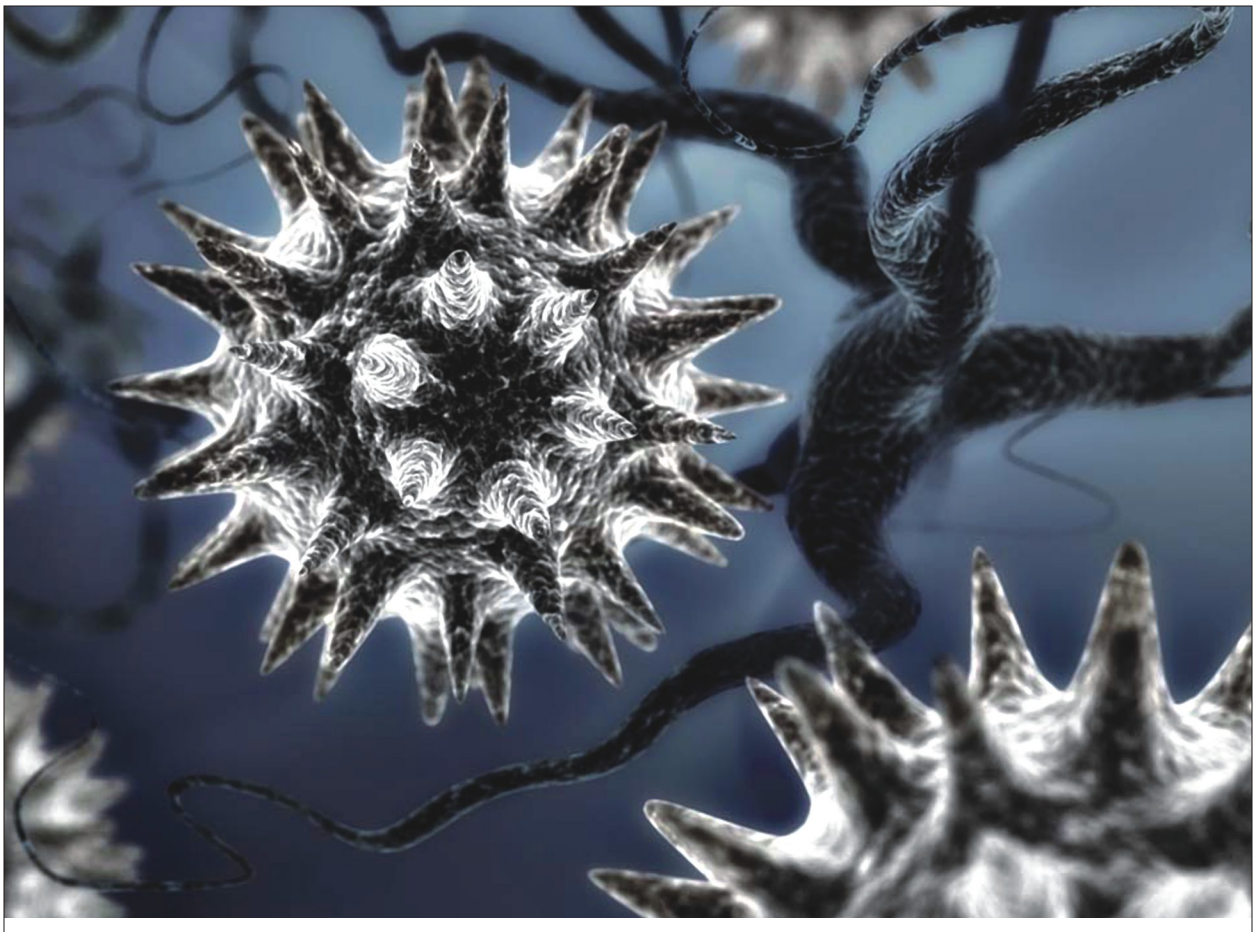

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Research Article
Assessment of Two Rapid Antigen Tests to Detect SARS-CoV-2 in Laboratory Setting in Ouagadougou, Burkina Faso

