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Evaluating the sensitivity and specificity of a rapid antigen screening test against real-time polymerase chain reaction for COVID-19 detection in northern Ethiopia

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ABSTRACT: Early diagnosis and treatment of COVID-19-positive patients are crucial to limiting complications and transmission. Despite real-time PCR being considered a gold standard for the diagnosis of COVID-19, its availability is still limited. There is a paucity of studies that show the sensitivity and specificity of rapid antigen tests (RAT) in Ethiopia. This study aimed to evaluate the sensitivity, specificity and predictive values of rapid antigen tests for the diagnosis of sever acute respiratory syndrome coronavirus type-2 (SARS-COV-2 among COVID-19 suspected patients. A hospital-based cross-sectional study was conducted on COVID-19-positive and negative individuals in the University of Gondar Comprehensive Specialized Hospital from December 26, 2022, to April 30, 2023. Socio-demographic, behavioral, and clinical data were collected using a structured questionnaire. All nasopharyngeal or throat swabs were tested using the Panbio™ COVID-19 rapid antigen test and the BIO-RAD CFX connect™ reverse transcription-polymerase chain reaction. Sensitivity, specificity, Kappa, and positive and negative predictive values were analyzed using the online Medcalc statistical tool to determine the diagnostic performance of the rapid antigen test using the RT-PCR reference method. Out of 120 participants, 53.3% were female. In this study, sensitivity, specificity, and accuracy of the Panbio™ rapid antigen test were 98.36%, 100%, and 98.33%, respectively The positive and negative predictive values were 100% and 99.17%, respectively. The Kohen's kappa statistic was 0.983 at 50% estimation of the disease prevalence. Panbio™ rapid antigen test results Showed outstanding agreement with RT-PCR using a nasopharyngeal or nasal swab from symptomatic patients. The RAT affordable and provides immediate outcome with in short period of time. In order to quickly identify the positive cases and put isolation and infection control measure in place, this test can be used in regions where the numbers SARS-CoV-2 cases is fast increasing.

KEYWORDS: COVID-19, diagnostic performance, Panbio™, rapid antigen test

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the agent of the pandemic coronavirus disease 2019 (COVID-19) is primarily a respiratory virus. It is a single-strand, positive-sense ribonucleic acid (+sRNA) virus that causes an incipient respiratory disease [1]. The disease was discovered in the Chinese state of Wuhan in December 2019 and has since become a global public health issue, prompting the World Health Organization (WHO) to declare it a pandemic disease on March 11, 2020 [2, 3].

The extremely spreadable characteristics of COVID-19 give it its harshness and riskiness and cause a high fatality rate and rapid spread of the virus from its origin to more than 210 countries around the world. That is why the WHO declared this problem a pandemic disease. The newly discovered virus was initially known as the "new coronavirus" (nCoV) [3], and the pathogen was eventually dubbed SARS-CoV-2 [4, 5]. The virus is transmitted from human to human through airborne droplets of respiratory secretions from infected patients and direct contact with contaminated surfaces or individuals, with an incubation period of 2–14 days following exposures to the virus [6]. The virus can live on the surface of materials or the skin for a few hours to days. The most common symptoms of infected individuals are a dry cough, shortness of breath, and high-grade fever [7].

Statement of the problem

Globally, more than 535,863,950 confirmed cases of COVID-19, including 6,314,972 deaths, have been reported by the WHO as of May 17, 2022. Of these, Europe (223,780,003), the Americas (161,251,921), the Western Pacific (62,605,363), South-East Asia (58,305,870), the Eastern Mediterranean (21,850,741), and Africa (9,069,288) have been reported as having the most confirmed cases so far. These reports show that COVID-19 is continuing to be a major problem worldwide, and early identification and utilization of preventive materials are essential to catching up with the problem. Therefore, additional diagnostic techniques in conjunction with contact tracing modalities, are crucial [8]. Many COVID-19 diagnostic tools, including rapid diagnostic tests, have been released recently. They are created to find out if someone has ever been infected with SARS-CoV-2 by detecting the antigens or nucleic acids of the virus and antibodies against it. The gold standard test method for detecting SARS-CoV-2 is the reverse transcription-polymerase chain reaction (RT-PCR) assay, which finds viral RNA from nasopharyngeal or oropharyngeal swabs or other upper respiratory tract samples [9].

