



Research article

Clinical evaluation of SARS-CoV-2 antigen-based rapid diagnostic test kit for detection of COVID-19 cases in Bangladesh



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ABSTRACT

The rapid and early detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections is key to control the current Coronavirus disease 2019 (COVID-19) pandemic. The present study was conducted to clinically evaluate a rapid diagnostic test (RDT) kit, Standard Q COVID-19 Ag Test (SD Biosensor®, Republic of Korea), with reference to the standard real-time RT-PCR for detection of COVID-19 cases in Bangladesh. Nasopharyngeal swabs were taken from 900 COVID-19 suspected patients. Among them, 34.11% (n = 307) were diagnosed as COVID-19 cases by RT-PCR assay, of which 85% (n = 261) were also detectable using the RDT. The overall sensitivity and specificity of the RDT compared to RT-PCR were 85.02% and 100%, respectively, regardless of age, sex, and type of SARS-CoV-2 variants. Most of the RT-PCR positive cases (94%) were found within the first five days of disease onset, and the sensitivity of RDT was 85.91% for the same samples. The positive predictive value (PPV) of the RDT was 100%, and the negative predictive value (NPV) was 92.8%. The Cohen's kappa value of 0.882 indicated excellent agreement between the RDT and RT-PCR assays. The findings of this study showed the potential use of SARS-CoV-2 antigen-based RDT to expedite the diagnostic process and onward COVID-19 management in Bangladesh.

1. Introduction

On 8th March 2020, the Institute of Epidemiology, Disease Control, and Research (IEDCR) in Bangladesh reported the first case of COVID-19 [1]. Bangladesh is facing multiple challenges for adequate COVID-19 testing facilities, timely transmission containment, and emergency healthcare services to combat the COVID-19 pandemic [1]. Only a fraction of the large population is under the COVID-19 surveillance; many cases go unreported in Bangladesh. Therefore, an alarming strategic gap exists between the testing coverage, resource availability, and the probable number of COVID-19 cases in the country. Moreover, the emergence of novel variants of SARS-CoV-2 will also impact transmission rates, clinical manifestation, and vaccine effectiveness, complicating the situation further [2].

Real-time reverse transcriptase-polymerase chain reaction (RT-PCR) is considered the gold standard for the detection of SARS-CoV-2 [3, 4]. It is a precise and sensitive molecular technique that quantitatively detects viral RNA from clinical specimens [4]. In Bangladesh, all the laboratories providing emergency testing support for COVID-19 rely on real-time RT-PCR. RT-PCR is a time-consuming, expensive process that requires specialized laboratory staff, highly sophisticated equipment, and a dedicated laboratory environment [5, 6, 7]. Still, a long way to go through with this COVID-19 pandemic. There is a pressing need to introduce less expensive, point-of-care (POC), rapid diagnostic tests (RDTs) for the early diagnosis, and isolation of infected individuals to control the transmission of SARS-CoV-2 infections. In May 2020, SARS-CoV-2 RDTs were approved by the US Food and Drug Administration (FDA) to improve containment strategies worldwide [8]. After that, various COVID-19 antigen-based RDTs have been introduced in the

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testing platforms [9, 10]. The RDTs have the advantage of generating results within 15–30 min that can be interpreted without any specialized instrument. Therefore, it has the potential to relieve the workload in diagnostic hospitals or laboratories and improve the overall turn-around time as well as patient management [11]. Evaluation of RDT performance at different settings is necessary for its validation and widespread use [12].

This study evaluated the performance and effectiveness of an antigen-based RDT, Standard Q COVID-19 Ag (SD Biosensor®, Republic of Korea), for the detection of SARS-CoV-2 in comparison with RT-PCR as the reference method. The RDT is a nucleocapsid protein (N) based rapid chromatographic immunoassay that qualitatively detects SARS-CoV-2 antigen in human nasopharyngeal specimens. As the “N” antigen is independent of spike (S) protein mutations, the RDT should detect different SARS-CoV-2 variants [8]. However, RDT-performance may vary geographically depending on the disease prevalence of the target population, and the kit was not evaluated for the Bangladeshi population [11, 12, 13, 14]. We conducted this study to clinically evaluate the RDT for on-site and standard POC detection of COVID-19 cases confirmed by RT-PCR in Bangladesh.

