A Comparative Study of Rapid SARS-Cov-2 Antigen Detection Assay against RT-PCR Assay for Diagnosis of COVID-19 in a Tertiary Hospital of Kathmandu

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ABSTRACT

Background

The Coronavirus disease 2019 (COVID-19) pandemic caused by the severe acute respiratory syndrome coronavirus (SARS-CoV-2) has spread worldwide since its first recorded case in the city of Wuhan, China, in December 2019. SARS-CoV-2 infection causes asymptomatic to sever pneumonia. Severe cases may develop acute respiratory disease symdrome (ARDS), with an average mortality rate of 6.9%. Real Time Reverse Transcriptase Polymerase Chain Reaction (rRT-PCR) assay is the current reference standard laboratory method for the diagnosis of SARS-CoV-2 infection. However, it takes around 6-8 hours to get the result and is time consuming. Therefore, rapid and accurate tests for SARS-CoV-2 screening are essential to expedite disease prevention and control. Lateral flow immunoassay using monoclonal anti SARS-CoV-2 antibodies which target for SARS-CoV-2 antigen can be complimentary screening test if their accuracy were comparable to that of the real time reverse transcription-polymerase chain reaction (RT-PCR) assay.

Objective

To find the sensitivity and specificity of a rapid antigentest kit in comparison to reverse transcription-polymerase chain reaction (RT-PCR).

Method

A cross-sectional hospital based study was carried out at Shree Birendra Army Hospital, Kathmandu for a period of four months.

Result

Our finding shows sensitivity and specificity of rapid diagnostic tests (RDT) Ag kit as 60.6% and 96.4% respectively. Positive and negative predictive value was 83.7% and 89.0%. Likewise, positive and negative likelihood ratio was 17.0 and 0.4. The overall accuracy of the antigen kit was 88.1% in comparison to reverse transcription-polymerase chain reaction (RT-PCR) as the gold standard.

Conclusion

Our study concluded the use of rapid antigen kit is mainly useful for screening purposes.

KEY WORDS

RDT Ag Kit, RT-PCR, SARS-CoV-2

INTRODUCTION

In December 2019, severe pneumonia cases of unknown origin were identified from Wuhan, China.[1] This pathogen was initially identified as novel coronavirus(CoV).[2] Then World Health Organization (WHO) named it as Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) and disease as Coronavirus Disease-2019 (COVID-19). Later, WHO declared it as Pandemic on 11th March 2020.[3] SARS-CoV-2 infection causes asymptomatic to severe pneumonia. Severe cases may develop acute respiratory distress syndrome (ARDS).[4]

According to WHO, the crude mortality rate was about 6.9%.[4] At the end of December 2020 according to WHO it had been reported death of 1,813,188 while in Nepal only reported death were 9.[5]

The Real Time Reverse Transcriptase Polymerase Chain Reaction (rRT-PCR) assay is the current reference standard method for the diagnosis, require at least four hours of operation if performed by skilled technicians.[6] Therefore, rapid and accurate tests for SARS-CoV-2 screening are essential to expedite disease prevention and control as well as screening during pre-operative management for invasive procedure. These testing methods require minimum time without requirement for sophisticated laboratory. Thus result into reduction of economic burden in low income country like ours. At the same time skilled technicians are not required. Immunochromatographic techniques using monoclonal anti-SARS-CoV-2 antibodies which targets SARS-CoV-2 antigen can be used as screening test.[7] This test can be complimentary screening test if accuracy were comparable to that of the real time RT-PCR assay.[8]

Therefore, this study was carried out to find the sensitivity and specificity of the Rapid Diagnostic Test (RDT) antigen in comparison to RT-PCR.

METHODS

A hospital based cross-sectional study was conducted among suspected cases of COVID-19 from the month of September to December 2020 at Shree Birendra Hospital Chhauni, Kathmandu, Nepal. The hospital is a tertiary care center and a teaching Hospital of Nepalese Army Institute of Health Sciences (NAIHS). A total of 731 cases attending Acute Respiratory Infection (ARI) clinic of Shree Birendra Hospital were enrolled in this study. All the suspected cases of COVID-19 infection as per WHO guidelinewere included in the study who had visited ARI clinic during first wave of COVID-19 infection in Nepal.[9]

Ethical approval was obtained from Institutional Review Committee of Nepalese Army Institute of Health Sciences (Reg. no. 392). We collected a pair of nasopharyngeal swab from each individual suspected of COVID-19 visiting ARI clinic at Shree Birendra Hospital. Trained and registered laboratory health professionals were employed for the purpose of sample collection. All samples for RT-PCR were collected using a kit containing nylon-flocked nasopharyngeal swab and a tube containing viral transport medium (Nodford International Co. Ltd). For the purpose of antigen testing, sample was collected using nylon-flocked swab that was directly inserted into the diluent provided by the manufacturer. The VTM was transported to the molecular laboratory in ice-box where aliquot was prepared in two cryovial tubes. First one was used for RT-PCR test immediately while the second tube was stored at -80° C for future use.