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Abstract

Background and Objective: Reliable rapid antigenic detection tests for SARS-CoV-2 may be an alternative diagnostic test for COVID-19 especially in resource-limited settings. The objective of this study was to evaluate the performance of two rapid tests namely Standard™ Q COVID-19 Ag and Standard™ F COVID-19 Ag compared to RT-PCR of SARS-CoV-2. **Materials and Methods:** This was an evaluation on 156 nasopharyngeal and/or oropharyngeal specimens collected on viral transport medium (VTM) from January to May, 2021. The "Standard™ Q COVID-19 Ag" and "Standard™ F COVID-19 Ag FIA" antigenic tests were compared with the "FastPlex™ Triplex SARS-CoV-2 Detection" RT-PCR assay on at least 86 positive and 70 negative specimens. **Results:** The assays showed a sensitivity of 57.00% and a specificity of 98.60% for the Standard™ Q COVID-19 Ag. Sensitivity and specificity were 52.33 and 94.29%, respectively, for the Standard™ F COVID-19 Ag test. In general, for each of the two antigenic tests, the results show that the lower the Ct value, the higher the sensitivity of the antigenic RDTs is (Ct≤33:55.6%, Ct≤29:63.6%, Ct≤25: 78.6% for the Standard™ Q COVID-19 Ag test), (Ct≤33:57.8%, Ct≤29:69.7% and Ct≤25:85.7% for the Standard F COVID-19 Ag test). **Conclusion:** The sensitivity of both antigenic RDTs compared to RT-PCR for SARS-CoV-2 is generally low, but this sensitivity improves with high viral load. The use of these RDTs in areas without RT-PCR may be an alternative in patients with high viral load, especially those with symptoms during the first days of the disease.

Key words: COVID-19, SARS-CoV-2, rapid antigen test, RT-PCR, evaluation

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The end of 2019 saw the emergence of a pandemic called COVID-19 disease due to a virus called SARS-CoV-2[1,2] which spread very quickly around the world. The first cases were identified in Wuhan, China in December 2019[3,4]. The World Health Organization (WHO) had a meeting on 30 January, 2020 and they declared the coronavirus outbreak from China a public health emergency of international concern[5]. As of 24 January, 2022 there are 342,928,762 cases with 5,576,274 deaths worldwide[5].

Despite true efforts made by governments from Sub-Saharan countries, Southern and Northern Africa have currently the highest number of official cases and deaths, but Western Africa is the most impacted by the death toll in Sub-Saharan zone, followed by Eastern and Central Africa[6]. Burkina Faso, a West African country, experienced its first cases in early March, 2020[7].

This global pandemic has forced firms and laboratories to develop molecular and antigenic diagnostic tests to facilitate case diagnosis and mass screening. Nowadays, several tests exist with different sensitivities and specificities[8]. Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) remains the reference technique for the diagnosis of SARS-CoV-2 due to its ability to directly detect the virus RNA by a gene amplification process[9]. In Burkina Faso, as in most countries with limited resources, RT-PCR is only available in equipped laboratories. It is costly and requires long delays for results. These constraints limit not only the decentralization of COVID-19 diagnosis but also the availability of diagnosis in healthcare settings. High-performance Rapid Antigen Detection Tests (RDT-Ag) may be a faster, cheap and reliable expensive alternative to nucleic acid amplification tests for diagnosing active SARS-CoV-2 infection[10,11].

