Evaluation of the performance of the COVID-19 rapid antigen tests in a tertiary level hospital in Bangladesh.

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Abstract

Background: While the COVID-19 pandemic is a worldwide crisis, tests with high sensitivity and specificity are essential for identifying and managing COVID-19 patients. Globally, several rapid antigen tests RATs for COVID-19 have been developed, but their clinical efficacy has not been well established. This study aimed to evaluate the performance of several rapid antigen tests (RATs) to diagnose SARS-CoV-2 infection.

Methods: This prospective observational study was conducted at Shaheed Suhrawardy Medical College hospital from February 2021 to April 2021 in Dhaka, Bangladesh. This study included the patients admitted in this hospital at the COVID-19 isolation unit or referred from the triage facility of the outdoor department of this hospital suspected as COVID-19 case. Two nasopharyngeal samples were collected simultaneously. one sample was used on the spot for the RAT. The other was sent to the adjacent Shaheed Suhrawardy Medical College COVID-19 RT-PCR laboratory for real-time reverse transcription-polymerase chain reaction (qRT-PCR). The performance of the RAT was evaluated using the results of qRT-PCR as a reference.

Results: A total of 223 patients were included in this study, and the real-time RT-PCR detected SARS-CoV-2 in 84 (37.7%) patients. Of these 84 patients, 9 (10.7%) were asymptomatic. The overall sensitivity and specificity of RATs were 78.6% and 99.3%, respectively. The sensitivity was 81.3% in symptomatic cases and 55.6% in asymptomatic cases. False-negatives were observed in 18 patients, 3 of whom were asymptomatic and had a low viral load (cycle threshold (Ct) > 30). The detection rate of RATs was 100% when the Ct value was up to 24. The detection rate was 42.3% when the Ct was >29. The detection rate of RATs was 92.3% when the onset of symptoms was within three days. The detection rate was 33.3% when the onset of symptoms was >7 days.

Conclusions: RATs for COVID-19 used in this study delivered an acceptable performance in patients with high viral load and within the first week of the onset of symptoms. They can be used as a supplementary method to RT-PCR for the diagnosis of COVID-19 patients.

Keywords: COVID-19, SARS-CoV-2, rapid antigen tests (RATs), RT-PCR, nasopharyngeal swab

Introduction

Since SARS-CoV-2 surfaced in China, it has become a major public health issue worldwide. There are 233,251,109 confirmed COVID-19 cases detected worldwide with 4,772,794 deaths, and in Bangladesh, there are 1,553,873 cases with 27,470 deaths to date (1). The incubation period of SARS-CoV-2 infection is approximately 2-5 days, and fever, cough, and fatigue are the most common primary symptoms (2). Diagnosis of COVID-19 becomes more complicated by the high overlap between the clinical symptoms of SARS-CoV-2 infection and those of other respiratory diseases and by the asymptomatic carriers (3). Rapid and effective diagnostic methods are required for the isolation and early management of SARS-CoV-2 infected patients (4). The mainstay for identifying infected individuals has been nucleic acid amplification tests (NAATs) for upper respiratory samples (5). The highest viral load is found in the upper respiratory tract early in the infection (6). While these assays are regarded as gold-standard evaluations, their clinical utility has been limited due to limited availability, significant turnaround time, and the requirement of highly trained personnel (7). In outbreak situations, the number of patients eligible for these tests may outnumber the testing capacity.

Several rapid antigen tests (RATs) for COVID-19 are currently available on the market. They could be used to detect acute infection instead of RT-PCR. RATs are less expensive, more accessible point-of-care tests that take less time to obtain findings; as long as they consistently detect SARS-CoV-2, they can be more effective in low-resource situations. The RATs' accuracy, on the other hand, is questioned (6). The World Health Organization

(WHO) issued interim recommendations on RATs for patient management where they must have a minimum of 80% sensitivity and 97–100% specificity as a minimum requirement (8). They could be used as triage tests to quickly identify people who are very likely to have COVID-19, decreasing or eliminating the need for costly nucleic acid amplification testing. In Bangladesh, most COVID-19 RT-PCR laboratories remain in urban areas. Rural people sometimes don't get a chance to perform RT-PCR tests or get the result timely. It delays the isolation of the patient and contact tracing; as a result, the virus spreads rapidly. Issues also remain regarding reagent supply, cost, and inadequate testing capacity. The government of Bangladesh already sets up RAT facilities for COVID-19 from tertiary to thana level hospitals.