2. Materials and methods

2.1. Study design and participants

From December 2020 to April 2021, two nasopharyngeal swab specimens were collected per individual from 900 COVID-19 suspected cases, one in 2 mL viral transport media (VTM) and another one in 200 μ L RDT buffer solution supplied with the STANDARD Q COVID-19 Ag test kit at the same time point from the Dhaka Hospital of the International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), Institute of Epidemiology, Disease Control and Research (IEDCR) and Institute for Developing Science and Health Initiatives (ideSHi). COVID-19 suspected individual was defined, as per the National Guidelines on Clinical Management of COVID-19, as a person with acute onset of fever and cough or any three or more of the following symptoms: general weakness/fatigue, headache, myalgia, sore throat, coryza, dyspnea, anorexia/nausea/vomiting, diarrhoea, and altered mental status [15]. This clinical information was recorded for each individual. RDT was performed on-site upon sample collection. Samples collected in VTM were transported to the lab maintaining temperature control (4.0 ± 2.0 °C) in a cool box and were tested within six hours by RT-PCR. Individuals were informed about the study, and consent was received before specimen and data collection. The study was approved by the Research Review Committee (RRC) and Ethical Review Committee (ERC) of icddr,b (Protocol no: PR-20102).

2.2. SARS-CoV-2 antigen detection assay

The RDT uses a lateral flow test format and is available as a cassette with a nitrocellulose strip. As per the manufacturer's instructions, 3 drops of the extracted specimen (collected in supplied buffer) were poured into the well of the test device, and the result was observed within 15–30 min. For positive SARS-CoV-2 antigen results, control (C) and test (T) lines appeared as colored in the result window. In the absence of SARS-CoV-2 antigen, the test line was color-free; however, a line appeared in the control line.

2.3. SARS-CoV-2 RNA extraction and detection via RT-PCR

Total RNA was extracted from 200 μ L of nasopharyngeal swab samples, collected in VTM, using the Chemagic viral NA/gDNA kit (PerkinElmer, MA, USA), as per the manufacturer's instructions. The presence of SARS-CoV-2 RNA was detected by RT-PCR targeting the RdRp and N-genes as per protocol described by the Chinese Center for Disease Control and Prevention (China CDC) and recommended by the World Health Organization (WHO) [16, 17]. RT-PCR reaction mixtures were prepared

with iTaq™ Universal Probes and One-Step Reaction Mix (Bio-Rad Laboratories, Inc., Hercules, CA, USA) and CFX96 Touch™ Real-time PCR Detection System (Bio-Rad Laboratories, Inc., Hercules, CA, USA) was used. Sample with a cycle threshold (Ct) value of <37 for any of the targets (RdRp and N) was considered as a positive case [18]. Based on Ct values, specimens were categorized as strong (Ct < 25) moderate (25 < Ct < 30) and weak (Ct > 30) positives.

2.4. SARS-CoV-2 variants of concern (VOC) detection

To validate the RDT for detecting different SARS-CoV-2 VOCs, RDT positive samples having real-time RT-PCR Ct values < 25 (n = 205) were selected for Sanger sequencing. Sanger sequencing was done in an automated ABI 3500 XL genetic analyzer (Applied Biosystems, Foster City, USA) [2]. The ARTIC amplicon primer sets were used to amplify the S gene of SARS-CoV-2 [19].

