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Comparison of the SARS-CoV-2 Rapid Antigen Test with Real-Time RT-PCR Assay for Laboratory Diagnosis of COVID-19 in Bangladesh

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Abstract

Background: The authenticity and importance of RT-PCR testing cannot be overstated, for resource-limited settings like Bangladesh, especially in places where RT -PCR facilities are unavailable, rapid antigen testing could be an important supportive tool in SARS-CoV-2 diagnosis. **Objective:** The aim of this study was to compare rapid SARS-CoV-2 antigen detection test with RT-PCR for viral gene detection assay. **Methodology:** This cross-sectional study was performed at National Institute of Laboratory Medicine and Referral Center (NILMRC), Dhaka between March and April 2021. The nasopharyngeal swab samples were obtained from COVID-19 suspected cases collected from NILMRC virology lab. RT-PCR testing was conducted by Novel Coronavirus Nucleic Acid Diagnostic Kit. Rapid antigen testing was conducted using Standard Q COVID-19 Ag test. **Results:** The median age was 35.2 years. Among the confirmed cases, 63.0% were male patients. A total of 68 samples came out as positive and 226 were negative using both methods. Additionally, four more positive cases were detected by the Rapid Antigen Testing method. The sensitivity and specificity of the rapid antigen test was found to be 94.0% and 99.0% respectively. **Conclusions:** Rapid antigen test and rt-PCR showed almost similar sensitivity and specificity. [*Bangladesh Journal of Infectious Diseases, April 2022;9(suppl_1):S24-S27*]

Keywords: COVID-19; RT PCR; Antigen test

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Introduction

The quick spread of novel SARS CoV-2 over the world has created a significant danger to the civilization[1]. First detected towards the end of 2019, the virus is causing a continuing pandemic across the world. Correct and timely identification of SARS-CoV-2 infection is of paramount importance, considering the frequent movement and mixing of people around the world.[2]

Till date, more than 178 million cases of COVID-19 infections have been detected around the world, including approximately 3.9 million deaths. With unavailability of effective vaccines or therapeutic drugs in most countries, and considering the unusual disease course in individuals, rapid disease detection and isolation of infected individuals still remains the most effective mode of disease prevention.[3]

The gold standard for COVID-19 diagnosis, even after one and half year of first case detection, remains reverse transcription-quantitative PCR (RT-qPCR) using nasopharyngeal swabs, throat swabs, or saliva, which requires at least three to four hours of lab work performed by skilled technicians, and requires costly instruments are careful handling of specimens.[4,5]

But in countries like Bangladesh, with a struggling healthcare system and unavailability of state of the art lab facilities in most areas, RT-PCR is almost impossible to perform in point of care (POC) settings or as a main mode of diagnosis. This has been documented by the failure of authorities to ramp up PCR testing even after repeated warnings from experts and other stakeholders. Besides, specimens are transported to the laboratories or clinics, often to distant districts, that have RT -PCR machines and other facilities. There are several issues to deal with here –due to long transportation time sample quality degrades significantly, and due to excessive workloads and turnaround time, test results are delayed resulting in utter chaos. To improve this situation rapid antigen tests for COVID-19 have been approved in many other countries. It is not expensive and there is no need of skilled personnel, and the sensitivity is already compared with that of qPCR in previous studies.[6-9]Taking all things into account, Bangladesh started performing antigen tests from 5th December, 2020.[10]

Rapid antigen test, which is mainly lateral flow immunoassays, uses monoclonal anti-SARS CoV-2 antibodies that is targeted against SARS-CoV-2 antigens, and can be performed in less than 30 minutes.[5-7] So, the aim of this study was to we compare rapid SARS-CoV-2 antigen detection test which is done by Standard™ Q COVID-19 Ag kit (SD Biosensor®, Republic of Korea) with widely used RT-PCR for viral gene detection assay done by *SANSURE* BIOTECH INC (China).