RT-PCR has many limitations, such as expense, comparatively long turnaround times, and the need for specialised testing equipment [10]. The RT-PCR test detects small amounts of SARS-CoV-2 nucleic acid fragments and can remain positive for a long time after the illness has passed. As a result, an RT-PCR test-based strategy is not recommended to cease isolation for the majority of patients [11, 12]. As a result, there is a need for new testing techniques to quickly stop the virus's spread, which would benefit patients and the healthcare system [13]. Rapid antigen tests, a subset of fast diagnostic procedures based on lateral immune chromatography, have been demonstrated to be useful for the point-of-care detection of SARS-CoV-2 antigens [13]. Many companies developed point-of-care promptly antigen testing in response to RT-PCR's limitations [14, 15].

Objective of the study

To evaluating the Sensitivity, Specificity and accuracy of a Rapid Antigen Screening Test against Real-Time Polymerase Chain Reaction for COVID-19 Detection in Northern Ethiopia.

Significance of the study

Comprehensive testing, contact tracing, and infectious case isolation remain critical strategies for reducing the spread of SARS-CoV-2 and preserving healthcare system integrity [16]. To date, RT-PCR has been considered the gold standard of test modalities for the diagnosis of COVID-19, but it is not accessible everywhere. In Ethiopia, RT-PCR is widely used in some centers, and tests were performed abroad (in South Africa) at the start of the pandemic [17]. Mass testing could only be performed in shorter turnaround time (TAT) tests using RAT, allowing for prompt public health actions to be implemented and the chain of infection to be broken. Therefore, RATs should be taken as one of the best opportunities to screen COVID-19-suspected individuals and alternative means of diagnosis that could be easily addressed in both private and governmental health facilities, particularly in rural areas. However, there were a limited number of published articles that showed the sensitivity and specificity of the Panbio™ COVID-19 RAT in comparison to RT-PCR in the Ethiopian population.

MATERIALS AND METHODS

Study design and period

A hospital-based cross-sectional study was conducted from December 26, 2022, to April 30, 2023.

Study Area

The study was carried out at UoGCSH in Gondar town, northwest Ethiopia. Gondar is located in the northwest part of Ethiopia, 739 km from Addis Ababa, the capital city of Ethiopia. It is the administrative capital of the Central Gondar Zone, with a population estimated to be 207,044 in the 2007 Ethiopian census report [18]. Currently, the town has one referral hospital, eight health centers, and several private health facilities. The UoGCSH is one of the largest hospitals in the country. It is a tertiary teaching hospital with a catchment area of over five million people in the Central Gondar zone and the surrounding districts. The hospital has a well-established outpatient department, inpatient wards, and clinics. The hospital is serving as the center for COVID-19 diagnosis and treatment, starting from the beginning of the pandemic up to date. It has arranged separate wards for diagnosis, follow-up, and treatment for COVID-19 patients.

Source of population

All COVID-19 suspect individuals who were attending the COVID-19 center at UoGCSH were the source population.

Study population

All COVID-19 confirmed positive and negative individuals who attended the COVID-19 center at UoGCSH during the study period were the study population.

Inclusion criteria

All patients attending UoGCSH were included in this study.

Exclusion criteria

Critically ill patients who were unable to respond were excluded in the study.

Sample size determination and sampling techniques

The sample size was determined using the Clinical & Laboratory Standards Institute recommended sample size calculation for method comparison, which is 60–100 per group of method [19]. The total sample size was 120 (60 RAT positive and 60 RAT negative). The study participants were selected through a non-randomized purposive sampling technique.

Data collection and processing

A semi-structured interviewer questionnaire was used to collect data. The questionnaire was prepared based on similar studies conducted in Ethiopia and worldwide. The questionnaire included socio-demographic, behavioural, and clinical characteristic questions. It was initially prepared in English and translated into a local language (Amharic), and then the data was retranslated back to English to check for consistency and completeness. Data were collected by professional health workers such as health officers, nurses, and laboratory technologists.