In Burkina Faso, at the beginning of the pandemic, RT-PCR was only available in a few national and regional laboratories. Today, however, the Ministry of Health, with the support of its partners, has been able to install PCR equipment in several localities in the country. In spite of this, access to screening and diagnosis remains a major constraint for the population in peripheral health care settings[12]. This accessibility could be improved with the introduction of efficient and reliable rapid and point-of-care diagnostic tests[13,14]. However, their implementation in health care settings requires prior validation of their characteristics at the local level. The objective of this study was to evaluate the biological performance of two rapid diagnostic tests (RDT), namely the Standard™ Q COVID-19 Ag and the Standard™ F COVID-19 Ag FIA , in comparison with the RT-PCR of SARS-CoV-2, with a view to use them for a rapid diagnosis of COVID-19.

MATERIALS AND METHODS

Type and study population: This was a cross-sectional study conducted from January to May 2021 in the Biomedical Research Laboratory (LaReBio) of the Institute for Research in Health Sciences (IRSS) in Burkina Faso.

Origin of the tests in evaluation: All tests under evaluation, as well as the reference tests used in this study, were obtained free of charge. The RDTs and RT-PCR tests were provided by the Ministry of Health for validation and routine diagnosis of COVID-19 in Burkina Faso, respectively.

Nature and sources of reference specimen: The samples used in this technical validation were human nasopharyngeal and/or oropharyngeal samples collected on 3 mL of viral transport media (VTM) and received at the biomedical research laboratory of the IRSS in Ouagadougou for the diagnosis of COVID-19 by RT-PCR. The use of samples collected on 3 mL of viral transport media (VTM) could result in additional sample dilution compared to samples directly collected with the manufacturer's collection kit containing 1 mL of antigen lysis solution (SD Biosensor Inc., Republic of Korea). However, this influence of dilution was optimized by considering Ct values in the estimates. A total of 156 samples were collected after RT-PCR analysis from candidates for COVID-19 diagnosis. This panel consisted of 86 RT-PCR positive samples and 70 negative samples.

Detection of SARS-CoV-2 by RT-PCR (gold standard): The SARS-CoV-2 RNA was extracted with the QIAGEN "QIAmp RNA mini" kit according to the manufacturer's instructions (QIAGEN, USA). Amplification and detection of the virus was performed from the extracts using the "FastPlex™ SARS-CoV-2 detection (RT-PCR)" kit (PreciGenome LLC, San Jose, USA) on the HumaCycler thermocycler (Human GmH, Germany). This is an assay designed for real-time detection of SARS-CoV-2 with high accuracy. The primers and probe have been designed to detect SARS-CoV-2 RNA in patient samples. It is an assay that targets 2 genes: ORF1ab and N with a detection limit of 285.7 copies/mL and a Ct threshold of 39 cycles. This test includes in addition to the target genes, an internal control gene (RPP30) which allows the result to be validated. The result was "positive" if the $Ct \leq 39$ for each of the genes or for both genes and "negative" if the Ct value >39 according to the manufacturer's instructions (PreciGenome LLC, San Jose, USA).

Detection of SARS-CoV-2 antigens by rapid tests: After RT-PCR, all samples were analyzed with each of the two antigenic assays "Standard™ Q COVID-19 Ag" and "Standard™ F COVID-19 Ag FIA" (SD Biosensor, Inc, Republic of Korea). The manufacturer's recommendations were adapted for use with samples collected on viral transport medium.

Standard™ Q COVID-19 Ag test is a rapid lateral flow immunoassay for the SARS-CoV-2 specific antigens qualitative detection. It is in cassette format with control line and result line. The SARS-CoV-2 specific antigens present in the sample form an antibody-antigen color particle complex by interaction with mouse monoclonal anti-SARS-CoV-2 antibodies conjugated. If antigens are present, a colored line appears in the results window after 15-30 min. The control line must appear for the test to be validated. The sensitivity and specificity of the Standard™ Q COVID-19 Ag test were 96.52 and 99.68%, respectively according to the manufacturer (SD Biosensor Inc., Republic of Korea).