The few field tests of SARS-CoV-2 RATs that have been published previously have yielded conflicting results. The COVID-19 Ag Respi-Strip (Coris Bioconcept, Gembloux, Belgium) showed high specificity (99.5–100%) but low sensitivity (30.2–57.6%) when compared to qRT-PCR in three different studies (9) (10). The BIOCREDIT COVID-19 Ag test (RapiGEN Inc., Gyeonggido, 14119, Korea) also had low sensitivity (11.1–45.7 percent with specimens from the nasopharynx, throat, saliva, and sputum) (11). The authors concluded that this test should only be used to supplement the qRT-PCR test due to the risk of false-negative results. However, two other investigations found that another antigen test, the Fluorescence Immunochromatographic SARS-CoV-2 Antigen Test (Bioeasy Biotechnology Co., Shenzhen, China), performed well. The overall sensitivity and specificity were 93.9 percent and 100 percent, respectively, in a trial of 127 participants in Chile (12), while the accuracy was 96.1 percent. The total sensitivity and specificity were 67.8% and 100%, respectively, in the second investigation of 239 people in China (13). The rapid antigen test can be performed in triage (with later selective testing by qRT-PCR) in scenarios requiring quick isolation of cases, such as symptomatic individuals or "high-risk" truck drivers at border crossings people from abroad in airports or seaports. Because a few antigen false positives are not infected, all individuals

4

classified as COVID-19 positive would require customized isolation until the qRT-PCR results are available and prevent contact with true positives until the qRT-PCR results are available. Suspected individuals who become negative in the COVID-19 rapid antigen test should be tested sequentially by RT-PCR to rule out infection. In contrast, a positive test should be considered as a true positive for high-risk groups in containment zones or hotspots and healthcare settings". In this study, the performance of several SARS-CoV-2 rapid antigen test kits was evaluated compared to the RT-PCR as the gold standard method.

Methods

2.1 Study population

This prospective observational study was conducted at Shaheed Suhrawardy Medical College hospital from February 2021 to April 2021 in Dhaka, Bangladesh. This study included the patients admitted in this hospital at the COVID-19 isolation unit or referred from the triage facility of the outdoor department of this hospital suspected as COVID-19 case.

2.2 Specimen collection

Paired nasopharyngeal swabs were taken from each patient by trained medical technologists. One swab sample was used for the rapid antigen test. Another was used for RT-PCR, which was transferred to Shaheed Suhrawardy Medical College COVID-19 RT-PCR laboratory in viral transport media by maintaining the cold chain. A rapid antigen test was performed on-site, and the RT-PCR was done within 24 hours of sample collection for the case of every participant. A trained medical technologist filled a data collection sheet before sample collection.

2.3 Rapid antigen test

In this study, there are eight manufacturers' Rapid antigen tests are used (Table 1). Each test was performed according to each manufacturer's information for use (IFU), and the visual interpretation obtained the results within 30 minutes. They are membrane-based immunochromatography assays that detect SARS-CoV-2 nucleocapsid antigen (N-protein) in nasopharyngeal samples over monoclonal antibodies. The manufacturers provide all necessary reagents to perform the assay, and no assay-specific, specialized equipment is needed. According to the IFU, the assay kits were stable when stored at room temperature.

Table 1. Characteristics of rapid antigen tests used in this study

S.no.	Name	Manufacturer	Target	Technology	Procedure
1	STANDARD Q COVID-19 Ag Test	SD BIOSENSOR	nucleocapsid	Immunochromatography	15-30 mins
2	AMS COVID-19 Antigen Rapid Test	AMS UK (NI) Limited	nucleocapsid	Immunochromatography	15-30 mins
3	StrongStep SARS-C0V-2 Antigen Rapid test	LIMING BIO	nucleocapsid	Immunochromatography	15-30 mins
4	VivaDiag SARS-C0V-2 Ag Rapid test	VivaCheck Biotech Co. Ltd	nucleocapsid	Immunochromatography	15-30 mins
5	BIOCREDIT COVID-19 Ag	RapiGEN INC	nucleocapsid	Immunochromatography	5-8 mins
6	NEWGENE COVID-19 Antigen detection kit	NEWGENE Bioengineering Co. Ltd	nucleocapsid	Immunochromatography	15-30 mins
7	VERI-Q COVID-19 Ag Rapid test	MicoBioMed Co, Ltd	nucleocapsid	Immunochromatography	20-30 mins
8	Humasis COVID-19 Ag test	Humasis Co, Ltd	nucleocapsid	Immunochromatography	15-30 mins

2.4 Real-time RT-PCR assays for the detection of SARS-CoV-2 RNA

Sample preparation and RT-PCR were done by Sansure Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing), which targeted two SARS-CoV-2 genes, namely, the ORF1ab and N genes. QuantStudio 5 (Applied Biosystems) was

used for genome amplification. The interpretation of the results was made according to the manufacturer's instructions. An RNA internal extraction control and an amplification control were included in the assay. Samples showing an exponential growth curve and a cycle threshold (Ct) value < 37 were considered positive.

