Performance Characteristics and Utility of the Standard Q COVID-19 Antigen Test for Emergency Admissions to Healthcare Facilities

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ABSTRACT

This study evaluated the performance of the COVID-19 Ag-RDT compared to the real-time reverse transcription-polymerase chain reaction (rtRT-PCR) for SARS-CoV-2 detection and its use among patients referred for emergency admission.

A total of 120 nasopharyngeal swabs were collected from patients referred for emergency admission and immediately preceded for testing to the Unit of Clinical Microbiology. Out of 60 Ag positive tests, 53 (88.3%) were confirmed by rtRT-PCR, while 7 (11.7%) tested negative (false positives). Out of 60 Ag negative tests, 56 (93.3%) were confirmed negative by rtRT-PCR, and 4 (6.7%) were positive (false negatives). Ct value comparison was performed for 53 samples that were positive by both methods: 8 (15.1%) isolates had Ct value up to 20; 37 (69.8%) 21 to 30 and 8 (15.1%) 31 to 40, respectively. The sensitivity of the analyzed rapid Ag test was 92.9%, and specificity 88.9%. The accuracy of the Ag test was 90.8%.

This study has shown that rapid Ag tests can be used in emergency admissions to healthcare facilities. However, rtRT-PCR should be considered after negative antigen test results in symptomatic patients, and after positive antigen test results in asymptomatic persons.

KEYWORDS

SARS-CoV-2; rapid antigen test; RT-PCR; emergency admission

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INTRODUCTION

Since its first notification, SARS-CoV-2 has spread around the world in a very short time, besides many attempts to control the disease. Laboratories have been constantly increasing the number of tests performed, and there was a need to shorten the time to obtain results. This is especially related to patients who require hospital admission, since early detection of positive patients is of particular importance for preliminary rapid triaging, timely isolation, and limiting the spread of the virus in hospital settings (1).

Therefore, the control strategy in hospitals is based on the availability of fast and reliable diagnostic tests that aim at early detection of virus in respiratory materials (2).

Until today, the reverse transcription-polymerase chain reaction (RT-PCR) assay was the gold standard in the diagnosis of SARS-CoV-2 infection since such an assay has excellent sensitivity and specificity. However, these assays are often too slow to inform patient placement in emergency departments (EDs) and require specialized instruments and educated and trained personnel (3). Isolation rooms are often limited in capacity, requiring the cohorting of COVID-19-positive patients. Due to all the above, options for additional non-PCR-based testing such as rapid antigen-based diagnostic tests (Ag-RDT) are receiving increasing attention and are being widely implemented in national test strategies. In principle, such assays are supposed to provide rapid and reliable information on the SARS-CoV-2 infection status, e.g. in emergency departments (ED) or other health care facility settings. They could help to improve the flow of patients through the ED into "COVID-19-positive" cohorts and reduce pressure on limited hospital isolation rooms (4, 5).

In the interim guidance of September 11, 2020, WHO has presented this option as a new technology for COVID-19 detection that is simpler and faster to perform than the currently recommended nucleic acid amplification tests (6).

This document has been updated to incorporate new findings concerning test performance across Ag-RDT brands and sample types. These tests have become a useful tool since they provide faster results in situations when PCR capacity is limited. Upper respiratory specimens or saliva are used for testing to detect SARS-CoV-2 proteins (e.g., nucleoproteins) and results are obtained within 30 minutes (7).

Although these tests could be used in diagnostic algorithms for emergency admissions to healthcare facilities, their performance data, especially from asymptomatic persons, is still limited.

This study evaluated the performance characterization of the SARS-CoV-2 Ag-RDT compared to the real-time reverse transcription-polymerase chain reaction (rtRT-PCR) for SARS-CoV-2 detection and its utilization among patients referred for emergency admission to the Clinical Center of the University of Sarajevo (CCUS), Bosnia and Herzegovina.