We measured for the presence or absence of SARS-CoV-2 antigen in samples using BIOCREDITCOVID-19 Ag kit CE-IVD Assay (Cat. No. G61RHA20, RapiGEN INC, South Korea). All assays were performed following manufacturer's protocol. BIOCREDIT COVID-19 Ag is a lateral flow assay that uses the principle of immunochromatography to detect COVID-19 antigen.[10] Briefly, nasopharyngeal swab specimen was inserted into the diluent tube provided by the manufacturer and swirled for 5-10 times. The swab was removed and the tube was closed with a filter cap. Three to four drops of the mixture was applied to the lateral flow device and the result was read out after incubation at room temperature for 5-8 minutes. Sample showing both control line and test line was considered positive for SARS-CoV-2 antigen. Sample showing only one control line was regarded as negative. No invalid results were observed during the study period. Invalid result was described if the control line C did not appear or only test line T appeared.

The SARS-CoV-2 nucleic acid extraction was performed using XABT (Beijing Applied Biological Technologies Co., Ltd.) nucleic acid extraction kit following manufacturer's guideline. Mole Bioscience nucleic acid test kit(Jiangsu Mole Bioscience Co. Ltd.), a fluorescent probe-based RT-PCR assay was used to prepare amplification reaction solution. The kit was able to detect ORF1ab gene, N gene and E gene of SARS-CoV-2 with the given cyclic condition (Table1). The amplification was performed using QuantStudio Real-TimePCR system (Thermo Fisher Scientific). The result was interpreted based on cycle threshold value strictly adhering to the protocol of manufacturer.

Table 1. Cyclic condition for amplification of reaction mixtures into the RT-PCR thermocycler

Step	Cycles	Temperature (°C)	Time (mm:sec)
1	1	55	15:00
2	1	95	00:30
3	5	94 54 72	00:10 00:15 00:20
4	40	94 58	00:10 01:00

The data was primarily entered into MS Excel, which was then transported to Statistical Package for Social Sciences (SPSS) and analyzed using the same SPSS version 20.0 (IBM Armonk, NY, USA). The result of SARS-CoV-2 nucleic acid detection by RT-PCR was used as reference standard to estimate the sensitivity, specificity and other technical performance of BIOCREDIT COVID-19 Ag kit. Chi-square test was employed to test for significance and the p-value less than 0.05 was considered statistically significant for the purpose of this study.

RESULTS

Out of total 731 suspected cases, 440 (60.19%) were male and291 (39.81%) were female. Participant's age ranged from 5 to 74 years. Higher number of them belonged to age group 18 - 60 years (Table2).

Table 2. Demographic characteristics of patientswith SARS-CoV-2 test by different method

		SARS-CoV-2 Antigen		SARS-CoV-2 RT PCR	
		Positive (%)	Negative (%)	Positive (%)	Negative (%)
Sex	Male	79 (64.2)	361 (59.4)	103 (60.6)	337 (60.1)
	Female	44 (35.8)	247 (40.6)	67 (39.4)	224 (39.9)
Age group	< 18 years	2 (1.6)	14 (2.3)	4 (2.4)	12 (2.1)
	18-60 years	56 (45.5)	357 (58.7)	92 (54.1)	321 (57.2)
	≥ 60 years	65 (52.8)	237 (39.0)	74 (43.5)	228 (40.6)

Analytical performance of SARS-CoV-2 antigen kit compared with RT-PCR was as shown (Table 3).

Table 3. Analytical performance of SARS-CoV-2 antigen kit with respect to SARS-CoV-2 RT PCR

	SARS-CoV-2 RNA positive	SARS-CoV-2 RNA negative
SARS-CoV-2 antigen positive	103	20
SARS-CoV-2 antigen negative	67	541

Higher number of both male and female showed cycle threshold value in between 20 – 30. The data shows no significant difference among the compared variables. The sensitivity and specificity of BIOCREDIT COVID-19 Ag kit obtained in this study was 60.6% and 96.4%, respectively. Data shows that the sensitivity of antigen kit increases when the patients has severe infection with higher number of viral particles (low cycle threshold value) and, decreases when the number of viral particles drops (Table 4).

Positive and negative predictive value obtained in the study was 83.7% and 89.0%. Likewise, positive and negative likelihood ratio obtained was 17.0 and 0.4. The overall accuracy of the antigen kit was 88.1% in comparison to RT-PCR as the gold standard (Table5).

