

RAPID ANTIGEN DETECTION KIT AS A DIAGNOSTIC TOOL FOR SARS COV-2

Clinical Microbiology

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ABSTRACT

Background: The first case of SARS CoV2 was detected in December 2019 from Wuhan city of China and in January 2020 from India. Since then, COVID-19 disease was rapidly spread out to all over the world and presented as severe pandemic. Real time RT-PCR is the gold standard frontline test for diagnosis of COVID-19 disease, caused by SARS-CoV-2 virus but it is time consuming and requires specialized laboratory facilities in terms of equipment, biosafety & biosecurity. **Material & Methods:** This retrospective study was undertaken from duration of June 2020 to October 2021 at B.J Medical college, Ahmedabad. In this study four rapid antigen detection test kits were evaluated for acceptance and fourteen rapid Ag Detection kits were validated (batch validation) as per ICMR guidelines and compared with gold standard real-time RT-PCR test at BJMC Ahmedabad. Considering real-time RT-PCR as the gold standard test, the sensitivity and specificity of the rapid antigen test kits were calculated.

Result: One out of four rapid Ag detection kits for evaluation, and all out of Fourteen in use approved rapid Ag detection kits for batch validation, were showing sensitivity more than 50% and specificity 100% and fulfilled the acceptance criteria defined by ICMR. **Conclusion:** Though gold standard test is real time PCR, rapid Ag tests help in early detection and containment of infection, allay the anxiety and fear of healthcare workers and aid in better clinical management of the patients.

KEYWORDS

SARS-CoV-2; rapid antigen test; specificity; sensitivity

INTRODUCTION

The Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) emerged in Wuhan, China, on December 31, 2019 and was declared pandemic by World Health Organization (WHO) on March 11, 2020 [1]. The search for rapid, accurate and affordable Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) diagnosis tools has become a global priority during Coronavirus-related Disease (COVID 19) pandemic. The real-time reverse transcription polymerase chain reaction (RT-PCR) obtained from nasopharyngeal or oropharyngeal swab samples is currently the most used diagnosing-test and it is considered the gold standard specimen for SARS-CoV-2 RNA testing. It is a very sensitive method and when used quantitatively, analyzing the cycle threshold (Ct), it also gives information on the viral load and the infectiousness. However, sometimes its use is limited, as it requires long turnaround times, skillful operators, biosafety measures, expensive instrumentation and a laboratory setting, resources not always available in most geographical areas [2]. Rapid diagnosis tests based on antigen detection are faster, easier to perform and cheaper. They can be used outside the laboratory and with fast turnaround times, therefore they could be essential when implementing population policies. These rapid tests, through a rapid chromatographic immunoassay technique, are used for the qualitative detection of SARS-CoV-2 antigens generally in nasopharyngeal swabs, they can be easily interpreted without specialized instrument and in less than 30 min. Most rapid antigen test have been designed and validated for the detection of SARS-CoV-2 in patients with COVID-19 symptoms in hospital settings, and there is currently little evidence on the accuracy of these tests in widespread testing of asymptomatic populations and in different settings [3].

Aims & Objectives

- To evaluate the rapid antigen kit for diagnosis of COVID-19 disease by comparing it with gold standard PCR test.
- To validate (batch validation) in use approved rapid Antigen detection kit of COVID-19.

MATERIALS & METHODS

This retrospective study was undertaken from duration of June 2020 to October 2021 at B.J Medical College, Ahmedabad. Clinical specimens are Respiratory samples, mainly nasopharyngeal and throat swabs, were collected from 1591 suspected COVID-19 cases at Civil Hospital, Ahmedabad from June 2020 to October 2021. Samples were mixed in 2 mL of viral transport media (VTM). Rapid Antigen testing was performed on site at sample collection and RT PCR testing should

performed at laboratory and compare With Rapid antigen test result for kit sensitivity and specificity.

For Batch validation of in use rapid Antigen detection kit at least 10 samples were tested as per ICMR guideline. Four rapid Ag detection kits were evaluated and Fourteen rapid Ag detection kits were validated (batch validation) at center.

RESULT

- Amongst the 1591 samples, the RT PCR detected 871 as Positive and 720 as Negative, the rapid antigen test detected 432 as positive, showing an overall sensitivity of 54.01%. All the samples detected positive with the antigen rapid test were also detected positive by RT-PCR.
- 288 Patient who were negative by RAT were detected positive by RT-PCR.
- The 720 samples with a negative result with the RT-PCR technique were also negative with the rapid test, giving an overall specificity of 100%.

Table 1: Sensitivity & Specificity of Rapid Antigen Kit for evaluation.

Kit Name	Number of patients recruited	Rap id Ag +ve	Rap id Ag -ve	RT PC R +ve	RT PC R -ve	Sensiti vity	Specifi city
YBIO RAPITEST COVID-19	202	58	144	91	111	63.74%	100%
	197	57	140	86	111	66.28%	100%
TRURAPID COVID 19	219	73	146	156	63	46.79%	100%
	209	72	137	146	63	49.31%	100%
Seloi Healthcare Insta covid 19	200	38	162	88	112	43.18%	100%
	174	37	137	83	91	44.57%	100%
SARS COV2 Antigen IVD(Mankind)	210	42	168	122	88	34.43%	100%

As shown in Table 1 Out of four rapid Ag detection kits for evaluation, only one kit had sensitivity more than 50% and specificity 100%.