MATERIAL AND METHODS

The study was conducted from November 2020 to February 2021. The nasopharyngeal swab samples were collected in a Citoswab Collection and Transport Kit (nal von Minden, GmbH, Moers, Germany) containing 3 ml of the virus transport medium (VTM) from patients referred for emergency admission and immediately proceeded for testing to the Unit of Clinical Microbiology situated at the Clinical Center of the University of Sarajevo, Bosnia and Herzegovina.

All patients were screened by using the Ag-RDT SARS-CoV-2 test (Standard Q COVID-19 Ag test; SD Biosensor, Inc. Gyeonggi-do, Republic of Korea) for the early detection of potential infection. The 350 µl of the specimen from VTM was tested by Ag-RDT in a Class II Microbiology Safety Cabinet and according to the manufacturer's instructions. In principle, mouse monoclonal anti-SARS-CoV-2 antibody is coated on the test line region, and mouse monoclonal anti-Chicken IgG antibody is coated on the control line region. Mouse monoclonal anti-SARS-CoV-2 antibodies conjugated with color particles are used as detectors for the SARS-CoV-2 antigen device. During the test, SARS-CoV-2 antigen in the specimen interacts with monoclonal anti-SARS-CoV-2 antibody conjugated with color particles, making an antigen-antibody color particle complex. This complex migrates on the membrane via capillary action until the test line, where it will be captured by the mouse monoclonal anti-SARS-CoV-2 antibody. A colored test line would be visible in the result window if SARS-CoV-2 antigens are present in the specimen. If SARS-CoV-2 antigens are not present in the specimen, then no color appears in the test line. The control line should always appear if the test procedure is performed properly and the test reagents of the control line are working.

Ag-RDT test was compared with PhoenixDX SARSCoV-2 Multiplex rtRT-PCR test (Procomcure Biotech; Thalgau, Austria) performed simultaneously (within two hours after the sample was received in the laboratory), using Applied Biosystems 7500 Realtime PCR System (Thermo Fisher Scientific, Waltham, MA, USA), to assess its performance characteristics.

The PhoenixDx SARS-CoV-2 Multiplex test is based on rtRT-PCR technology for the qualitative detection of the RNA genome of SARS-CoV-2 from the patient sample. Nucleic acids were extracted from 200 µL of VTM using a fully automatic system, Nextractor NX-48, utilizing the NX-48 Viral NA Kit (Genolution, Seoul, Republic of Korea) according to the manufacturer's instructions. Samples were eluted in 50 µL of elution buffer. The isolated nucleic acids (RNA) were immediately used for rtRT-PCR.

External controls (positive and negative) were included in the kit and processed in the same way with each run. The PhoenixDx SARS-CoV-2 Multiplex master mix contains detection probes for the two SARS- CoV-2 ORF1ab and N genes (FAM-labeled) and one for the internal RNaseP (HEX/VIC labeled). Each reporter dye is measured at defined wavelengths, which enables simultaneous detection and discrimination of the amplified coronavirus targets. The total of 20 ml of RT-PCR reaction mix contained the kit-specific RT Enzyme mix (1 ml), the SARS-CoV-2 Multiplex mix (15 ml), and 4 ml of nucleic acids (RNA) of the sample, positive or negative control, respectively. The rtRT-PCR program was set up as follows: reverse transcription (50 °C, 5 minutes), initial denaturation (95 °C,

5 minutes), and amplification (40 cycles of the steps: 95 °C, 5 seconds, and 60 °C, 30 seconds—data collection step). Positive Ct value for virus-specific targets considered to represent the positive SARS-CoV-2 result with or without the presence of internal RNase P signal.

Descriptive statistics used for data analysis included mean, median, mode, standard deviation, minimum, maximum, count, and confidence level. Performance of the Ag-RDT test compared to rtRT-PCR was evaluated by sensitivity, specificity, accuracy, positive and negative likelihood ratio, prevalence, and positive and negative predictive values (PPV, NPV).

RESULTS

The SARS-CoV-2 Ag-RDT test (Standard Q COVID-19 Ag test; SD Biosensor, Gyeonggi-do, Republic of Korea) was compared with the PhoenixDX SARS-CoV-2 Multiplex rtRT-PCR test (Procomcure Biotech; Thalgau, Austria) using the ABI 7500 Realtime PCR System.