The "Standard™ F COVID-19 Ag FIA" test, on the other hand, is a fluorescent immunoassay and should be used with Standard™ F analyzers (SD Biosensor Inc., Republic of Korea). It has a test line that is coated with SARS-CoV-2 monoclonal antibodies. The SARS-CoV-2 antigen will react with the europium-conjugated SARS-CoV2 monoclonal antibodies in the conjugation buffer and form a fluorescence particle complex. The SARS-CoV-2 antibodies capture this complex on the test line and produce a fluorescence signal. Analysis of the intensity of the fluorescence signal generated on the membrane is done by the Standard F200 analyzer using pre-programmed algorithms. The results is "positive" if COI$\geq$1.0 and "negative" if COI<1.0. The COI is a numerical representation of the fluorescence signal measurement according to the manufacturer's instructions. The result appears on the analyzer after 30 min. The sensitivity and specificity of the Standard™ F COVID-19 Ag FIA test is 100 and 100%, respectively according to the manufacturer.

Statistical analysis: Data were entered into Excel and analyzed using OpenEpi software. For each RDT, the results obtained were compared with those of the RT-PCR test performed in the laboratory and the main performance characteristics of the RDT were determined. For this purpose, the results of each RDT were classified as positive or negative in relation to the known results of the RT-PCR method. The sensitivity and diagnostic specificity of each test were estimated with their 95% confidence intervals. Sensitivity was calculated based on the following Ct values: Ct$\leq$25 and Ct>25; Ct$\leq$29 and Ct>29; Ct$\leq$33 and Ct>33. The agreement between the antigenic RDTs and the RT-PCR was calculated using Kappa Coefficient[15].

RESULTS

Characteristics of the participants: Males were the most represented with a rate of 68.6% (107/156) and the majority were SARS-CoV-2 positive (55.1%). The majority of participants were asymptomatic (88.5%) with a positive rate of 55.1%. (Table 1).

Results of SARS-CoV-2 antigenic tests: The assay results yielded one false positive (FP) with the Standard™ Q COVID-19 Ag test versus 4 false positives with the Standard™ F COVID-19 Ag FIA test. The number of false negatives (FN) was less with the Standard™ Q COVID-19 Ag test than with the Standard™ F COVID-19 Ag FIA test (FN: 37 vs 41) (Table 2). For both tests, the lower the Ct, the higher the number of positives.

Overall, the analyses showed a sensitivity of 57% (95% CI: 46.4-66.9%) and specificity of 98.6% (95% CI: 92.3-99.8%) for the Standard™ Q COVID-19 Ag test. In contrast, the sensitivity and specificity of the Standard™ F COVID-19 Ag FIA test were 52.3% (95% CI: 41.9-62.5) and 94.3% (95% CI: 86.2- 97.8) respectively. Diagnostic accuracy was 75.6% and 71.1% for the Standard™ Q COVID-19 Ag test and Standard™ F COVID-19 Ag FIA test, respectively. The LRP was higher (39.9%) for the Standard™ Q COVID-19 Ag test than for the Standard™ F COVID-19 Ag FIA test (9.2%) (Table 3 and Table 4).

Table 1: Gender and clinical status of the participants

	n (%)		
Characteristics	Number (n = 156)	RT-PCR positive samples (n = 86)	RT-PCR negative samples (n = 70)
Gender			
Female	45 (28.8)	23 (51.1)	22 (48.9)
Male	107 (68.6)	59 (55.1)	49 (44.9)
Missing	4 (2.6)	4 (100)	0 (0.0)
Clinical			
Symptomatics	18 (11.5)	10 (55.6)	8 (44.4)
Asymptomatics	138 (88.5)	76 (55.1)	62 (44.9)

Table 2: Results of the Standard™ Q COVID-19 Ag rapid test and Standard™ F COVID-19 Ag FIA