As shown in Figure 1 Out of Fourteen rapid Ag detection kits for batch validation all kits had sensitivity more than 50% and specificity 100%. All 14 kits have 100 % Positive predictive value and 67% Negative predictive value.

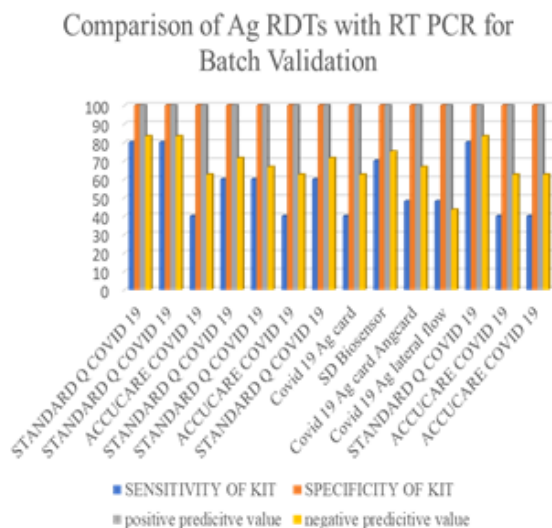


Figure 1: Comparison of Ag RDTs with RT PCR for Batch Validation

Kits which have sensitivity more than 50% with comparison to gold standard RT PCR test was accepted as per ICMR criteria for diagnostic use. At center one kit fulfilled such criteria and accepted by ICMR. All the kits for batch validation were showing sensitivity within the range hence accepted.

DISCUSSION

This observational study, designed to analyze the efficacy of the Rapid Antigen Test, showed the limitations of this test when used as a screening massive tool for the diagnosis of acute SARS-CoV2 infection in symptomatic as well asymptomatic populations. Positive results indicate the presence of viral antigens, but clinical correlation with patient history and other diagnostic information is necessary to determine infection status. The test can detect both viable and non-viable SARS CoV2 antigenic material.

A false negative test result may occur if the level of antigen in a sample is below the detection limit of the test or if the sample was collected or transported improperly; therefore, a negative test result does not eliminate the possibility of SARS CoV2 infection. Individuals with CT values greater than or equal to 34 are unlikely to have replication – competent virus.

The rapid spread of SARS-CoV-2 demands the availability of a rapid, accurate, and affordable diagnostic test. Rapid diagnosis can limit the spread of the infection in the initial stages and can be very beneficial in asymptomatic contacts of symptomatic patients and super-spreaders. In a country with a huge population like India, SARS-CoV-2 testing by RT-PCR is a challenging task. Thus, RAT can be utilized for rapid SARS-CoV-2 detection as a point-of-care test. The nucleocapsid protein of the virus is present in abundance in the nasopharyngeal samples; thus it is a preferred target analyte for antigen detection.

Compared to the RT-PCR test, this antigen test greatly reduces the turnaround time from 6 h to 30 min and can be used as a routine in a high-throughput setting, especially during a pandemic where infection control is the highest priority and there is an urgent need to isolate positive cases.

In this study overall sensitivity of rapid Ag test Kit was 54.01% and specificity of 100 %. Out of four rapid Ag detection kits for evaluation, only one kit (Y Bio) had sensitivity more than 50% and specificity 100%. This was further justified when a study by Gupta et al. from India exhibited a much higher sensitivity (81.8% (95% CI: 73.2–90.4)) and the Rapid Ag test kit used in this study was in fact manufactured in India [4,5]. Chaimayo et al. in their study mentioned higher sensitivity of the rapid SARS-CoV-2 antigen test (98.33% by Standard Q COVID-19 Ag test) than other rapid antigen tests [6]. J clin et al shown sensitivities ranged from 76.2% (95% CI: 65.4–85.1%) to 88.8% (95% CI: 79.7–94.7%) while the specificity ranged from 96.3% (95% CI: 90.8–99.0%) to 99.1% (95% CI: 95.0–100%) [3].

Till date, 95 Antigen based Rapid Test Kits have been validated

(including 17 revalidated Kits), and the following are found to be satisfactory. Kits which have sensitivity more than 50% with comparison to gold standard RT PCR test was accepted as per ICMR criteria for diagnostic use. At center one kit fulfilled such criteria and accepted by ICMR. All the kits for batch validation were showing sensitivity within the range hence accepted.[7]

In this study we had validate 14 Rapid Ag test kits for SARS-Cov2 detection, among 14 all kits had sensitivity more than 50% and specificity 100% and all 14 kits had 100 % Positive predictive value and 67% Negative predictive value. A. Fernandez-Montero et al. in their study shown 71.43% sensitivity, 99.68 % specificity, 81.40% Positive predictive value and 99.44% Negative predictive value [2].

Antigen-detecting diagnostic tests are designed to directly identify SARS-CoV-2 proteins produced by replicating virus in respiratory secretions (or oral fluid/saliva) and have been developed as both laboratory-based tests and rapid diagnostic tests intended for near-patient use. The diagnostic development landscape is dynamic, with over two hundred tests for SARS-CoV-2 antigen detection on the market, of which 85% can be delivered at the point of care and the other 15% for use on high throughput machines in laboratory-based settings [8].

CONCLUSIONS

Though gold standard test is real time PCR, rapid Ag tests would help in early detection and containment of infection, allay the anxiety and fear of healthcare workers and aid in better clinical management of the patients. It is very helpful in mass screening during abrupt increase in number of cases when testing with RTPCR cannot be copied. However, further prospective studies are required for its implementation in the best clinical setting.

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