Laboratory procedures

Specimen collection for RAT and RT-PCR methods

For all study participants, both RAT and RT-PCR tests were analyzed using the Panbio™ COVID-19 antigen rapid test device and the BIO-RAD CFX connect™ RT-PCR, respectively. The principal investigator and the privileged laboratory technologists at the COVID-19 diagnostic Centre of UoGCSH conducted the examinations. The tests were done as per the manufacturer's guidelines. Before sample collection, the participants were instructed to clear all nasal secretions for the appropriate identification of the antigen. The nasopharyngeal swab was collected from the posterior part of the nasopharynx using a sterile nasopharyngeal swab that was given with the test kit. The sterile nasopharyngeal swab was inserted into the nasal cavity at an angle of 90° in an extended neck position to collect the swab, which was retained in the nasopharynx for about five seconds and was removed gently while rotating it. Then, the swab was allowed to insert into the extraction buffer tube provided with the kit and was dipped into it five to six times before squeezing it, and then the swab was appropriately discarded following the protocol. A nozzle was positioned firmly to close the extraction buffer tube, and it was tremble for about 20 seconds, and then two drops was put onto the specimen well that we had prepared [20]. The RT-PCR tests were analyzed at the center of COVID-19 diagnosis at UoGCSH.

COVID-19 Real time PCR tests

Nucleic acid extraction

The extraction of viral RNA was performed to obtain sufficient target RNA available for reverse transcription and complementary DNA (cDNA) amplification. Nasopharyngeal or throat swabs were collected from each study participant for RAT and RT-PCR test analysis. The samples were collected using nylon-flocked swabs and stored in a 3 ml Viral Transport Medium Kit III (VTM). Lysis solution contains a strong protein denaturant that can quickly dissolve proteins and dissociate nucleic acids. With the existence of lysis solution and ethyl alcohol, the dissociated acid compositions could be combined on the silicone membranes, and then by the actions of an inhibitor remover and a deionizer, the protein, inorganic salt ions, and many organic impurities were removed. Then eluent was added to elute nucleic acid.

PCR master mix

The technique was based on one-step RT-PCR technology. The novel coronavirus (2019-nCov) Open Reading Frame 1ab (ORF1ab) and N genes (N-gene probes were labeled with FAM and ORF1ab probes were labeled with VIC) were designed to detect the RNA of the novel coronavirus (2019-nCov) sample. This technique also includes an endogenous internal control detection system. The internal control probe was labeled with Cy5 for sample collection monitoring. Real-time PCR was carried out by using multiplex real-time PCR technology, ORF1ab, and the nucleocapsid gene (N) of the SARS-CoV-2 genome. RNA isolated and purified from upper and lower respiratory

tract specimens is reverse transcribed to cDNA and amplified in a real-time PCR instrument using a one-step master mix. Probes consist of a reporter dye at the 5′ end and a quenching dye at the 3′ end. The fluorescent signals emitted from the reporter dye were absorbed by the quencher. During PCR amplification, probes hybridized to amplified templates are degraded by the Taq DNA polymerase with 5′-3′ exonuclease activity, thereby separating the reporter dye and quencher and generating fluorescent signals that increase with each cycle. The PCR instrument automatically drew a real-time amplification curve for each optical channel based on the signal change and calculated cycle threshold (Ct) values (the point at which fluorescence was detectable above the background) that were interpreted by the operator to determine the presence or absence of SARS-CoV-2 RNA [21].

Panbio™ COVID-19 antigen rapid test device

Panbio™ COVID-19 antigen rapid test device contains a membrane strip, which is pre-coated with immobilized anti - SARS-CoV-2 antibody on the line and mouse monoclonal anti-chicken IgY on the control line. Two types of conjugates (human IgG specific to SARS-CoV-2 antigen gold conjugate (binds to the nucleo capsid protein) and chicken IgY gold conjugate) moved forward on the membrane chromatographically and reacted with anti-SARS-CoV-2 antibodies and pre-coated specific to SARS-CoV-2 antigen gold conjugate and anti SARS-CoV-2 antibodies formed a test line in the result window. Neither the test line nor the control line was visible in the result window before prior to applying the patient specimen. A visible control line was required to determine if the test result was valid.

Data processing and analysis

Data were entered into EpiData version 3.1 to ensure completeness before being analyzed with SPSS version 25 which was used for analysis of the data. A Shapiro-Wilk test was conducted to check the normality of the data. Categorical variables were analyzed and presented as frequency and percentage, and continuous variables were describes using mean. To detect the accuracy, reliability, and generalization of the RAT method in comparison to RT-PCR, the specificity, sensitivity, Kohen's Kappa statistics, positive and negative predictive values (PPV and NPV) were analyzed. An online Medcalc statistical tool was used to assess the diagnostic performance of the RAT method [22]. The statistical significance of the variables was declared at p-value less than 0.05 and the confidence interval of 95%.