A total of 120 nasopharyngeal swabs from patients for emergency admission were analyzed (Figure 1).

Comparison of Ag-RDT and rtRT-PCR results showed the difference in rtRT-PCR Ct values (Table 1). Actually, the mean Ct value for Ag-RDT positive results was 24.85 ± 4.24 (STDEV), while the higher Ct values were observed for Ag-RDT negative results (mean 31.25 ± 2.22 STDEV). Ag-RDT positivity was more prevalent in persons at the mean age of 58 years. Persons with negative Ag-RTD tests showed a slightly lower mean age (52 years). Although the days of onset of symptoms were not available for all tested persons, the mean of 4 days of Ag-RDT testing was recorded regardless of Ag-RDT result.

According to the presence of COVID-19 symptoms among patients admitted to the emergency hospital department, 26/60 (43.33%) of Ag-RDT positive persons were symptomatic and 34/60 (56.67%) of them were asymptomatic (Table 2). The Ag-RDT positivity of 25/26 (96.15%) symptomatic persons was confirmed by rtRT-PCR. Among COVID-19 asymptomatic persons, 28/34 (82.35%) of Ag-RDT positive results were confirmed by rtRT-PCR. The 56/57 (98.25%) of Ag-RDT and rtRT-PCR negative patients, had no COVID-19 signs. These patients were tested as a preventive measure upon entry to the Clinical center. All 3/3 (100.00%) symptomatic patients with Ag-RDT negative test were positive by rtRT-PCR assay.

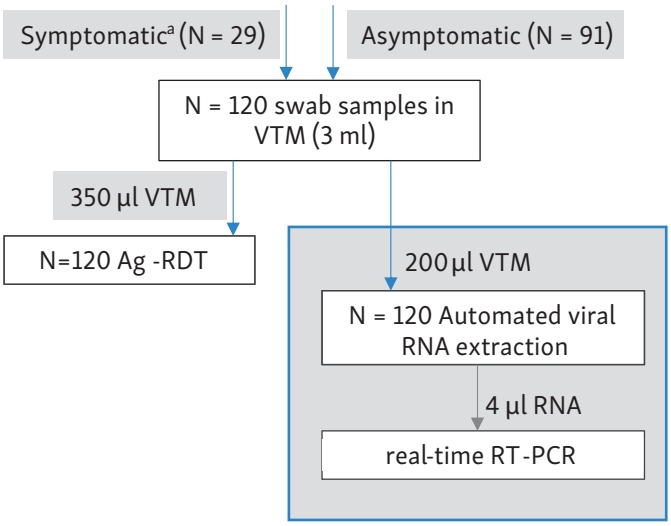

Fig. 1 Flowchart of the study.
[a] Symptoms were linked to the COVID-19.

Performance characteristics of the Ag-RDT assay are summarized in Table 3. Namely, Ag-RDT positive tests were confirmed by rtRT-PCR in 53/60 (88.33%) samples, while 7/60 (11,67%) tested rtRT-PCR negative (false positives). Ag-RDT negative results matched rtRT-PCR in 56/60 (93.33%) cases, except for 4/60 (6.67%) samples that tested Ag-RDT negative and rtRT-PCR positive (false negatives). The sensitivity of Ag-RDT was 92.98% and the specificity was 88.95%.

Tab. 1 Comparison of Ag-RDT and commercial rtRT-PCR assay.

	Age (years)[a]		**Real-time RT-PCR (Ct value)**		**Testing after onset of symptoms (days)**[b]	
*Ag-RDT test result**	*Ag-RDT (+)*	*Ag-RDT (-)*	*Ag-RDT (+)*	*Ag-RDT (-)*	*Ag-RDT (+)*	*Ag-RDT (-)*
Mean	58.83	52.02	24.85	31.25	4.19	4
Median	63	57	24	32	3	4
Mode	57	55	22	32	1	N/A
Standard Deviation	20.60	21.87	4.24	2.22	5.19	N/A
Minimum	1	7	15	28	0	4
Maximum	89	91	34	33	20	4
Confidence Level (95.0%)	5.32	5.65	1.17	3.53	2.36	N/A
Total number of respondents	*60*	*60*	*53*	*4*	*21*	*1*