2.5. Data analysis

The statistical tests were done using SPSS software, version 20.0 (SPSS Inc., Chicago, IL, USA) and Microsoft Excel 2019. To assess the diagnostic accuracy of the RDT and the degree of its comparability with RT-PCR results, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and negative likelihood ratio were determined using web based diagnostic test evaluation tool [20]. The PPV and NPV showed the probability of positive and negative results among true cases and healthy individuals, respectively [21]. Positive percent agreement, negative percent agreement, and Cohen's kappa statistic were calculated to measure the level of agreement beyond chance between the two diagnostic tests. A value of $k \leq 0.40$ shows poor agreement, $0.40 < k < 0.75$ indicates fair or good agreement, and $k \geq 0.75$ denotes excellent agreement [22, 23]. The continuous variables were used to draw scatter plots and compared by using an unpaired T-test with $p < 0.05$ considered as statistically significant. CT values were shown as mean, median, and interquartile range (IQR) values.

3. Results

A total of 900 COVID-19 suspected individuals were enrolled in this study; 307 (34.11%) tested real-time RT-PCR-positive, and 593 (65.89%) were negative. Among 307 RT-PCR positive cases, 261 (85.02%) were RDT-positive. For the 593 RT-PCR negative cases, all of them were RDT-negative. The detection of the SARS-CoV-2 Alpha (B.1.1.7) and Beta (B.1.351) variants among RDT positive COVID-19 cases confirmed the variant-independent testing potential of the RDT. Out of the 205 subjected to sequencing, only 179 sequences were retrievable of which 12 (6.7%) were Alpha (B.1.1.7), 109 (60.89%) were Beta (B.1.351) and 58 (32.40%) were Wuhan-Hu-1 like.

Table 1. Overall performance of the STANDARD Q COVID-19 Ag kit.

STANDARD Q COVID-19 Ag Test Result	
Characteristics	Value (95% CI)
Overall Sensitivity	85.02% (80.52%–88.82%)
Overall Specificity	100% (99.38%–100.00%)
Negative Likelihood Ratio	0.15 (0.11–0.20)
Disease prevalence	34.11% (31.01%–37.31%)
Positive Predictive Value (PPV)	100.00%
Negative Predictive Value (NPV)	92.8% (90.81%–94.39%)
Accuracy	94.89% (93.24%–96.23%)
Positive percent agreement	85.02% (80.52%–88.82%)
Negative percent agreement	100.00% (99.38%–100.00%)
Overall percent agreement	94.89% (93.24%–96.23%)
Cohen's kappa (K)	0.882 (0.85–0.91)

The overall sensitivity of the RDT was 85.02% (95% CI, 80.52%–88.82%) with a specificity of 100% (95% CI, 99.38%–100.00%) (Table 1). The sensitivity of the RDT was 100% for the strong positives ($n = 205$ and $n = 213$ for RdRp and N genes targets, respectively). However, it decreased with the increase of Ct values as per RT-PCR assays. The sensitivity was more than 80% and 17% for the moderate and weak positives, respectively (Table 2). The diagnostic accuracy of the RDT was 94.89% (95% CI, 93.24%–96.23%) (Table 1). The Cohen's kappa value was 0.882 (95% CI, 0.85–0.91, $p < 0.001$), showing excellent agreement between the two assays. The PPV and NPV of the RDT were 100% and 92.8% (95% CI, 90.81%–94.39%), respectively. COVID-19 daily disease prevalence among the study population, calculated from the current study between December 2020 and April 2021, was 34.11% (95% CI, 31.01%–37.31%). The highest disease prevalence data were obtained from April (57.65% (95% CI, 46.45–68.30%)), and the lowest was in February (22.88% (95% CI, 15.65–31.52%)).

The maximum RDT-performance for diagnosis of COVID-19 was registered up to the fifth day after the onset of symptom/s (Figure 1). Most of the RT-PCR positive cases (98%) were found within the first five days of disease onset, and the RDT showed 85.91% (95% CI, 81.43%–89.65%) sensitivity for the same samples (Table 1). The mean number of days since the onset of symptoms to get an RDT-positive test result was 3.06 days (95% CI, 2.94–3.18 days) and median 3 days (standard deviation 1.82, minimum 1, maximum 30). Analysis of demographic characteristics revealed that the median age of study participants was 35 years (standard deviation 13.94, minimum 0, maximum 91), and mean age was 37.05 years (95% CI, 36.14 to 37.96). Samples from female patients showed the sensitivity of 82.58% (95% CI, 75.01%–88.62%), whereas, for male patients, it was 86.86% (95% CI, 80.93%–91.48%) (Table 1). The sensitivity of the RDT with patients aged >18 years was 85.76% (95% CI, 81.25%–89.54%). It was 66.67% (95% CI, 34.89%–90.08%) for those <18 years of age as the time course for positivity possibly varies in children (Table 1).