Data quality assurance

A two-day training was provided for all data collectors, and the session covered the detailed aspects of the study that cover the objective, methodology, risk and benefit analysis, and preparation of COVID-19 prevention mechanisms according to the guidelines of WHO by the principal investigator. The quality of the study was ensured at each phase of the study. The general approachability and feasibility of the questionnaires were addressed in the pilot study. Before the actual data collection, the questionnaires were pretested with 10% of the sample size. The overall quality of laboratory analysis was maintained by strictly following the manufacturers' instructions and standard operational procedures (SOP) in the pre-analytic, analytic, and post-analytic stages. The principal investigator was also strictly monitored the data and sample collection. The collected data were double-checked for completeness, consistency, accuracy, and clarity on a daily basis.

Ethical considerations

Ethical approval was obtained from the Ethical Committee of the Institute of Biotechnology, University of Gondar, and Ref. No: IBO/158/12/2022. A permission letter was also obtained from the UoGCSH before starting data collection. In addition, all study participants were requested for their written informed consent after a detailed explanation of the study purpose, procedures, benefits, and risks using their mother tongue (Amharic). If the study participants were illiterate, the data collector had read and takes the sign or thumb impression when they agreed. All the principles of ethics, such as informed consent, confidentiality, and privacy, were maintained. The clinical and laboratory information of the study participants was collected using codes rather than personal identifiers. Study participants who had been suspected of any other infections were linked to UoGCSH for further diagnosis and appropriate treatment.

RESULTS

Socio-demographic characteristics of study participants

One hundred twenty patients (60 rapid antigen diagnosis (RAD) test positive and 60 RAD test negative) fulfilling the eligibility criteria, were enrolled. Of the total 120 enrolled study participants, 64 (53.3%) were female,

and the mean age was 43.38 years. The majorities of the study participants (75.8%) were urban residents and married (65%), as shown on the (Table 1).

Distribution of COVID-19 per different age groups

The RT-PCR COVID-19 positive study participants were relatively higher among younger age groups (21.3%) in 30-39 years, followed by 18% of each age group (20–29) and 40–49 years. The frequency of COVID-19 positive and negative RT-PCR confirmed cases by age group was shown on the (Figure 1).

Table 1. Socio-demographic characteristics of the study participants at UoGCSH 2023.

Characteristics		RAD positive	RAD negative	Total
Age mean ± SD		41.58 ± 15.772	45.17±15.559	43.38 ± 15.703
Sex n (%)	Male	32 (53.3)	24 (40.0)	56 (46.7)
	Female	28 (46.7)	36 (60.0)	64 (53.3)
Education status n (%)	Illiterate	10 (16.7)	34 (56.7)	43 (35.8)
	Primary	2 (3.3)	4 (6.7)	6 (5.0)
	Secondary	17 (28.3)	13 (21.7)	30 (25.0)
	University	31 (51.7)	9 (15.0)	41 (34.2)
Occupation n (%)	Unemployed	1 (1.7)	2 (3.3)	3 (2.5)
	Farmer	6 (10.0)	11 (18.3)	17 (14.2)
	Government	23 (38.3)	10 (16.7)	33 (27.5)
	Private	16 (26.7)	21 (35.0)	37 (30.8)
	Other	14 (23.3)	16 (26.7)	30 (25.0)
Marital status n (%)	Single	16 (26.7)	13 (21.7)	29 (24.2)
	Married	41 (68.3)	37 (61.7)	78 (65.0)
	Divorced	2 (3.3)	2 (3.3)	4 (3.3)
	Widowed	1 (1.7)	8 (13.3)	9 (7.5)
Resident n (%)	Urban	55 (91.7)	36 (60.0)	91 (75.8)
	Rural	5 (8.3)	24 (40.0)	29 (24.2)