	Standard™ Q COVID-19 Ag rapid test			Standard™ F COVID-19 Ag FIA rapid test		
	RT-PCR			RT-PCR		
	Positive	Negative	Total	Positive	Negative	Total
All samples tested						
Ag rapid test positive (n)	49	1	50	45	4	49
Ag rapid test negative (n)	37	69	106	41	66	107
Total	86	70	156	86	70	156
RT-PCR positive samples with a cycle threshold Ct≤33						
	Ct≤33	Ct>33	Total	Ct≤33	Ct>33	Total
Ag rapid test positive (n)	25	24	49	26	19	45
Ag rapid test negative (n)	20	17	37	19	22	41
Total	45	41	86	45	41	86
RT-PCR positive samples with a Ct≤29						
	Ct≤29	Ct>29	Total	Ct≤29	Ct>29	Total
Ag rapid test positive (n)	21	28	49	23	22	45
Ag rapid test negative (n)	12	25	37	10	31	41
Total	33	53	86	33	53	86
RT-PCR positive samples with a Ct≤25						
	Ct≤25	Ct>25	Total	Ct≤25	Ct>25	Total
Ag rapid test positive (n)	11	38	49	12	33	45
Ag rapid test negative (n)	3	34	37	2	39	41
Total	14	72	86	14	72	86

Table 3: Performance of the Standard™ Q COVID-19 Ag rapid test compared to RT-PCR

Parameters	Set (95% CI)	Ct≤33 (95% CI)	Ct>33 (95% CI)	Ct≤29 (95% CI)	Ct>29 (95% CI)	Ct≤25 (95% CI)	Ct>25 (95% CI)
Sensitivity	57 (46.4-66.9)	55.6 (41.2-69.1)	58.5 (43.4-72.2)	63.6 (46.6-77.8)	52.8 (39.7-65.6)	78.6 (52.4-92.4)	52.8 (41.4-63.9)
Specificity	98.6 (92.3-99.7)	-	-	-	-	-	-
Accuracy of diagnosis	75.6 (68.3-81.7)	55.6 (41.2-69.1)	58.5 (43.4-72.2)	63.6 (46.6-77.8)	52.8 (39.7-65.6)	78.6 (52.4-92.4)	52.8 (41.4-63.8)
LRP	39.9 (5.4-291.8)	-	-	-	-	-	-
LRN	0.4 (0.4-0.5)	-	-	-	-	-	-
kappa coefficient	0.5 (0.4-0.7)	-	-	-	-	-	-

Ct: Cycle threshold, LRP: Likelihood ratio of positive test and LRN: Likelihood ratio of negative test

Table 4: Performance of the Standard™ F COVID-19 Ag rapid test compared to RT-PCR

Parameters	Set (95% CI)	Ct≤33 (95% CI)	Ct>33 (95% CI)	Ct≤29 (95% CI)	Ct>29 (95% CI)	Ct≤25 (95% CI)	Ct>25 (95% CI)
Sensitivity	52.3 (41.9-62.5)	57.8 (43.3-71.0)	46.3 (32.1-61.2)	69.7 (52.70-82.6)	41.5 (29.3-54.9)	85.7 (60.1-96.0)	45.8 (34.8-57.3)
Specificity	94.3 (86.2-97.8)	-	-	-	-	-	-
Accuracy of diagnosis	71.1 (63.6-77.7)	57.8 (43.3-71.0)	46.3 (32.1-61.2)	69.7 (52.7-82.6)	41.5 (29.3-54.9)	85.7 (60.1-96.0)	45.8 (34.8-57.3)
LRP	9.2 (5.4-15.5)	-	-	-	-	-	-
LRN	0.50 (0.48-0.53)	-	-	-	-	-	-
kappa coefficient	0.4 (0.3-0.6)	-	-	-	-	-	-

Ct: Cycle threshold, LRP: Likelihood ratio of positive test and LRN: Likelihood ratio of negative test