4. Discussion

RDT is often cost-effective, user-friendly, and can generate result within 15–30 min [24]. The STANDARD Q COVID-19 Ag Test is an RDT that detects SARS-CoV-2 antigen in nasopharyngeal specimens [11, 12, 13, 25]. We determined the performance characteristics of the RDT to

detect SARS-CoV-2 antigen in respiratory samples collected from COVID-19 suspected cases in Bangladesh. The STANDARD Q COVID-19 Ag Test showed sensitivity and specificity of 85.02% and 100%, respectively, and had an accuracy of 94.89% among the Bangladeshi population. Tests with diagnostic accuracy above 90% are known to have high diagnostic value [26]. WHO has set the limit of sensitivity $>80\%$ and specificity $>97\%$ for SARS-CoV-2 antigen-based RDTs compared to RT-PCR assay [24]. The 100% PPV indicated that all individuals with a positive RDT result had the disease. PPV was expected to be high in the study population as COVID-19 daily disease prevalence was 34.11% during the study period. RDT clinical performance depends on disease prevalence of the targeted populations at a given time, for example, studies from Pakistan and Serbia that evaluated the same RDT reported 52% and 58.1% sensitivity respectively during low prevalence ($\leq 5\%$) of SARS-CoV-2 [7, 26]. The range of Ct values in relation to the days of disease onset suggested that a higher viral load of SARS-CoV-2 was present during the first few days of infections and was detected mostly using the RDT. The findings of this study were in line with the previously reported studies of SARS-CoV-2 antigen-based RDTs [6, 24, 27].

The sensitivity of the RDT kit was $\sim 10\%$ lower than the declared value of 96.52% sensitivity and 99.68% specificity [28]. A sample containing a viral load below the limit of detection for the RDT can generate a false-negative result. Therefore, an additional RT-PCR assay for the patients, if tested antigen-negative, should be considered confirmatory diagnosis [15, 28, 29, 30]. However, chances for false-negative RT-PCR should also be taken under consideration. By targeting two SARS-CoV-2 genes RdRp and N gene, as conducted in this study (WHO recommended protocol), chances for false-negative RT-PCR results can be minimized. The RDT used in this study demonstrated higher sensitivity within 5 days of onset of symptom/s. Hence, it is better not to rely on RDT results after 5 days of illness [24]. These findings are synonymous with the Government of Bangladesh's guidelines [27]. The performance of the RDT kit might be over-estimated as the present study was limited to symptomatic cases with ≤ 5 days of illness (97.1%, 298/307). One of the limitations of the study was the exclusivity of asymptomatic cases. Unidentified asymptomatic cases may also accelerate the spread of SARS-CoV-2 in the community as they shed the same viral load as symptomatic individuals [31, 32]. In the future, we can aim to evaluate the performance of RDTs for POC diagnosis of SARS-CoV-2 in asymptomatic cases. Besides, we could not recover the virus or its genetic information from all the

Table 2. RDT performance with demographic, clinical, and laboratory characteristics of study participants.