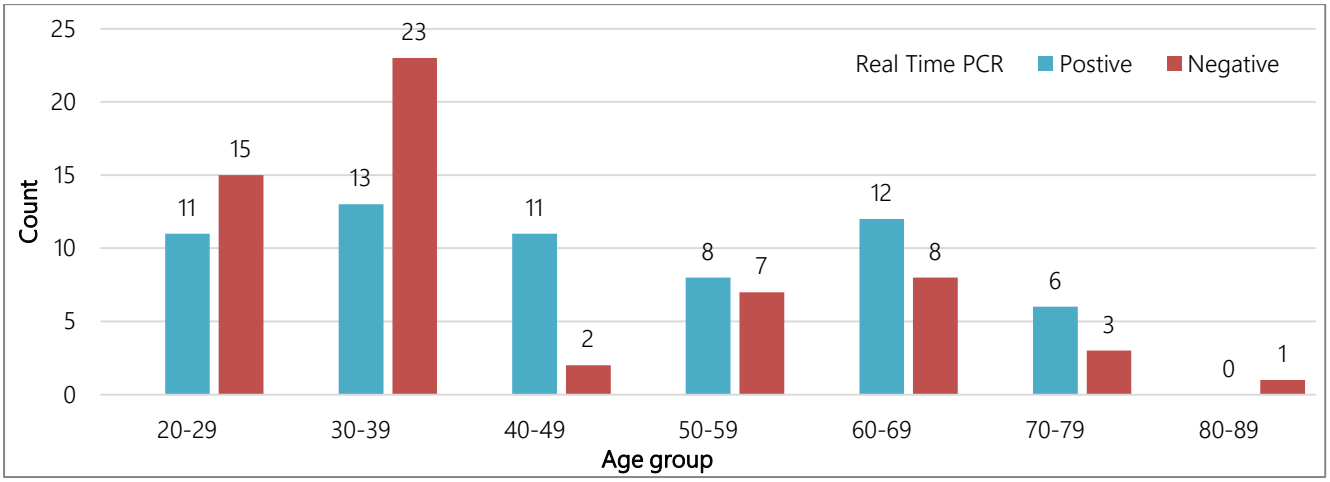

Figure 1. Distribution of COVID-19 per age group of the study participants at UoGCSH 2023.

Behavioral characteristics of the study participants

Majority of the study participants had no habit of using sanitizer and mask with the proportion of 112 (93.3%) and 111 (92.5%), respectively. Only few of them had the behavior of using alcohol, cigarettes or chat chewing as shown on (Table 2).

Clinical manifestation of the study participants

The majority of the study participants had no habit of using sanitizer or masks, with a proportion of 112 (93.3%) and 111 (92.5%), respectively. Only a few of them had the behavior of using alcohol, cigarettes, or chewing, as shown on (Table 3).

Sensitivity and specificity of RAD test

For the analysis of sensitivity and specificity, the gold standard diagnostic method, RT-PCR, was used as a reference. All of the study participants who had positive results for the RAD test showed positive results for the RT-PCR test. However, one of the study participants who had negative results for the RAD found positive for the RT-PCR given the sensitivity and specificity of RAT 98.36 and 100%, respectively (Table 4).

Accuracy of rapid antigen test using the ROC curve analysis

The diagnostic accuracy of the rapid antigen test was evaluated using ROC curve analysis and determined using the area under curve (AUC), taking the RT-PCR results as a reference. The AUC obtained for the rapid antigen test was AUC = 0.992, p <0.001 (Figure 2).

Table 2. Behavioral characteristics of the study participants suspected with COVID-19 at UoGCSH 2023.

Behavior		RAD positive n(%)	RAD negative n(%)	Total n(%)
Sanitizer usage	Yes	0(0.0)	8 (13.3)	8 (6.7)
	No	60 (100.0)	52 (86.7)	112 (93.3)
Mask usage	Yes	1 (1.7)	8 (13.3)	9 (7.5)
	No	59 (98.3)	52 (86.7)	111 (92.5)
Alcohol drinking	Yes	12 (20.0)	13 (21.7)	25 (20.8)
	No	48 (80.0)	47 (78.3)	95)79.2)
Cigarette smoking Yes	Yes	10 (16.7)	4 (6.7)	14 (11.7)
	No	50 (83.3)	56 (93.3)	106 (88.3)
Chat chewing	Yes	3 (5.0)	1 (1.7)	4 (3.3)
	No	57 (95.0)	59 (98.3)	117(6.7)

Table 3. Clinical manifestations of the study participants suspected with COVID-19 at UoGCSH 2023.

Clinical manifestations	RAT positive n=60 n (%)	RAT negative n=60 n (%)	Total n=120 n (%)
Cough	50 (83.3)	47 (78.3)	97 (80.8)
Fever	42 (70.0)	22 (36.7)	64 (53.3)
Fatigue	39 (65.0)	18 (30.0)	57 (47.5)
Headache	43 (71.7)	26 (43.3)	69 (57.5)
Shortness of breath	27 (45.0)	4 (6.7)	31 (25.8)
Stuffy nose	36 (60.0)	7 (11.7)	43 (35.8)
Myalgia	30 (50.0)	12 (20.0)	42 (35.0)
Sore throat	15 (25.0)	5 (8.3)	20 (16.7)
Loss of sense of smell/taste	27 (45.0)	9 (15.0)	36 (30.0)

Table 4. Comparison for sensitivity and specificity between RAT and real-time RT-PCR test results of COVID-19 at UoGCSH 2023.