2.5 Data analysis

The sample was described as age (median in both assays) and sex percentage. The performance of Covid-19 Rapid antigen tests was assessed, calculating its sensitivity and specificity. RT-PCR was considered the "gold standard" for evaluating the positivity or negativity of the samples under analysis. All analyses were performed using SPSS v. 22.0.

Results

In total, 223 COVID-19 cases were included in this evaluation. The majority were males (60%), and the overall mean age was 34 years (95% CI: 32–35 years; Table 2). 37% of cases were positive among the total cases. Among them, 64% were male, and 36% were female. Positive cases belong to the 18-64 years age group. Maximum people were symptomatic (89%). All demographic data of the study population are shown in Table 2. Among the symptomatic SARS-CoV-2 RT-PCR positive cases, fever was the most common symptom (90.7%), followed by loss of smell (72.0%) and cough (72.0%). The study subjects were also present with sore throat, breathlessness, fatigue, and diarrhea (Table 3). The overall sensitivity, specificity, PPV and NPV of RATs was 78.6%, 99.3%, 98.5% and 88.5% respectively. The sensitivity was found high (81.3%) in symptomatic cases and low (55.6%) in asymptomatic cases (Table 4). The cycle threshold (Ct) values ranged between 20.13 and 36.46. The positive samples, Ct 20-24, detection by rapid antigen tests was 100%. When the Ct was >29, the detection rate was only 42.3% (Figure 1). Sensitivities of rapid antigen tests by the onset of

symptoms were different according to time. When symptoms were within three days, the detection rate was 92.3%, 4-7 days. The detection rate was 86.8%. But when symptoms were >7 days, the detection rate was 33.3% (Figure 2).

Table 2: Demographic data of the study population.

Total		SARS-CoV-2 RT-PCR	
		Positive	Negative
	223 (100)	84 (37.7)	139 (62.3)
Age (years), Median	31	30.5	31
<18 (%)	14 (6.3)	8 (9.5)	6 (4.3)
18-64 (%)	205 (91.9)	74 (88.1)	131 (94.3)
>64 (%)	4 (1.8)	2 (2.4)	2 (1.4)
Sex			
Male (%)	134 (60.1)	54 (64.3)	80 (57.6)
Female (%)	89 (39.9)	30 (35.7)	59 (42.4)
Symptomatic patients (%)	178 (79.8)	75 (89)	103 (57.9)
Asymptomatic patients (%)	45 (20.2)	9 (11)	36 (80.0)

Table 3: Characteristics of symptoms of SARS-CoV-2 RT-PCR positive and SARS-CoV-2 RT-PCR negative patients.

Total		SARS-CoV-2 RT-PCR	
		Positive	Negative
n	178	75	103
The onset of symptoms (days) Median	5	5	5
Symptoms (%)			
Fever	141 (79.2)	68 (90.7)	73 (70.9)
Fatigue	126 (70.8)	52 (69.4)	74 (71.9)
Cough	125 (70.2)	54 (72.0)	71 (68.9)
Sore throat	67 (37.7)	20 (26.7)	47 (45.6)
Loss of smell	66 (37.0)	54 (72.0)	12 (11.7)
Breathlessness	33 (18.5)	17 (22.7)	16 (15.5)
Diarrhea	16 (8.9)	12 (16.0)	4 (3.9)

Table 4: Sensitivity and specificity of rapid antigen tests among all participants.