Methodology

Specimens: This cross sectional study was conducted at the Virology Laboratory at National Institute of Laboratory Medicine and Referral Center (NILMRC). After obtaining proper consent from participants, nasopharyngeal swabs were collected from 300 suspected COVID-19 patients by the team of National Institute of Laboratory Medicine and Referral Center, Dhaka from March and April 2021. Samples were mixed in 2 mL of viral transport media (VTM). Samples were then transported in the cooler box (2–8 °C) to the virology laboratory, NILMRC. All specimens were processed in bio safety level-2 (BSL-2) facilities taking necessary precautions.

SARS-CoV-2 RNA detection using RT-PCR: Extraction was performed according to the manufacturer's instructions. Sample Lysis buffer provided by Sansure Biotech Inc., which targets RNA dependent RNA polymerase (RdRp) and nucleocapsid (N) genes of SARS-CoV-2, was used to lysate crude samples. Briefy, 20μL of extracted RNA was added to 30μL of master mix (26 μL 2019-nCoV-PCR Mix + 4 μL 2019- nCoV-PCR-Enzyme Mix). The Applied Biosystems *Quant Studio-5* Real-Time PCR System was used for amplification. The thermal conditions consisted of 1 cycle of 30 min at 50°C, 1 min at 95 °C followed by 45 cycles of 15s at 45 °C, and 10 s at 25 °C. A Ct value of <40 for either gene was considered a positive result.

SARS-CoV-2 Antigen Detection: Standard Q COVID-19 Ag test (SD Biosensor®, Republic of Korea) is a rapid chromatographic immunoassay for the detection of SARS-CoV-2 antigen in respiratory samples. Control (C) and test (T) lines (Pre coated) are seen in the result window of the test device. A colored test line would be visible if SARS-CoV-2 antigens are present in the specimen. The intensity of color depends upon mainly the amount of SARS-CoV-2 antigen present in the specimen. No color appears in the test line, if SARS CoV-2 antigens are not present in the specimen. Without the presence of colored control line the test will be declared as invalid, and should be repeated. Specimens were mixed by vortex mixer and 350 μL of swab

specimen were added to the extraction buffer provided in the kit. The filter nozzle cap was pressed tightly onto the extraction tube. Three drops of the extracted sample were put on a test device and after 15–30 min results were taken.

Positive and Negative Cases Detection: The negative results were declared when Ct-value is higher than 40 for both target genes (RdRp, N). The results of rapid test were interpreted as positive when both control (C) and test (T) lines appeared within 30 min.

Ethical approval: The study was conducted maintaining all the ethical issues. Informed written consent was obtained from each participant and all data were anonymized. This study was approved by the Institutional Review Board at the NILMRC.

Statistical Analysis: Data were analyzed by SPSS version 20, USA. Sensitivity & specificity was calculated using an online statistical tool.[11] The degree of agreement between two tests was determined by Cohens kappa coefficient (κ) values with 95% confidence intervals and expressed as k-value. Kappa values express the agreement between two tests i.e. rt-PCR and Rapid Antigen test result. k value is interpreted as follows:<0.20= poor, 0.21-0.40= fair, 0.41-.60= moderate, 0.61-0.80=good and 0.81-1.00= indicates a very good agreement.[12]

Results

Of the samples tested for COVID-19 (n=300) by real-time RT-PCR assay, 22.66% (n=68) were positive, while 75.33% (n=226) were negative (Figure 1). The median age of participants was 35.2 years (range 25–70). A total of 63% of the confirmed cases (n=43) were male.