Demographic Characteristics		STANDARD Q COVID-19 Ag Test Result		RT-PCR Test Result		Sensitivity (%)
		Positive	Negative	Positive	Negative	
Age	Adults (>18)	253	608	295	566	85.76% (95% CI, 81.25%–89.54%)
	Children (<18)	8	31	12	27	66.67% (95% CI, 34.89%–90.08%)
Sex	Male	152	353	175	330	86.86% (95% CI, 80.93%–91.48%)
	Female	109	286	132	263	82.58% (95% CI, 75.01%–88.62%)
Onset of symptom/s	1 to 5	256	594	298	552	85.91% (95% CI, 81.43%–89.65%)
	6 to 10	5	42	8	39	62.50% (95% CI, 24.49%–91.48%)
	Day >10	0	3	1	2	0.00% (95% CI, 0.00%–97.50%)
RdRp Ct values	Ct < 25	205	0	205	0	100% (95% CI, 98.22%–100.00%)
	25 $<$ Ct < 30	49	12	61	0	80.33% (95% CI, 68.16%–89.40%)
	Ct > 30	7	34	41	0	17.07% (95% CI, 7.15%–32.06%)
N gene Ct values	Ct < 25	213	0	213	0	100% (95% CI, 98.28%–100.00%)
	25 $<$ Ct < 30	37	5	42	0	88.10% (95% CI, 74.37%–96.02%)
	Ct > 30	11	41	52	0	21.15% (95% CI, 11.06%–34.70%)
All Ct Values (RdRp)	Mean	-	-	22.54	-	-
	Median	-	-	21.51	-	-
	IQR	-	-	17.81 to 26.65	-	-
All Ct Values (N gene)	Mean	-	-	22.27	-	-
	Median	-	-	21.04	-	-
	IQR	-	-	17.27 to 26.22	-	-