Overall			
	RT-PCR positive	RT-PCR negative	Total (%)
Antigen test positive (%)	66 (29.6)	1 (.4)	67 (30)
Antigen test negative (%)	18 (8.1)	138 (61.9)	156 (70)
Total (%)	84 (37.7)	139 (62.3)	223 (100)
Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
78.6 (68.3 to 86.8)	99.3 (96 to 99.9)	98.5 (90.3 to 99.8)	88.5 (83.6 to 92.0)
With symptoms			
	RT-PCR positive	RT-PCR negative	Total (%)
Antigen test positive (%)	61 (34.3)	1 (.5)	62 (34.8)
Antigen test negative (%)	14 (7.9)	102 (57.3)	116 (65.2)
Total (%)	75 (42.2)	103 (57.8)	178 (100)
Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
81.3 (70.7 to 89.4)	99.0 (94.7 to 99.9)	98.4 (89.6 to 99.8)	87.9 (82.0 to 92.1)
Without symptoms			
	RT-PCR positive	RT-PCR negative	Total (%)
Antigen test positive (%)	5 (11.1)	0 (0)	5 (11.1)
Antigen test negative (%)	4 (8.9)	36 (80)	40 (88.9)
Total (%)	9 (20.0)	36 (80)	45 (100)
Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
55.6 (21.2 to 86.3)	100 (90.3 to 100.0)	100	90.0 (78.8 to 97.5)

Sensitivity, specificity, positive, and negative predictive values are provided with 95% confidence intervals.
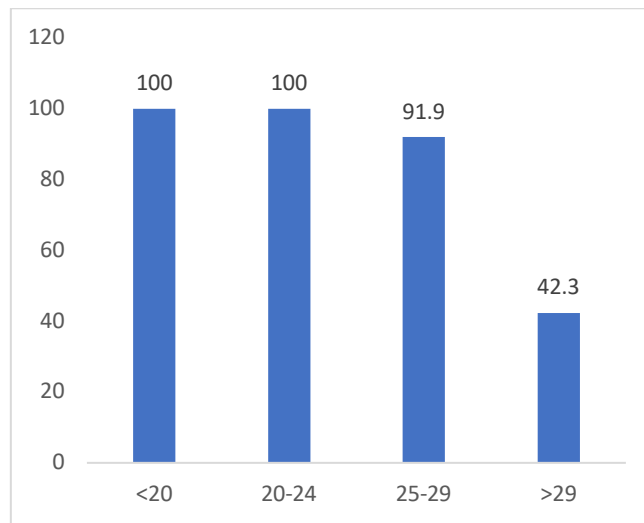
Figure 1: Sensitivities of rapid antigen test stratified by Ct value. Sensitivity is provided with 95% confidence intervals, Ct = cycle threshold.
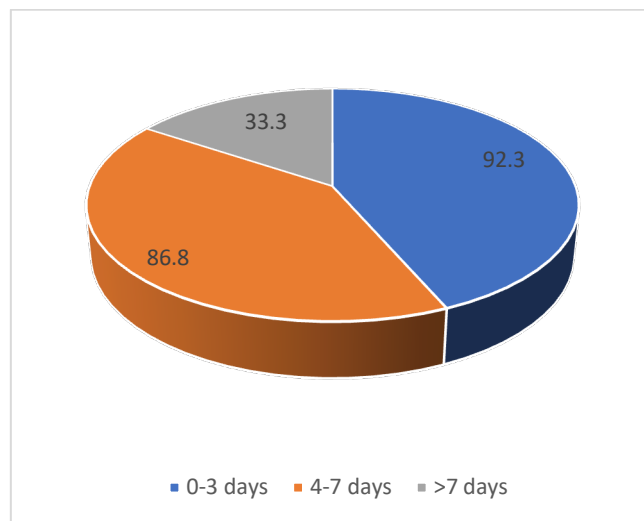

Figure 2: Sensitivities of rapid antigen test stratified by symptoms onset. Sensitivity is provided with 95% confidence intervals Ct = cycle threshold.

Discussion

From the beginning of the COVID-19 pandemic, it appears that the actual number of COVID-19 cases in many countries is much higher than reported due to the limited testing (14), (15). Though RT-PCR is the gold standard for diagnosing COVID-19, this technique needs biosafety level-2 laboratories with sophisticated analytical instruments and trained staff personnel (16), (17). The low-cost RATs for rapid COVID-19 with good diagnostic performance have become a global healthcare requirement. The rapid diagnostic test performed by a minimally trained healthcare worker close to a patient and outside of central laboratory testing thus widen the testing facility (18).

