Original Article

Haematol J Bangladesh 2023;7(1):26-31
https://journal.hematologybd.org/index.php/haematoljbd
ISSN: 2523-1219 (print)
ISSN: 2707-1405 (online)

Comparative Study between Gold Standard Real-time RT-PCR Assay and Rapid Antigen Test for Detection of COVID-19 in Sylhet CMH

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Citation: Nurunnabi M, Mamdu MK, Sddika A. Comparative study between gold standard real-time RT-PCR assay and rapid antigen test for detection of COVID-19 in Sylhet CMH. Haematol J Bangladesh. 2023;7(1):26-31.

DOI: https://doi.org/10.37545/haematoljbd202398

Received: 17 July 2022

Accepted: 13 February 2023

Published: 20 March 2023

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ABSTRACT

Background: *The notorious pandemic coronavirus disease 2019 (COVID-19) has been spread all over the world. Its third pandemic wave has been completed and now a fourth wave is running over many countries. Almost 60 million people have been infected and more than 5.7 million people have died. Till now almost 1.2 million people have been infected and about 30000 people have died in Bangladesh from Covid-19. It has not only taken away human lives but also has put a tremendous impact on our society and economy. So, the early detection of this virus and isolation of the patients have a significant role to control the disease. Henceforth there is an urgent need for simple, accurate and rapid identifications of C0VID -19. Rapid antigen tests can provide timely results, which is of particular importance in a primary setting. So, the Performance of the Rapid antigen detection test (RAT) should be evaluated and compared with the gold standard real-time reverse transcription-polymerase chain reaction (RT-PCR) test for diagnosis of COVID-19.* ***Methods:*** *Specimens were collected from both nasopharyngeal and oropharyngeal regions from 1000 patients who reported to the flu centre in CMH Sylhet with complaints of fever, cough & headache for detection of COVID virus-2 RNA by rapid antigen (RAT) and RT-PCR test. We used a real-time RT-PCR kit (Sansure, Biotech china, gene RdRP & N) and a COVID-19 Ag Kit (Wondfo, Republic of China) for the detection of COVID-19 RNA.* ***Results:*** *Out of 1000 samples 158 were positive and 842 were negative by real-time RT-PCR assay where 154 samples were positive, and 846 cases were negative by rapid antigen test for severe acute respiratory syndrome (SARS) CoV-2. The duration from the onset of symptoms to laboratory tests in all suspected cases ranged from 0 to 02 days (with a median of 01 days). The rapid SARS-CoV-2 antigen detection tests sensitivity and specificity were 97.29% (95% CI, 90.06-99.89%) and 97.94% (95% CI, 97.26-98.57%), respectively. Seven samples were found negative in RAT but were found positive in RT-PCR, the other three samples were found positive in RAT while they were found negative in RT-PCR.* ***Conclusions:*** *It is observed that most rapid antigen tests for COVID-19 are significantly comparable with RT-PCR tests and had enough sensitivity and specificity for 158 individuals, infected with severe acute respiratory syndrome-CoV-2.*

Keywords: *COVID-19, SARS-CoV-2, Rapid antigen, RT-PCR, CMH Sylhet.*

Introduction:

The first case of COVID-19 was detected in the city of Wuhan, China, in December 2019. Thereafter already the third wave of the coronavirus disease (COVID-19), a delta variant, spread all over the world and had a devastating impact on our health, society and economy. Now its fourth wave is spreading in many countries including Bangladesh. As of first July 2022, almost 600 million people have been infected and near about 06 million have died.[1-3] Several millions of people have been infected with loss of thousand lives also in Bangladesh. As of now, there is no specific available therapy for controlling SARS-CoV-2 disease so a key component for controlling this devastating pandemic disease is rapid identification of SARS-CoV-2 and containment of infected individuals.[4,5] Relatively inexpensive and easily accessible Rapid antigen test (RAT) offers an easy-to-use diagnostic tool to quickly identify such patients. Moreover, they are useful in primary and emergency care settings since they do not require any laboratory equipment.[6,7] On the other hand, the real-time reverse transcription-polymerase chain reaction (RT-PCR) assay, which is the gold standard test for laboratory diagnosis of COVID-19, needs skilled technicians to perform and also requires at least four hours for getting the results, and hence, it is very necessary to do the COVID-19 screen test accurately and rapidly for controlling and preventing the disease.[8-10] We analysed a rapid SARS-CoV-2 antigen detection test, (Wondfo COVID-19 Ag kit), by using 1000 respiratory specimens. The result of this lateral flow immunoassay was compared with the RT-PCR assay for viral gene detection, (Sansure 2019-nCoV assay, China).[11-13]

Methods:

A total of 1000 samples mainly from nasopharyngeal and oropharyngeal were collected from suspected COVID-19 individuals, simultaneously for RT-PCR assay and rapid antigen tests (RAT). Later on, 2ml of viral transport media (VTM), consisting of an antibiotic, 0.4% foetal bovine serum, antifungal and HEPES agents mixed with the collecting samples and transported at 2-80C to the RT-PCR laboratory, CMH Sylhet.