RAT	RT-PCR		
	Positive	Negative	Total
Positive	60	0	60
Negative	1	59	60
Total	61	59	120
Performance parameters	**Value**		**CI 95%**
Sensitivity	98.36%		(91.20 - 99.96)
Specificity	100%		(93.94 - 100)
PPV	100%		(94.51-100)
NPV	98.33%		(89.41 - 99.76)
Accuracy	99.17%		(95.44 - 99.98)
Kappa	0.983		P=0.0001
SE of kappa	0.017		

NA= not applicable, PPV= Positive predictive values, NNP= Negative predictive values

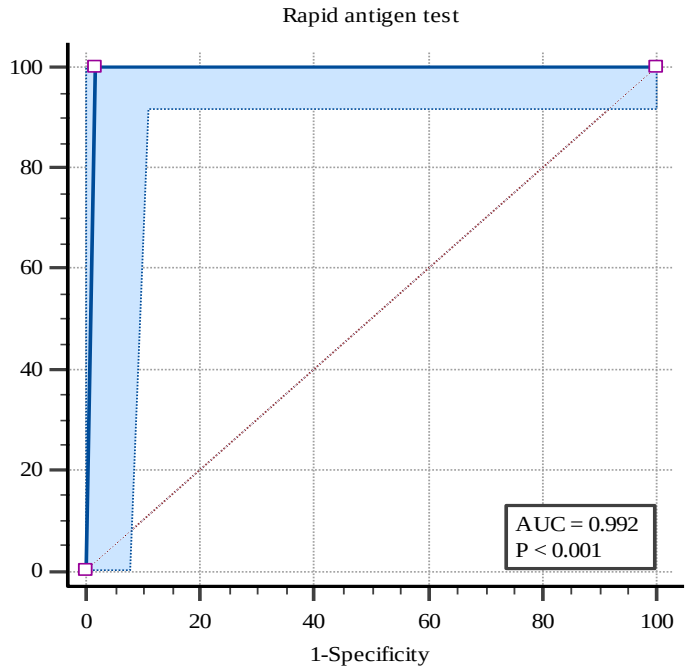

Figure 2. ROC curve constructed for the rapid antigen test for the COVID-19 diagnosis by using the real-time PCR as reference at UoGCSH 2023.

DISCUSSION

The reverse transcriptase PCR remained the gold standard for the diagnosis of COVID-19 in sputum, nasopharyngeal swabs, bronchoalveolar lavage fluid, and nasal oral fluids [23]. It is not a rapid test, has a longer TAT, and also requires specialized laboratory equipment and materials. In addition, it requires trained technicians, so there is a need for a rapid test kit that can be used for screening and diagnosis. As a result, a focus on the use of RAT kits was necessary to overcome diagnostic gaps and mass-screen suspected individuals [24]. In this study, the performance of Panbio™ COVID-19 Ag Rapid Test, an antigen-based rapid test kit, was compared with real-time PCR.

The rapid antigen test kit evaluated in this study (Panbio^{TM}) showed a sensitivity of 98.36% (95% CI: 91.20–99.96) and a specificity of 100% (95% CI: 93.94–100) with a kappa coefficient value of 0.983 (SE: 0.017) (P = 0.0001) and an accuracy of 99.17% (95% CI: 95.44–99.98). The results showed that the RAT kit can be employed at the point of healthcare. The rule of screening tests is to reduce the possibility of finding more false negative reports, particularly in circumstances when an RT-PCR test is not available. However, RT-PCR cannot be replaced with RAT to diagnose and monitor SARS-CoV-2 because of its varying sensitivity and specificity [25]. The sensitivity and specificity results of this study are consistent with the WHO minimum requirements that recommend RAT techniques have at least 80% sensitivity and 97% specificity for COVID-19 detection [26].