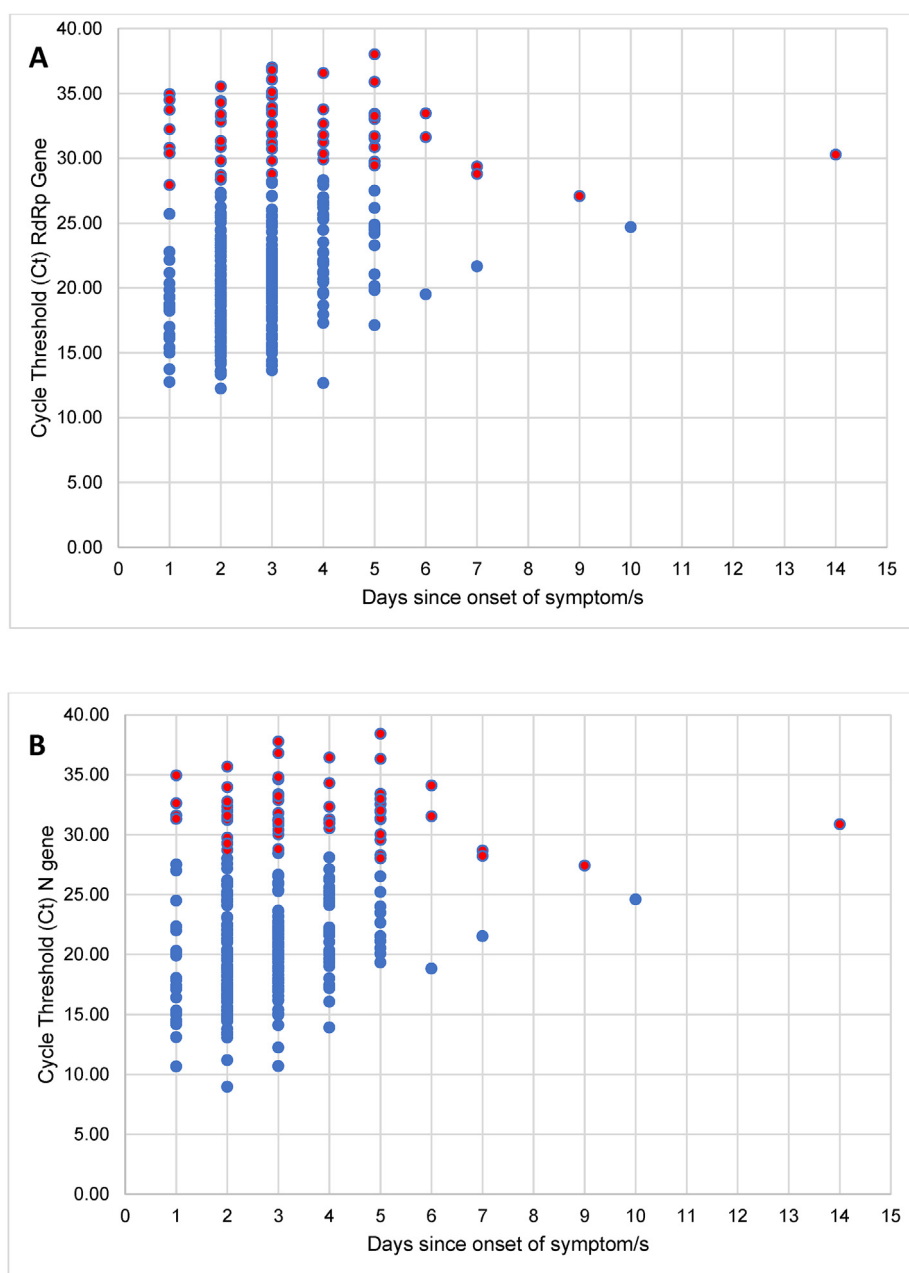


Figure 1. Cycle threshold (Ct) values of RT-PCR-positives (n = 307) targeting A) RdRp and B) N gene on different days since the onset of symptom/s. Dot colors represent false-negative (red, n = 46) and true-positive (blue, n = 261) results by the STANDARD Q COVID-19 Ag Test.

PCR-positive samples, due to the unavailability of virus culture facilities and the low nucleic acid content of the sample.

The emergence of SARS-CoV-2 variants raises concerns about the effectiveness of current diagnostic assays to identify COVID-19 cases [33, 34]. We investigated the potential use of RDT to detect the VOCs, and sequenced positive samples (Ct < 25) to validate the RDT against circulating SARS-CoV-2 variants. The VOCs B.1.1.7 (Alpha-variant) and B.1.351 (Beta-variant) were known to be circulating in Bangladesh during the study period [2]. The RDT used in this study also detected the B.1.1.7 (n = 12) and B.1.351 (n = 109) SARS-CoV-2 VOCs along with 58 Wuhan-Hu-1 like sequences in respiratory samples. However, this study did not assess the VOC's effects for low viral load samples as Ct values ≥ 25 were not sequenced.

The RDT showed strong clinical performance in detecting SARS-CoV-2 infections among the COVID-19 suspected cases in this study. In areas with high transmission rates, RDTs can reduce the RT-PCR burden of

laboratories while improving the accuracy and biosafety of the remaining laboratory work. Schools, offices, and industries can deploy RDTs after reopening to identify symptomatic and asymptomatic cases and isolation of the infected individuals. The RDT showed strong clinical performance in detecting SARS-CoV-2 infections among the COVID-19 suspected cases in this study. The findings of this study provide an insight into the usefulness of RDTs in the testing algorithm of COVID-19 diagnosis along with RT-PCR assay. We propose incorporating RDTs would enhance nationwide laboratory and POC diagnosis in Bangladesh to allow fast detection and isolation of COVID-19 cases.

Institutional review board statement

The study was approved by the Research Review Committee (RRC) and Ethical Review Committee (ERC) of icddr,b (Protocol no: PR-20102).

Informed consent statement

All the study participants gave written informed consent before specimen and data collection.

Declarations

Author contribution statement

Md. Mahfuzur Rahman: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Ananya Ferdous Hoque: Performed the experiments; Wrote the paper.

Yeasir Karim, Zannat Kawser, Abu Bakar Siddik, Mariya Kibtiya Sumiya, Ayesha Siddika, Md. Shaheen Alam: Performed the experiments.

Ahmed Nawsher Alam, Muntasir Alam, Mohammad Enayet Hossain: Analyzed and interpreted the data.

Sayera Banu, Firdausi Qadri, Tahmina Shirin, Mustafizur Rahman: Conceived and designed the experiments.

Mohammed Ziaur Rahman: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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