Inclusion criteria:

a. Suffering from fever, Cough and sore throat recently last 01 to 05 days.
b. Positive contact history.
c. Preoperative cases.

Exclusion criteria

a. Age below 5 yrs.
b. Individuals previously suffered from COVID-19.
c. Individuals suffered from other upper respiratory tract infections like flu and dengue.

According to the manufacturer's instructions, Sansure 2019-nCoV Assay (China), which targets RNA-dependent RNA polymerase (RdRp) and nucleocapsid (N) genes of CoV-2, was used for CoV-2 RNA detection. The real-time thermal cycler, CFX-96 (Bio-Rad Laboratories) was used for amplification. Thereafter the result was analysed, and a positive result was defined in which a cycle threshold value (Ct value) was <40 for all two target genes.

In this study, the Rapid COVID-19 antigen test (Wondfo, Republic of China) kit was used which is a rapid lateral immuno-chromatographic assay for the detection of nucleocapsid (N) antigen of a severe acute respiratory syndrome (SARS)-CoV-2 in respiratory samples. This test device has control (C) and test (T) lines on the result window. Here the control (C) region is coated with mouse monoclonal anti-chicken IgG antibody whereas the test (T) region is coated with mouse monoclonal anti-SARS-CoV-2 antibody against SARS-CoV-2 N antigen, conjugated with colour particles. This antigen-antibody colour particle complex migrates to the test (T) region by capillary force. The intensity of the colour on the test (T) lines depends on the concentration of SARS-CoV-2 N antigen presented in the sample.

On the test device, three drops of the extracted sample were given and in 15-30 min the test result was analysed. Two coloured lines of control (C) and test (T) lines appeared for positive results as shown in Figure 1. All Specimens were processed in biosafety level-2 facilities.

Figure 1: Figure shows: Strip A= invalid (no mark in control & test region), B= Negative (Red mark in control region) and C= Positive (Red mark in both control & test region).

All Statistical results such as percentages, 95% confidence interval (95% CI), sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were obtained by using a statistical tool, SPSS-20.[14]

Results:

A total of 1000 specimens from nasopharyngeal and oropharyngeal/Throat swabs were collected from suspected COVID-19 individuals from March to December 2021 and were laboratory-confirmed by the gold standard RT-PCR assay.

The patients' median age was 32.6 yrs. Among the samples tested for COVID-19 by real time PCR assay, (Ensure nCoV-2019, assay), 15.8% (n=158) were positive, while 84.2% (n=842) were negative for SARS CoV-2 RNA as shown in Figure 2.

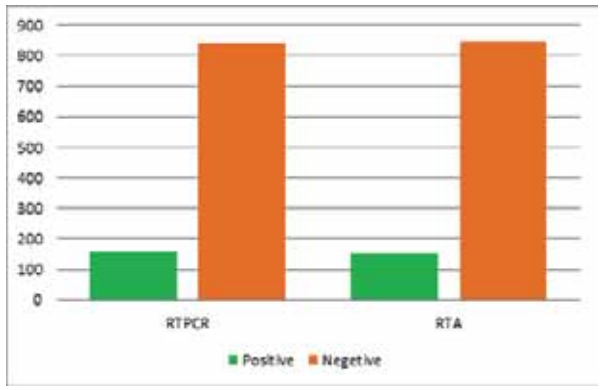

Figure 2: Number of positive and negative cases in RT-PCR and RAT.

The Figure shows: Out of 1000 samples, 158 cases were positive by RT-PCR but in the case of RAT the number of positive was 156. On the other hand, 842 samples were found negative by RT-PCR assay. Whereas 844 samples were found negative by the Rapid Antigen test (RAT)

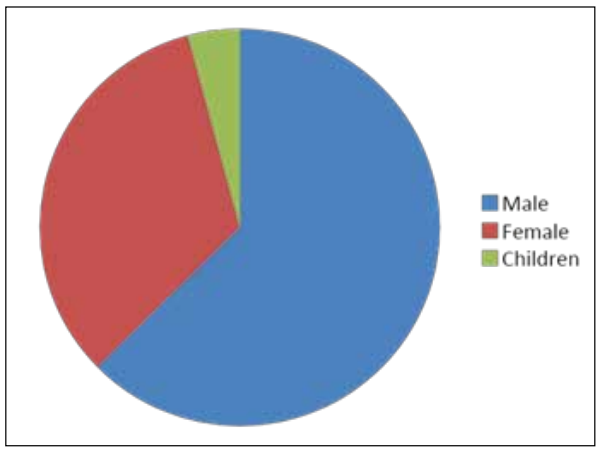

Figure 3: Presentation of sample size according to gender.

The Figure 3 shows, out of 1000 samples, males were 62.6%, females were 33.2 % and children were 4.2%. 79.6% of patients had direct contact with a variety of confirmed cases. Almost 90% of patients had symptoms of upper respiratory tract infections. The median time for onset of symptoms and performing both RT-PCR and RAT laboratory tests were 2 days.