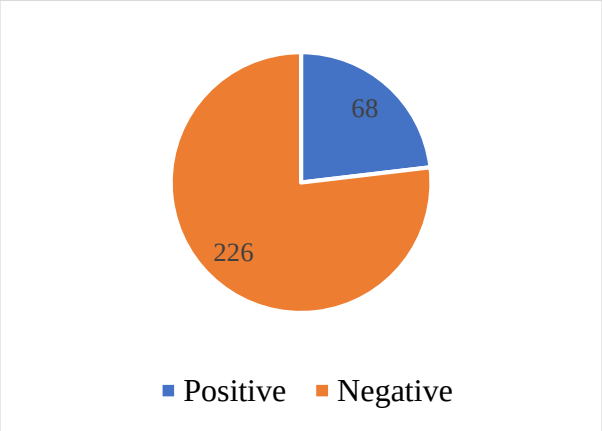

Figure 1: Positive and Negative status of Total Study Population

The average cycle threshold (Ct) values in COVID-19 positive cases were 25.73±6.43 (min 14.41, max 37.20) for RdRp gene and 24.71±6.69 (min: 12.49, max: 35.02) for N gene. Comparing SARS CoV-2 antigen detection with RT-PCR assay, the sensitivity and specificity of rapid SARS-CoV-2 antigen detection to identify COVID-19 were 94% and 99% respectively (Table 1).

Table 1: Sensitivity and specificity of Standard Q Covid-19 Antigen test

SARS-CoV-2 antigen	SARS-CoV-2 RNA		Total
	Positive	Negative	
Positive	68	2	70
Negative	4	226	230
Total	72	228	300

Sensitivity 94.44 (95% CI, 86.38% to 98.47%); Specificity 99.12% (95% CI, 96.87% to 99.89%)

Of six samples discordant with RT-PCR results, four were false negative, and two were false positive. The false negative sample's Ct-values were >34 for RdRp, and >35 for N genes. The high viral load (Ct < 25) specimens showed 100% sensitivity when tested with rapid antigen kits. The specimens of medium viral load that is (Ct 25-<30) was 94% sensitive. But low viral load that is (Ct 30-<35) and very low (Ct >35) was calculated to 40 %, and 18 % respectively. Comparing with RT PCR, a good agreement was observed between these two tests (k value= 0.98, 98%; p value <0.001).

Discussion

In acute respiratory infections resembling COVID-19, RT-PCR is routinely performed to detect SARS-CoV-2 from respiratory secretions in COVID-19 diagnostic laboratories. At present, there are 128 clinical laboratories in Bangladesh conducting RT - PCR testing on suspected COVID-19 patients' samples. Rapid antigen test, a simple and quick alternative for nucleic acid amplification assays will accelerate disease screening, and as of today, 295 centers around the country are using rapid devices in Bangladesh.[1]

Sensitivity and specificity of the SARS-CoV-2 Rapid Antigen Test were published in the manufacturer's recommendations to be 96.5 % and 99.7 %. Our results showed almost similar sensitivity and specificity and reconfirm the claims made by the manufacturer.

Two samples were tested negative in RT PCR but positive in Antigen test. Although it is unknown

what caused the mismatched result, maybe it was tested very early. These 2 samples were given at day 1 of fever. This was further observed in other studies where samples obtained on the day of symptom initiation had lower detection sensitivity and specificity.[13]And 4 samples were positive in RT -PCR but came back negative in antigen test. Possible Reasons may be high Ct value of those samples (>35). This was further validated in other studies where higher Ct value samples were often undetected using rapid antigen kits.[14]

A study in China with participation of the manufacturer found an overall sensitivity of 68% in 208 RT -PCR-positive nasopharyngeal swabs. However, when analyzing the subgroup of samples with Ct values less than 30, the sensitivity of the assay increased to 98%. In the present study, a reduction of the sensitivity to 94% was also observed in samples with higher Ct values. Accordingly, antigen tests would be more sensitive in the early stages of symptomatic infection.[15]

Conclusion

In conclusion, the antigen tests might be useful in disease detection and also can be used as a screening test for COVID-19 as its procedure is simple and turnaround time is quick. RT -PCR for SARS-CoV-2 is still more sensitive and specific and is still a standard test for COVID-19 diagnosis. Despite of all the limitations, the rapid SARS-CoV-2 antigen test can help, especially in resource limited areas where RT -PCR laboratory is not available.

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