This study result is highly consistent with the sensitivity and specificity results of the manufacturer. The sensitivity and specificity of the standard Panbio^{TM} COVID-19 Ag Rapid Test Device for rapid detection of SARS-CoV-2 antigen provided by the manufacturer were 98.1% (95% CI: 93.2–99.8%) and 99.8% (95% CI: 98.6–100.0%), respectively, among symptomatic cases (from a total of 506 nasal swab specimens; positive n = 104; negative n = 404) [20].

The findings of this study are also consistent with the study conducted on COVID-19-suspected individuals in Bangkok, Thailand, with 98.33% and 98.73% of sensitivity and specificity, respectively [15]. However, this study sensitivity result does not agree with the studies conducted in India 81.8% [27] and 61% [28], and Italy 66.82% [29]. Conversely, the diagnostic success specificity result is consistent with the previously mentioned studies conducted in India with 99.6% and 94.4%, and Italy with 99.89%.

The sensitivity, specificity, accuracy, and kappa coefficient of this study are also consistent with the findings from the study conducted in Switzerland which aimed to evaluate the detection of a novel antigen-based rapid detection test with 93.9%, 100%, 96.1%, and 0.9 respectively [30]. The sensitivity performance of RAT using nasopharyngeal samples in this study is significantly higher than the studies conducted in African countries with consistent specificity results in Uganda [31], and Egypt [32].

The specificity of this study result is also consistent with the studies conducted in different areas of Ethiopia. However, the sensitivity of these study results is also not consistent with multiple studies conducted in Ethiopia, Harar 77.5% [33], Addis Ababa 79.4% [34], Wollega 81% [9] and Addis Ababa 61.18%, 74.12% and 83.53% [35]

The 0.983 kappa coefficient value of this study demonstrates complete agreement between the two diagnostic methods since the k value above 0.9 is considered to be almost perfect agreement [36]. The kappa value is also higher than the previous studies, which reported 0.735, 0.80, and 0.81 in Addis Ababa, Wollega, and Harar, respectively.

Earlier studies examined the sensitivity of SARS-CoV-2 antigen testing compared to RT-PCR, leading some public health experts to place less trust in antigen testing than RT-PCR testing in ending the COVID-19 pandemic [27]. However, the findings of this study showed a promising result on the accuracy and applicability of RAT on a mass scale to screen multiple individuals early, at low cost, and in a shorter time in low-income countries like Ethiopia, where molecular analysis is expensive and has limited access.

When it takes longer to use RT-PCR for the identification of infectious individuals, the RAT may be advantageous when optimally deployed. High-frequency testing is also known to drastically minimize the COVID-19 burden by commencing early treatment of cases while limiting the source of infection and transmission in parallel. [37]. In addition, the diagnostic performance values of this study elucidate the advantage and relevance of the RAT to continued use for healthcare at the periphery level in the majority of symptomatic probable cases.

The positive and negative predictive values (PPV and NPV) of this study are 100% and 98.33%, respectively. The predictive values are calculated without taking into account the actual COVID-19 prevalence in the study area. Therefore, it was calculated by estimating the prevalence of 50% given by the online calculator tool. The disease prevalence influences the PPV and NPV of diagnostic tests. As the disease burden rises, so does the PPV, but the NPV decreases. Similarly, as prevalence decreases, the PPV decreases but the NPV increases [38]. The PPV and NPV results are consistent with the studies conducted in Italy 97.87% and 97.62%, respectively [29].

The slight difference in the diagnostic performance of Panbio^{TM} could be attributed to a variety of factors, including the batch of kit reagents, specimen quality in the pre-analytical phases, antigen level, sample handling and processing, and subjective reading of the RAT. The higher sensitivity of this study could also be because nearly all of the specimens were obtained from the upper respiratory tract. The SARS-CoV-2 viral load was found at a higher level in upper respiratory specimens soon after symptoms started [39], implying a larger possibility of positive antigen identification in the early phase. This SARS-CoV-2 antigen testing kit could be used for individuals in the early stages of symptoms when higher viral loads are expected.

In this study, the discrepancy was with the molecular RT-PCR method, with only one false-negative result (1.64%). A single false negative RAT result might be due to the level of antigen is lower than the detection limit of the test. The Ct value of this study participant was 36.3, which is a relatively lower viral load when compared with other study participants. A previous research [40] has demonstrated that lower Ct values reflect higher viral loads, resulting in much higher RAT sensitivity, antigen concentration, and closely associated Ct values .