Table I: The sensitivity and specificity of the Rapid COVID-19 Ag test (Wondfo)

	RT-PCR assay (Sansure 2019-nCoV Assay)		
	Positive	*Negative	Total
Rapid SARS-CoV-2 antigen assay (Wondfo COVID-19 Ag kit)			
Positive	154	2	156
Negative	4	840	844
Total	158	842	1000
Sensitivity	97.29% (151/158; 95% CI, 90.06–99.89%)		
Specificity	98.94% (840/ 842; 95% CI, 97.26–98.57%)		

Ct-values of RdRp, and N genes more than 40 are defined as* Negative.

For detection of SARS-COV-2 RNA, RNA dependent RNA polymerase (RdRp) and Nucleocapsid (N) genes were used in real time RT-PCR assay. whereas in positive cases average cycle threshold (Ct) values were 25.54±5.65 (min 13.51, max 38.90) for the RdRp gene, and 25.09±6.37 (min 12.07, max 37.17) for the N gene. But Ct-value was higher than 40 for both genes (RdRp and N), was defined as a negative.

Rapid antigen tests (Wondfo COVID-19 Ag test kit) were interpreted as a positive when both control (C) and SARS-CoV-2 antigen (T) lines appeared within 10-15 min, if a line only appeared on the control (C) region, defined as negative as shown in Figure 1. The test would be declared invalid if the line appears only on the T region but not in control (C).

The sensitivity and specificity of the rapid antigen test were compared with the real time RT-PCR assay and showed 97.29% (151/158; 95% CI, 90.06-99.89%) and 98.94% (840/ 842; 95% CI, 97.26-98.57%), respectively, as shown in Table I.

A total of six samples were discordant with RT-PCR results, 04 were false negative, and 02 were false positive. The false negative sample's Ct-values were 36.4 for RdRp, and 38.54 for N genes.

Discussion:

SARS-COV-2 infection can be confirmed by RT-PCR assay. The disease screening in our study can be sped up by other rapid antigen immunoassays which have an almost similar sensitivity and specificity to real-time RT-PCR. The manufacturer mentioned that the sensitivity and specificity were 84.34% and 100% of the traditional COVID-19 antigen test which detects the SARS-COV-2 infection.[15,16]

A higher level of viral load in specimens of SARS-COV-2 infection is detected in the upper respiratory system immediately after the symptoms are visible so there is a higher possibility of detecting positive antigens at this early stage. The SARS-COV-2 detection kit can be very effective for patients at this early stage. Other considerations such as clinical manifestations, duration from disease onset to laboratory test, types of specimens and how the specimens are gathered and processed may influence the results.[17,18]

Compared to other previously reported rapid antigen tests, our test appears to have a higher sensitivity 97.29% (by Wondfo COVID-19 Ag test). Whereas Fluorescence immuno-chromatographic assay reported a sensitivity of 93.9% for the 2019-nCOV antigen test, COVID-19 antigen Respi- strip CORIS reported 50% and 11.1-45.7% was reported by BIOCREDIT COVID-19 Ag.

In this study without the present population prevalence of COVID-19, the positive and negative predictive values (PPV and NPV) of the assay cannot be calculated accurately. However, we found 02 false positive samples tested by the Wondfo COVID-19 Ag test. The PPV is low in a low COVID-19 prevalence area.

Theoretically, the PPV vs NPV of the Wondfo COVID-19 Ag test would be 43.81% (95% CI, 24.56-65.17%) versus 99.98% (95% CI, 99.87-100.00%) in the 1% COVID-19 prevalence rate, while in the 10% COVID-19 prevalence rate, the PPV

vs NPV of the Wondfo COVID-19 Ag test would be 88.79% (95% CI, 78.47-95.37%) versus 99.76% (95% CI, 98.72-99.97%). Therefore, the Wondfo COVID-19 Ag test may be beneficial in the high prevalence area. We have found similarities in our study with the research done in Thailand after comparison. In our study, the sensitivity and specificity of the rapid antigen test compared with the real-time RT-PCR assay showed 97.29% (154/158; 95% CI, 90.06-99.89%) and 98.94% (840/842; 95% CI, 97.26-98.57%), respectively. On the other hand, in research done in Thailand, in June 2020, where sensitivity and specificity were 98.19% (59/60; 95% CI, 91.06-99.89%) and 98.54% (389/394; 95% CI, 98.16-98.37%), respectively.[19,20]

The simple procedure and fast results with high NPV of Wondfo COVID-19 Ag test are what makes it beneficial, but it detects low PPV in a low prevalence region which is a drawback, So the RT-PCR test for SARS-COV-2 RNA detection is the pivotal test for COVID-19 because it has higher sensitivity and specificity.

Conclusions:

For detection of SARS-CoV-2, the rapid antigen test (Wondfo COVID-19 Ag kit) conveyed comparable sensitivity 97.29% (95% CI, 90.06–99.89%) and specificity 98.94% (95% CI, 97.26–98.57%) with real-time RT-PCR assay. Rapid antigen test (RAT) can be beneficial to health workers when dealing with infected individuals, particularly in an outbreak and rural areas where resources are limited, despites its limitation. It is believed that due to rapid, simple, and easy to do, RAT can be very promising as a screening assay for detection of SARS-COV-2.

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