Legend: * Data were divided based on Ag-RDT test results (positive +; negative –); [a] Age statistics of respondents participated in the study, provided in years. The columns give the brief insight into the age structure of the study population along with testing results and days after onset of symptoms (where available); [b] Number of days after onset of symptoms when Ag-RDT testing was performed. Results are shown for respondents with available data; N/A: Data not available. Statistics for the given parameters could not be calculated on the basis of one symptomatic person in whom the Ag-RDT test was negative upon admission, on the fourth day after the onset of symptoms; Ag-RDT: Standard Q COVID-19 Ag test (SD Biosensor, Gyeonggi-do, Republic of Korea); real-time RT-PCR: PhoenixDX SARS-CoV-2 Multiplex rtRT-PCR test (Procomcure Biotech; Thalgau, Austria).

Tab. 2 Concordance of Ag-RDT and commercial Real-time RT-PCR test with the presence of COVID-19 symptoms of patients in emergency hospital admission.

		COVID-19 Symptoms		
		Yes	*No*	*Total*
Ag-RDT (+)		26 (43.33%)	34 (56.67%)	60 (100.00%)
	w/ Real-time RT-PCR (+)	25 (96.15%)	28 (82.35%)	53 (88.33%)
	w/ Real-time RT-PCR (–)	1 (3.85%)	6 (17.65%)	7 (11.67%)
Ag-RDT (–)		3 (5.00%)	57 (95.0%)	60 (100.00%)
	w/ Real-time RT-PCR (–)	0 (0.00%)	56 (98.25%)	56 (93.33%)
	w/ Real-time RT-PCR (+)	3 (100.00%)	1 (1.75%)	4 (6.67%)

Legend: Ag-RDT test results (positive +; negative –); Real-time RT-PCR test results (positive +; negative –); Ag-RDT: Standard Q COVID-19 Ag test (SD Biosensor, Gyeonggi-do, Republic of Korea); real-time RT-PCR: PhoenixDX SARSCoV-2 Multiplex rtRT-PCR test (Procomcure Biotech; Thalgau, Austria).

Tab. 3 Performance characteristics of Ag-RDT evaluated by commercial real-time RT-PCR.

Real-time RT-PCR	**Ag-RDT**				**Performance characteristics of Ag-RTD**	
		Positive	Negative	*Total*	Sensitivity:	92.98%
	Positive	53	4	57	Specificity:	88.89%
	Negative	7	56	63	Accuracy:	90.83%
	Total	60	60	120	Positive likelihood ratio:	8.368
					Negative likelihood ratio:	0.07895
					Prevalence:	47.5%
					PPV:	88.33%
					NPV:	93.33%

Legend: PPV – positive predictive value; NPV – negative predictive value; Ag-RDT: Standard Q COVID-19 Ag test (SD Biosensor, Gyeonggi-do, Republic of Korea); real-time RT-PCR: PhoenixDX SARSCoV-2 Multiplex rtRT-PCR test (Procomcure Biotech; Thalgau, Austria).

The probability that a person with a positive screening test (Ag-RDT) truly has the disease (PPV) was 88.33%, while the probability that someone with a negative screening test (Ag-RDT) truly doesn't have the disease (NPV) was 93.33%. The accuracy of the Ag-RDT test was determined by the ratio of correct results (rtRT-PCR positive at the same time) to all the results of the Ag-RDT. It was 90.83%. Both positive and negative likelihood ratios describe the value of a test. The possibility that the person with the disease would test positive for Ag-RDT was 8.368 (positive likelihood ratio) and that the healthy person would test negative was 0.07895 (negative likelihood ratio), respectively. The prevalence of SARS-CoV-2 infection as determined by rtRT-PCR positives (true positives, N = 57) in a total group (N = 120) was 47.5% (Table 3).

