) Ab kit (IgM)				
Antigen kit	Negative		Positive	Total
Negative	26		28	54
Positive	12		9	21
Total	38		37	75
(C) Antibody kit (IgG)				
Antigen kit	Negative		Positive	Total
Negative	18		36	54
Positive	18		3	21
Total	36		39	75
(D) Antibody kit (IgG)				
Antibody kit (IgM)	Negative		Positive	Total
Negative	24		15	39
Positive	13		24	37
Total	37		39	76

Abbreviations: IgG, immunoglobulin G; IgM, immunoglobulin M.

twice that of antigen test. Present study shows sensitivity of combined testing is approximately three times than antigen testing alone, whereas this value is double when compared with only antibody testing. SARS-CoV-2 sensitivity increases when combined methods are used.

The reason for lower sensitivity of antigen tests is the dependance upon viral load. Also, RT-PCR may remain positive for longer duration, because it is a nucleic acid detection-based test. However, antigen will clear from the nasopharynx earlier. Higher viral load has correlated with higher sensitivity but it shows high specificity (99.3–100%).^{11,12}

The appearance of IgG antibodies correlates with clearance of antigen in various infectious diseases; surveilling symptomatic individuals or individuals with mild symptoms who are likely to be unaware of their disease status would be greatly helped by combining antigen to the test. However, the overall sensitivity of the kit is not very promising for diagnostic utility for COVID-19 and there is much scope of improvement.¹³

A major limitation of this study was small sample size. In addition, cycle threshold values were not followed up in the subjects.

Conclusion

To enhance the laboratory diagnostics in the most challenging time of COVID-19 pandemic supporting evidence-based

medicine, government guidelines, health-care policies should be formulated and prioritized. The efforts of microbiologist–clinician team should focus on implementing the most reliable diagnostic tools; however, because COVID-19 is a new disease, there are not enough data as of yet that would enable the determination of standards for the interpretation of serological point of care test. Serological tests should meet the standards to be put to use in patients having symptomatic and asymptomatic disease.

Conflict of Interest

None.

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