Laboratory confirmation of COVID-19 using rapid antigen tests depends on the viral load in samples (19), (20), (21). High viral loads (low Ct value) of SARS-CoV-2 is linked with the early respiratory symptomatic stage of COVID-19 (22), (23). SARS-CoV-2 viral RNA can be detected by RT-PCR as late as 83 days after the symptom onset, though detection of viral RNA by RT-PCR is not essentially related to infectiousness (24). When the SARS-CoV-2 virus was cultured from the upper respiratory tract samples PCR positive patients, growth was rarely positive after the ninth day of illness (25). This study revealed similar data which was found in previous studies. The RATs showed 100% sensitivity when the Ct value was <24. The sensitivity was highest (92.3%) when the onset of symptoms was within three days. Symptomatic patients showed the highest sensitivity, which was 81.3%. On the other hand, low viral load (high Ct value) was correlated with the negative RATs despite a positive RT-PCR test. Based on these findings, RATs can be used as a screening test for the patients who developed symptoms COVID-19 within 3-5 days. RATs are less effective when patients are in the late stage of the illness, which is consistent with WHO (26). Besides RATs and RT-PCR,

both test results could be false negative due to low viral load in nasopharyngeal specimens (26), (25), (27).

Previous studies have shown that, besides the lower sensitivity of RATs compared with PCR, they increase the turnaround time for results, which is vital to disrupt transmission chains to control the spread of this SARS-CoV-2 infection (28), (29), (30). Therefore, several diagnostic algorithms recommend the use of RATs as the first step for symptomatic patients within the first 5-7 days of symptom onset (31), (30), (32). The findings of this study were similar to the previous ones. Some authors have shown that RATs can be helpful to detect asymptomatic SARS-CoV-2 infection with high transmissibility (33). Based on these findings, RATs can be used as a screening test for masses people. This study found the sensitivity of RATs in asymptomatic patients is only 55.6%. So, more studies are required to ensure the effectiveness of RATs for that purpose.

A rapid diagnosis of suspected cases of SARS-CoV-2 infection is necessary for proper management of the infected patients and control of the spread of the virus. RATs deliver the result quickly, but factors such as patients' symptoms, the viral load, the quality of the specimen heavily influence their performance. In this study, the performance of several RATs was compared to that of the RT-PCR done by Sansure Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit, which is routinely used in the COVID-19 RT-PCR laboratory of Shaheed Suhrawardy Medical College. The data acquired suggest that the RATs perform well in the case of symptomatic patients when the viral load is high in the nasopharyngeal swab. The sensitivity found in this study is much lower than that claimed by the manufacturers. The overall (both symptomatic and asymptomatic patients) sensitivity was 78.6%, and specificity was 99.3%. The PPV and NPV were 98.5% and 88.5%, respectively, and accuracy

was 86.15%. Cohen's kappa value was 0.14, which indicates a poor agreement between the two assays. These data were similar to another recently published study (34). The poor sensitivity of the RATs is common and related to its technical design. RATs do not amplify their signal, so less amount of target protein can be skipped. WHO recommends that rapid antigen tests, which have a sensitivity of ≥80% and a specificity of ≥97%, feature close to those of a real-time PCR assay (35). RATs are not suitable as the sole screening tool for the diagnosis of COVID-19. RT- PCR should be performed on the negative cases that remain the gold standard for detecting SARS-CoV-2. As RATs were less sensitive than RT-PCR, a negative RAT result cannot exclude SARS-CoV-2 virus infection. The sample should be retested by RT-PCR further. So the RATs have the potential to replace the RT-PCR test to some magnitude but not in all circumstances (36).

This study has some limitations. It was a retrospective study which has been conducted in a single institution. RATs manufactured by eight companies have been used in this study, but more samples should be tested by each manufacturer. Thus, the conclusion of this study should not be generalized to other available RATs. More prospective multicentre studies and meta-analyses are required to establish the effectiveness of other RATs.

Conclusion

The RATs evaluated in this study showed an acceptable sensitivity in respiratory samples when the patient came in early days of infection or samples with low Ct values related to higher viral loads. Thus, it has the potential to become supplementary to qRT-PCR for the early diagnosis of SARS-CoV-2, especially in situations with inadequate access to molecular methods. But the results obtained from RATs should be confirmed by qRT-PCR.

List of abbreviations

COVID-19- Coronavrus Disease 2019)

RAT- Rapid Antigen Test

SARS-CoV-2- Severe Acute Respiratory Syndrome Coronavirus 2

qRT-PCR- Real-Time Reverse Transcription Polymerase Chain Reaction

NAAT- Nucleic Acid Amplification Test

IFU- Information for Use

CI- Confidence Interval

SPSS- Statistical Package for the Social Sciences

Ct- Cycle threshold

WHO- World Health Organization

Declarations

Ethics approval and consent to participate:

This study was approved by the Ethical Review Committee of Shaheed Suhrawardy Medical College, Dhaka, Bangladesh (No-ShSMCH/Ethical/2021/27).

Consent for publication:

Written informed consent was taken from each participant.

Availability of data and materials:

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

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