DISCUSSION

This study evaluated the diagnostic performance of two antigenic tests in the routine laboratory diagnosis of SARS-CoV-2. The results showed a low sensitivity for each of the two antigenic tests in general, but this sensitivity increases with the SARS-CoV-2 viral load in the sample. The specificity of both tests was good for the diagnosis of SARS-CoV-2. These low sensitivity values could be explained by the asymptomatic profile of the majority of our study population (81%). In a study comparing the performance of antigenic RDTs, the authors found that the sensitivity and specificity were 41.2 and 98.4%, respectively, in asymptomatic subjects, whereas they were 80% (Se) and 98.9% (Sp) in symptomatic participants[16]. Another study in Uganda showed a sensitivity of Standard™ Q COVID-19 Ag test of 70% (95% CI: 60-79%) and a specificity of 92% (95% CI: 87-96%) in patients recruited in a hospital setting[17]. According to Bruzzone *et al.*[18] the sensitivity of rapid SARS-CoV-2 antigen detection tests depends mainly on the viral load and this optimal sensitivity is at a Ct≤29 and is better at a Ct<25.

In general, for each of the two tests, results show that the lower the Ct value, the higher the sensitivity of each of the two antigenic RDTs (Ct≤33: 55.6%, Ct≤29: 63.6% and Ct≤25: 78.6% for the Standard™ Q COVID-19 Ag test), (Ct≤33: 57.8%, Ct≤29: 69.7% and Ct≤25: 85.7% for the Standard™ F COVID-19 Ag FIA test). This same finding was made in a study conducted by with the Standard™ Q COVID-19 Ag test. These authors had found sensitivities of 71.0 (51.96-85.78) for Ct≤35, 85.0 (62.11-96.79) for Ct≤30, 92.3 (63.97-99.81) for Ct≤25. Other studies led to the same finding with Ct<29; Ct: 29-36, Ct: 37-39 for the Standard™ Q COVID-19 Ag test[19], Ct≤30, Ct≤35 for the Standard™ F COVID-19 Ag FIA test[18].

The Uganda study reported that 92% of specimens with strong positive (Ct≤29) qRT-PCR results were positive by the STANDARD™ Q COVID-19 Ag assay and that Only 50% of specimens classified as moderately positive (Ct = 30-37) and weakly positive (Ct = 38-39) by qRT-PCR were positive with the antigen test[17]. Low Ct values suggest that more viral RNA was present in the specimen, while high Ct values correspond to specimens with lower viral load[20]. A low viral load implies that more cycles were required to amplify the viral target. The cycle threshold (Ct) value is the number of PCR cycles at which the fluorescence signal crosses the threshold for positivity and Ct values are inversely proportional to the amount of viral RNA present in the sample being tested[20]. The current study results was in agreement with the WHO statement that RDT-Ag performs better in individuals with high viral load and will be more reliable in settings where the prevalence of SARS-CoV-2 is ≥5%[14]. In addition, data from the literature show that the viral load of SARS-CoV-2 is highest at the onset of infection and especially during the first 7 days of symptom onset[21].

In current study, there is a better performance of the Standard™ Q COVID-19 Ag test compared to the Standard™ F COVID-19 Ag FIA test (Se: 57% and Sp: 98.6% versus Se: 52.3% and Sp: 94.3%). But these results are lower than those reported in other studies (Se: 65.8% (48.65-80.37) Sp: 100% (87.66-100)) for the Standard™ Q COVID-19 Ag[22] test; and (Se: 60.85% (53.74-67.52) Sp: 98.00 (95.71-99.08)) for the Standard™ F COVID-19 Ag FIA[23]. The sensitivity and specificity of the Standard™ Q COVID-19 Ag test was even better in a study conducted in Thailand on symptomatic patients with a median time to onset of symptoms of 3 days (0-14 days). These authors had found a sensitivity and specificity of 98.33% (91.06-99.96%) and 98.73% (97.06-99.59%) respectively[24]. In addition to the presence or absence of symptoms, as well as the time of onset of symptoms that affect the performance of antigenic RDT[16] variants may affect the receptor N[25,26]. Some authors had suggested that variants not detected by the rapid antigen test may be due to the T135I mutation in the N protein, posing a potential diagnostic risk for commercially available antigen tests[26].