Table 4. Characteristics of COVID-19 patients and analytical performance of the antigen kit

Variables		Cycle threshold value			Chi-square	p value
		< 20	20–30	>30		
Sex	Male	34 (61.8)	63 (57.8)	6 (100.0)	4.293	0.117
	Female	21 (38.2)	46 (42.2)	0 (0)		
Age-group	< 18 years	1 (1.8)	3 (2.8)	0 (0)	3.158	0.532
	18-60 years	35 (63.6)	54 (49.5)	3 (50.0)		
	≥ 60 years	19 (34.5)	52 (47.7)	3 (50.0)		
SARS-CoV-2 antigen	Positive	38 (69.1)	62 (56.9)	3 (50.0)	2.574	0.276
	Nega-tive	17 (30.9)	47 (43.1)	3 (50.0)		

Table 5. Technical evaluation of BIOCREDIT COVID-19 Ag test kit

Positive predictive value	83.7%
Negative predictive value	89.0%
Positive likelihood ratio	17.0
Negative likelihood ratio	0.4
Accuracy	88.1%

DISCUSSION

We evaluated the analytical performances of BIOCREDIT COVID-19 Agkit with reference to RT-PCR for SARS-CoV-2 nucleic acid detection as gold Standard. WHO recommends the SARS-CoV-2 antigen or antibody test to have minimum sensitivity of ≥ 80% and specificity of ≥ 97% for use in routine diagnosis.[11] Meanwhile, our study shows sensitivity of 60.6% and specificity of 96.4% for the test kit which is significantly lower as claimed by the manufacturer to be 90.3% and 100% respectively. Such a drop may have been resulted from improper or untimely collection of samples as the onset of symptoms could not been documented as in other studies and is often considered a crucial factor when comparing RDTs with RT-PCR tests. The sensitivity of antigen kit obtained in our study was concordance with study conducted in Pakistan by Saeed et al. (52%) in October 2020 and, the study conducted in China by Diao et al. (68%) for nasopharyngeal samples as the study was done with similar colloidal gold immunochromatography based SARS-CoV-2 Antigen Rapid Test Kit.[12,13] Our results differ from a similar study in Nepal by Shrestha et al. in 2020 which showed sensitivity of 85% and specificity of 100%.[14] The sample size of our study was relatively large in comparison to that of Shrestha et al. that only involved 113 subjects.[14] This might be a possible reason for the variation in data among many other factors.

Only one type of antigen detection kit (BIOCREDIT COVID-19 Ag) was tested due to logistic and financial shortcomings during the period which limits the comparison of our

findings with other superior antigen detection kits. Study by Porte et al has reported 93.9% sensitivity and 100% specificity using Bioeasy Ag kit (FIND,2020).[15] Comparable results were observed in study by Bouassa et al. using SIENNATM Covid 19 Antigen Rapid Test with sensitivity of 90% and specificity of 100%.[16] Such high sensitivity and specificity may be attributed to either low test numbers for comparison or factors like sample collection with a median duration of symptoms of 2 days.

Our study also compared the sensitivity of the kit with RT-PCR, it was found that sensitivity of the kit was 69.1% when Ct value < 20, subsequently sensitivity decreases to 56.9% when Ct value between 20 to 30 and to 50% when Ct value > 30. Lambert-Niclot also reported sensitivity of Ag-RDT as only 50% when compared with RT-PCR.[8] Likewise Gannon et al. also found sensitivity of Ag-RDT between 11.1% and 45.7%.[17] Scohy et al. found sensitivity as 30.2% only among those with high viral load.[18]

The accuracy of the Ag-RDT depends on several factors like way of sample collection, concentration of virus in collected sample, processing of collected specimen and the precise formulation of the reagents in the test kits.[19] The study conducted by Jeewandara et al. on two WHO approve kits also noted overall sensitivity of SD-Biosensor Ag kit was 36.5% and the Abbott Ag kit was 50.76% where as specificity of 99.4% by the Abbott and 97.5% by SD-Biosensor.[20] It has been stated by SARS-CoV-2 antigen-detecting rapid diagnostic tests, an implementation Guide by WHO, Ag-RDT for COVID-19 will be most often be positive when viral loads highest and patients are most infectious-typically 1-3 days prior to onset of symptoms and during the first 5-7 days after the onset of symptoms then subsequently become negative. Therefore, with appropriate timing of sample collection may increase the sensitivity of Ag-RDT kit.

This study has several limitations. The date of onset of symptoms was unknown. Study included only symptomatic patients with suspected SARS-CoV-2 infection and only a single type of kit was used.

CONCLUSION

Despite of low sensitivity observed with the antigen detection kit, the method can be used for screening purpose where RT-PCR facility is not available. The study recommends assessing the quality of antigen detection kits in conjunction with RT-PCR on local population before Implementing them for screening purpose.

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