DISCUSSION

Rapid and accurate identification of SARS-CoV-2 is crucial for emergency admissions to healthcare facilities, since the patients may be asymptomatic carriers, and if not promptly identified, could spread the infection within the hospital. The RT-PCR test is the gold-standard diagnostic for SARS-CoV-2 infection, but the results are often delayed and not suitable for the emergency department (ED) timing. Due to their quick performance and the timeliness of their results the rapid antigen test (RAT) could overcome the limitations of RT-PCR testing and improve the risk management of infection and transmission in the ED (8).

To examine the performance and utility of RAT in emergency hospital admissions, a study was conducted from November 2020 to February 2021 at the Clinical Center of the University of Sarajevo, Bosnia and Herzegovina. These data provide the first quantitative analysis of the performance characteristics of a rapid antigen detection kit when applied to an emergency department in our country.

120 patients referred for emergency admission and required hospitalization was tested with both a RAT and RT-PCR. The prevalence of SARS-CoV-2 infection as determined by rtRT-PCR positives (true positives, N = 57) in a total group (N = 120) was 47.5%. In SARS-CoV-2 positive patients, the RAT was positive in 88.33% of cases (53/60), while a false-positive RAT was in 11.67% (7/60) with a negative RT-PCR. Overall, the sensitivity and specificity of Ag-RDT in our study were 92.98% and 88.95% respectively, and the accuracy was 90.83%.

The average sensitivity reported in symptomatic individuals from 37 evaluations was 72.0% (95% CI: 63.7–79.0%), while that in asymptomatic individuals from 12 evaluations was 58.1% (95% CI: 40.2–74.1%) (9). The SD Biosensor RAT (Inc., Republic of Korea) manufacturer reported a higher sensitivity (96.52%; 95% CI: 91.33–99.04%) obtained in prospective, randomized, single-blinded studies conducted in Brazil and India in symptomatic and

asymptomatic individuals (SARS-CoV-2 Rapid Antigen Test Package Insert 2020-08, V 1.0).

WHO recommends the use of Ag-RDTs that meet minimum performance requirements of ≥ 80% sensitivity and ≥97% specificity (7). According to Centers for Disease Control and Prevention (CDC, 2021) the sensitivity of 69.86% indicates that RAT should not replace real-time RT-PCR in the diagnosis and surveillance of SARS-CoV-2 infection (10).

In our study, the mean Ct value for Ag-RDT positive results was 24.85 ± 4.24 (STDEV), while the higher Ct values were observed for Ag-RDT negative results (mean 31.25 ± 2.22 STDEV). After the acute phase when the viral load decreases, the use of Ag-RDTs might lead to high rates of false negatives, suggesting that the tests should be replaced by a combination of molecular and serological tests (11).

Ag-RDT was confirmed by rtRT-PCR in 96.15% (25/26) of symptomatic, and 82.35% (28/34) of COVID-19 asymptomatic persons. In the group of symptomatic persons, the performance of the RAT seems to be high enough to propose its use as an initial screening test directly upon arrival in triage. In this group, a positive RAT may accelerate the management of the infected patient.

A high percentage of our patients (98.25%) with Ag-RDT and rtRT-PCR negative results had no COVID-19 signs. In case of a negative test, the subsequent clinical management may depend on the degree of clinical suspicion. However, a negative RAT in patients with low clinical suspicion cannot completely exclude the presence of SARS-CoV-2 infection (8, 12).

The accuracy of the Ag-RDT test largely depends on the specificity of monoclonal antibodies. However, relatively high predictive values (our study showed PPV 88.33% and NPV 93.33% respectively), ease of use, low cost, and short turnaround time (15 ~ 30 minutes) give it an advantage to be used for triage of COVID-19 patients in areas such as ED. In countries with limited resources, it could be more suitable compared to more sensitive but expensive "on demand" Real time PCR platforms.

CONCLUSIONS

This study has shown that the use of RAT to assess the risk of infection directly in triage should be considered in emergency admissions to healthcare facilities. The short time to results might have a key role in the early placement of SARS-CoV-2-positive patients into COVID-19 units, reducing the risk of cross-transmission in the emergency department. However, rtRT-PCR should be considered after negative antigen test results in symptomatic patients, and after positive antigen test results in asymptomatic persons.

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