The monoclonal antibody specific for SARS-CoV-2 N antigen applied to the Standard Q COVID-19 Ag test was produced from the WUHAN-01 strain, which is genetically very similar to the SARS-CoV-2 strains detected in Thailand[27]. Note that most of the antigenic tests were produced during the first months after the start of the COVID-19 pandemic and would certainly be based on the wild-type strain of SARS-CoV-2 (WUHAN-01)[24]. Yet, the test evaluations were done at a time when there was wider genetic variability with the circulation of alpha, beta, gamma and delta strains[28,29].

A systematic review of the cochrane database reported that 8 evaluations of antigenic tests in 5 studies showed sensitivity ranging from 0.17 to 94% and specificity from 94 to 100%[30]. Several antigenic tests are available on the market, but only a few of them are reported to perform well enough to be used for population-based SARS-CoV-2 screening. The WHO recommends the use of Antigenic Rapid Diagnostic Tests (Ag-RDTs) that meet minimum performance requirements of sensitivity ≥80% and specificity ≥97%. The Ag-RDTs tests are less sensitive than RT-PCR tests, particularly in asymptomatic individuals, but careful selection of cohorts for testing can mitigate this limitation[14]. In the absence of transmission or in cases of low transmission, the positive predictive value of Antigenic Rapid Tests (Ag-RDTs) is low and, in this case, RT-PCR is preferable for first-line testing.

The likelihood ratio of the positive test of the Standard™ Q COVID-19 Ag Rapid test was good (39.9). This shows that there is a 39.9-fold greater chance of testing positive with this Ag-RT when a person is infected with SARS-CoV-2 than when they are not. In contrast, this chance of testing positive with the Standard™ F COVID-19 Ag FIA is only 9.2 times greater when a person is infected than when they are not. Cohen's kappa coefficient values were 0.5 and 0.4, respectively, for the Standard™ Q COVID-19 Ag test and the Standard™ F COVID-19 Ag FIA test. This reflects moderate agreement of the Standard™ Q COVID-19 Ag and poor agreement of the Standard™ F COVID-19 Ag FIA with RT-PCR, respectively. This shows that compared to RT-PCR as a reference test in the detection of SARS-CoV-2, antigenic RDTs lack reliability for their use in general population screening. However, in symptomatic subjects who generally have a high SARS-CoV-2 viral load at the onset of symptoms[31,32]. However, in symptomatic subjects with a generally high viral load of SARS-CoV-2 at the onset of symptoms, the use of these antigenic tests may be considered, especially in resource-limited settings with limited access to RT-PCR. These tests should not be used to screen asymptomatic persons who

usually have a low viral load. It is recommended that the manufacturer improve the diagnostic performance of these tests for better detection of SARS-CoV-2 antigen in subjects with low to moderate viral loads.

This study was conducted in the laboratory, which could be a limitation in the context of field use. Larger, population-based studies could provide more robust results on the real-world diagnostic performance of these RDTs.

CONCLUSION

This study shows that rapid antigenic tests perform poorly compared to RT-PCR in the detection of SARS-COV-2 in the laboratory from VTM samples, especially for those with particularly low viral load. Thus, their routine use in the asymptomatic general population could lead to false negative results. However, the increase in sensitivity in proportion to the SARS-CoV-2 viral load supports their use in symptomatic subjects during the first days of symptoms. The low cost and rapid turnaround of antigenic RDT results should be balanced against their limited sensitivity, especially in persons with low viral load, including asymptomatic persons.

SIGNIFICANCE STATEMENT

The sensitivity of both antigenic RDTs compared to RT-PCR for SARS-CoV-2 is generally low, but this sensitivity improves with high viral load. The use of these RDTs in areas without RT-PCR may be an alternative in patients with high viral load, especially those with symptoms during the first days of the disease.

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