Comparison of the Rapid Antigen Testing Method With RT-qPCR for the Diagnosis of COVID-19

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Abstract

Background: Coronavirus disease 2019 (COVID-19) has till now affected about 110 million people globally. It has not spared any country and has led to 24 lakh deaths. As a result, the testing had to be increased manifold leading to depletion in the number of the quantitative reverse transcription polymerase chain reaction (RT-qPCR) kits. Point-of-care rapid antigen-based tests were developed in order to meet the increasing demands. The objective of this study was to compare the performance of a rapid chromatographic test (index test) with a gold standard test (RT-qPCR).

Methods: A retrospective analysis was done at a tertiary care teaching hospital in Eastern Uttar Pradesh, India. Paired samples were taken from all patients reporting to the clinic for antigen-based rapid diagnostic testing (RDT) and RT-qPCR. The sensitivity and specificity were calculated to evaluate the performance of the RDT.

Results: The overall sensitivity and specificity of the RDT were observed to be 53.6% (79.7-67.0) and 97.35% (94.6-98.9), respectively. In symptomatic individuals, the sensitivity was higher 61.0% (44.5-75.8). The test positivity rates of RDT were found to be higher at a cycle threshold value <20.

Conclusion: RDT can be used as a screening test to rule in the infection especially in symptomatic patients who are more prone to spread the disease. It is an important weapon in the armamentarium of public healthcare for the containment of COVID-19.

Categories: Internal Medicine, Infectious Disease, Epidemiology/Public Health

Keywords: rdt, covid-19, immunochromatography, test characteristics, rt-qpcr

Introduction

Coronaviruses are enveloped non-segmented, positive-sense RNA viruses belonging to the family Coronaviridae and order Nidovirales [1,2]. They cause multiple system infections in animals but mainly lead to respiratory tract infections in human beings [3,4]. Although most of the human infections are mild in nature, the epidemics of the other two betacoronaviruses, severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome (MERS)-CoV, were fatal in nature and have caused more than 10,000 deaths in the past two decades. In December 2019, one more coronavirus disease emerged in Wuhan, China, and rapidly spread all over the world. After performing a deep sequencing analysis of the lower respiratory samples, this unknown virus was named as SARS-CoV-2 and disease named as coronavirus disease 2019 (COVID-19) [5,6]. Till date, India has reported 31,440,951 confirmed cases of COVID-19 and is ranked second only to the United States of America [7]. This growing trend and overwhelming increase in the total number of cases has overburdened our testing capacity and has led to a severe scarcity of molecular testing kits and reagents. Moreover, the quantitative reverse transcription polymerase chain reaction (RT-qPCR) testing requires a sophisticated biosafety level (BSL-2/BSL-3) laboratory and skilled technicians to perform the test. It takes a minimum of 8-10 hours for the generation of report from the receiving of the sample. Although it is the gold standard test for the diagnosis of COVID-19, it cannot be performed in each and every city/district due to lack of molecular virology facilities and difficulty in procuring reagents/viral transport medium (VTM) [8]. In view of the above, the need of the hour was to develop a point-of-care test that would detect and isolate positive molecular cases rapidly, diagnose them at an early stage and contain the spread. This could prove very useful in an emergency department where a quick triage could be done for patients who are more prone to spread the disease. It is an important weapon in the armamentarium of public healthcare for the containment of COVID-19.
The overall sensitivity and specificity of the antigen test came out to be 53.6% (95% CI: 39.7%-67.0%) and 97.3% (95% CI: 94.6%-98.9%), respectively. The positive predictive value (PPV) was 81.1% (95% CI: 64.8%-73.8%). The positive predictive value (PPV) was 81.1% (95% CI: 64.8%-73.8%). The agreement between the tests was moderate with a kappa value of 0.57 (p value: 0.000).
92.0%) and negative predictive value (NPV) 90.7% (95% CI: 86.7%-93.9%). The detailed test characteristics are represented in Table 1. The relation between the pretest probability and posttest probability is represented in the Figure 1. When tested individuals were segregated according to the symptoms, sensitivity of the RAT was found to be higher among the symptomatic individuals than the asymptomatic individuals whereas the specificity was found to be higher in asymptomatic individuals than the symptomatic. Thus, the RAT is a better test to rule out the infection than diagnosing the infection. This is also evident from the NPV value.