Moreover, the diagnostic accuracy of our study (99.17%) indicates the outstanding reliability of the RAT to correctly distinguish true positive and true negative results. Despite the diagnostic accuracy of a test is entirely dependent on the disease's prevalence [41], in this study prevalence of the disease was unknown, so estimated 50%. The higher accuracy might be due to higher viral load (low Ct-value) in our study's participant than the studies reported lower sensitivity. The complete specificity (absence of any false positive) result in our study demonstrate the absence of cross-reactivity with other viral or bacterial antigens which shared epitopes structure for the diagnostic immunoassay antibodies.

Moreover, the efficient diagnostic performance values might be due to the inclusion of only symptomatic cases which showed two or more of the most common COVID-19 clinical manifestations. Another cause could be related to testing times, such as in the early or late stages of infection. It has been demonstrated that the viral load is associated with the onset of symptoms, with a high viral load observed during the symptomatic phase. Rapid diagnostic procedures have the highest sensitivity for detecting infection in people who are potentially contagious and would contribute to the virus's transmission [10, 42].

The selection and utilization processes and interpreting test results of the RAT methods should be conducted cautiously. Unless it is done, devastating public health and economic consequences may occur. It has shown that false-positive results can impose a significant economic burden in terms of direct and indirect costs like repeated laboratory tests, inappropriate treatments, and subsequent quarantine. A false negative result, on the other hand, conceals the true prevalence of the disease. Furthermore, misleading negative results promote transmission, potentially increase morbidity and mortality [43].

Limitations of the study

Despite following the diagnostic evaluation sample size guideline, the small sample size might affect the conclusion of this study. This study included only symptomatic patient who presented to the health facility seeking treatment. Because SARSCo-V-2 infection occurs in a high proportion of the population with asymptomatic presentation, caution must be exercised when interpreting RAD test results in study participants. The cycle threshold level was not considered. The duration of illness was not considered because all of the study participants came to the institution after days of self-treatment thinking the illness was common cold.

CONCLUSIONS AND RECOMMENDATIONS

The Panbio™ RAT COVID-19 Ag test demonstrated excellent concordance with the RT-PCR using nasopharyngeal and nasal swab samples from symptomatic patients. Using the gold standard RT-PCR as a reference, the diagnostic performance of RAT demonstrated an almost complete sensitivity and specificity. The rapid antigen tests are a promising method for SARS-CoV-2 detection and infection control. Moreover, our findings suggest a Panbio™ RAT should continue using at the health facilities especially in remote and outbreak locations and the diagnostic performance of this test method should be evaluated on asymptomatic individuals. Additionally, it needs further research and recommends more assessment for level of viral load which demonstrates the lower threshold to be detected by RAT.

DECLARATIONS

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Author's contributions

Debaka Belete: conception and design of the work, data acquisition, analysis, and interpretation, drafting the manuscript, revising the manuscript; BA, GG and NB conception and design of the work, data acquisition and analysis; DB, BA and NB: data interpretation, manuscript revision, and supervision. All authors reviewed the manuscript.

Data availability

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

Ethics approval and consent to participate

We would like to confirm that the study protocol was thoroughly reviewed and approved by the Institutional Review Board of Institute of Biotechnology, University of Gondar Ref. No: IBO/158/12/2022, and an official letter of cooperation's was provided to the UoG-CSH. Written informed consent was obtained from all participants before their inclusion in the study. All the principles of ethics, such as informed consent, confidentiality, and privacy, were maintained. The clinical and laboratory information of the study participants were collected using codes rather than personal identifiers. Study participants who had been suspected of any other infections were linked UoGCSH for further diagnosis and appropriate treatment. We conducted the study following the Declaration of Helsinki [44].

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Competing interests

The authors declare that they have no competing interests in this work.

Abbreviations

ACE-2: Angiotensin Converting Enzyme-2; AUC: Area under the Curve; CI: Confidence Interval; cDNA: Complementary DNA; COVID-19: Corona Virus Disease 2019; Ct: Cycle of Threshold; MERS-CoV. Middle East Respiratory Syndrome Corona Virus; NPV: Negative Predictive Value; ORFlab: Open Reading Frame Lab; PPV: Positive Predictive Value; RAD: Rapid Antigen Diagnosis; RAT: Rapid Antigen Test; SARS-Cov-2: Severe Acute Respiratory Syndrome Corona Virus 2; SOP: Standard Operating Procedure; TAT: Turnaround Time; UoGCSH: University of Gondar Comprehensive Specialized Hospital; VTM: Viral Transport Medium; WHO: World Health Organization.

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