|                  | Overall | Symptomatic | Asymptomatic |
|------------------|---------|-------------|--------------|
| True positive    | 30      | 25          | 5            |
| True negative    | 255     | 85          | 170          |
| False positive   | 7       | 5           | 2            |
| False negative   | 26      | 16          | 10           |
| Sensitivity      | 53.6% (39.7-67.0) | 61.0% (44.5-75.8) | 33.3% (11.8-61.6) |
| Specificity      | 97.3% (94.6-98.9) | 94.4% (87.5-98.2) | 98.8% (95.9-99.9) |
| LR (+)           | 20.1 (9.3-43.3) | 11.0 (4.5-26.6) | 28.7 (6.1-135.0) |
| LR (-)           | 0.5 (0.4-0.6) | 0.4 (0.3-0.6) | 0.7 (0.5-1.0) |
| PPV              | 81.1% (64.8-92.0) | 83.3% (65.3-94.4) | 71.4% (29.0-96.3) |
| NPV              | 90.7% (86.7-93.9) | 84.2% (75.6-90.7) | 94.4% (90.0-97.3) |

**TABLE 1: Test parameters according to the type of patient (symptomatic vs asymptomatic)**

LR, likelihood ratio; PPV, positive predictive value; NPV, negative predictive value

**FIGURE 1: Relation between the pretest probability and posttest probability for the rapid antigen test in COVID-19**

COVID-19, coronavirus disease 2019

When we compared the test positivity at different cycle threshold (CT) values, we found that the antigen positivity increases from CT values <20 to 21-25 to achieve a highest value of 88% and then decreases gradually to almost 0% in patients with a CT value more than 35 (Figure 2).
Discussion

This study was a hospital-based study of the analysis of test characteristics of the rapid antigen test kit (STANDARD Q) when compared with the RT-qPCR (gold standard). The sensitivity of STANDARD Q was found to be 53.6% and specificity was found to be 97.3% with the PPV of 81.1% and NPV of 90.7%. Among symptomatic individuals, the sensitivity was found to be 61.0% and specificity was found to be 94.4% whereas among asymptomatic individuals, it was 33.3% and 98.8%, respectively.

Many studies conducted in India and abroad found the sensitivity and specificity to be higher as compared to the current study [11-15]. The reported sensitivity varied from as high as 93.9% to as low as 70.0% and the specificity varied from as high as 100% to as low as 92.0%. The severity of infection or antigen load may also be a critically determining factor. In the current study population, only 38.6% were symptomatic. When we classified the RT-qPCR results according to the CT values, we found the CT values ranged from 18 to 39. Only 5 cases (8.9%) reported to have CT values ≤20. Around 37.5% of the RT-qPCR-positive individuals had the CT values >30. When we compared the test positivity rate of RT-qPCR at various cutoffs of CT values, we found the test positivity to rapidly decline after the CT value of 25.1 to 30.0 from 88.2% to 61.5% (Figure 2). The test positivity further declined to 0% when the CT value was more than 35.0. This indicates the infection was low grade that might have resulted in the low sensitivity of the test results. A similar pattern was observed in the study conducted by Gupta et al. in New Delhi [15]. This indicates the antigen test is not a reliable in low-viral-load cases many of which are asymptomatic. Thus, rapid antigen test kits can be used as a screening test to rule out the infection but has lower significance when used in contacts who are asymptomatic. At the same time, in individuals with a high viral load, the sensitivity reaches as high as 88%. Thus, it can be used to screen the individuals with a high viral load (more likely to be symptomatic), thus preventing the spread of the disease in the community. The low sensitivity and specificity may be attributed to the low prevalence in the current study population as compared to the other studies. Although there is a wide variability in the sensitivity and specificity of the RDT, the kits are suitable for point-of-care testing and thus can have wide applicability in pandemic control [15].

The likelihood ratio was found to be around 20, which indicates there is a 20-fold increase in the likelihood of being COVID positive after RDT positivity. The posttest positivity increases from a pretest value of 18% to 82%, which shows that 82% times a person who is RDT positive is likely to be COVID positive, and 9% times, the RDT-negative patients may be COVID positive. Thus, highly susceptible cases like symptomatic contacts and cases from high-prevalence areas like hospital settings can be used to diagnose the cases that can be further confirmed by the RT-qPCR.

The study is strengthened by the fact that this is one of the few studies conducted in India that reported the test characteristics of the RDT kit for COVID-19. The study is limited by the fact that sample size has not been calculated for the study.

Conclusions

The rapid antigen test is an important tool for controlling the ongoing COVID–19 pandemic and early detection of cases, particularly in special situations. It was seen in this study that the rapid antigen test performed well in the patients with a low CT value, i.e., a high viral load. Thus, all such cases if diagnosed early can help in breaking the chain of transmission. There is further need to assess the performance of
various types of SARS-CoV-2 rapid antigen tests available commercially.

**Additional Information**

**Disclosures**

**Human subjects:** Consent was obtained or waived by all participants in this study. Institutional Human Ethics Committee (IHEC) issued approval IHEC/AAIMS-GKP/BMR/56/2020.

**Animal subjects:** All authors have confirmed that this study did not involve animal subjects or tissue.

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