Sensitivity and Specificity of Lateral Flow Antigen Test Kits for COVID-19 in Asymptomatic Population of Quarantine Centre of Province 3

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ABSTRACT

Background

Nearly after 6 months of the spread of Corona Virus Disease 19, along with the world Nepal is still trying to control the spread and prevent general population from acquiring it. With limited resources in manpower, technology and evidence it has been a difficult battle. But with time and more understanding of the virus new technology to detect the virus are coming up. It is a major breakthrough in the diagnostic field as this helps us in not only detecting the virus but also helps us to mobilize our human resources. This comes in a time where the cases are increasing at an alarming rate. Although numbers of Polymerase Chain Reaction testing have increased but due to the time consuming and the cost wise, we need a faster and equally reliable alternative. Antigen test approved by different countries can be used for point of care, screening and surveillance depending upon the requirements after calculating its sensitivity, specificity and accuracy.

Objective

To find out sensitivity and specificity of the Antigen test kit for COVID-19.

Method

Antigen tests were compared with Reverse Transcription Polymerase Chain Reaction as a reference standard in calculated sample size of 113 subjects in a high risk population. Both Reverse Transcription Polymerase Chain Reaction and antigen test were performed in a same subject with in maximum of 2 days' interval. Convenience sampling technique was used to select the subjects. Ethical approval was taken from Nepal Health Research Council before data collection. Study was done from August to September 2020 from Quarantine center of Province 3.

Result

There were total of 113 test carried out, among those 47 were positive and 66 were negative in Reverse Transcription Polymerase Chain Reaction. After preparing two by two table, Sensitivity and specificity of the tested was calculated which came out to be 85% and 100% respectively, with accuracy of 93.80%.

Conclusion

Even though the sensitivity and specificity came to be higher, this test should be interpreted cautiously depending upon the prevalence of Corona Virus Disease 19 in that particular community and the clinical and epidemiological context of the person who has been tested. When in doubt by clinical correlation should be confirmed with Reverse Transcription Polymerase Chain Reaction.

KEY WORDS

Asymptomatic, Coronavirus Disease 19, Lateral flow antigen test, Sensitivity

INTRODUCTION

In Wuhan city, a new form of viral infection emerged, now referred to as Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Cases of SARS-CoV-2 have been recorded in more than 210 countries.[1] Globally there have been 30,055,710 confirmed cases of Corona Virus Disease 19 (COVID-19).[2] Nepal detected its first case in January 2020 since then there has been a surge in the cases leading to 65,593 confirmed cases nationwide.[3,4] Testing early and detecting the virus and isolating them from the community is equally essential.[5]

Polymerase Chain Reaction (PCR) is the standard testing for COVID-19, it still struggles to reach the rural and remote parts of the country, and it lags behind due to its time-consuming process. A rapid device to facilitate testing outside of laboratory is now necessary. We need a faster and equally reliable alternative. Another advantage of antigen test is its affordability. Objective of this study was to find out sensitivity and specificity of the antigen test kit for COVID-19.

METHODS

An observational diagnostic study was conducted among 113 study subjects who were close contacts of confirmed cases identified through contact tracing residing in quarantine center from August 2020 to September 2020. Ethical Approval letter was obtained from Nepal Health Research Council (Ref No. 2868). On the 5th day of quarantine two nasopharyngeal swabs was collected from same individual by trained lab technician. One of the samples was transported in 3 ml Viral Transport Medium (VTM) and sent to molecular lab for RT-PCR test and other sample from the same individual was processed for the results as instructed by the manufacturing company of antigen kit. At the time of swab collection none of the individuals were symptomatic. Consent was obtained from the participants. Data collection was done from province 3 quarantine, collection was done with the help of nasopharyngeal secretion, both sample for RT-PCR and Antigen RDT. For the detail procedures of nasopharyngeal swab collection, preservation, transportation, performing RT-PCR and antigen RDT, we followed the standard protocol regulated by WHO, instruction manual of company and as per NHTC training regarding sample collection and transport. One hundred and thirteen study subjects were selected using convenient sampling technique. Nasopharyngeal swabs were collected and assay performed following the instructions recommended by the manufacturer (fig. 1).

Results of RDT as the reference method were compared with those of RT-PCR. Tests were repeated for samples with indistinct outcomes. The demographic and clinical data were obtained and analyzed in an anonymous manner from the national contact tracing forms A and B. The statistical analysis considered sensitivity, specificity, PPV, NPV and accuracy. Data collected were entered and analyzed using MS Excel.

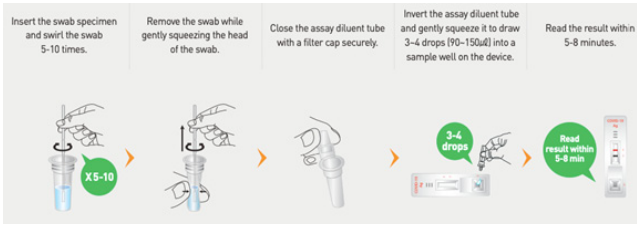

Figure 1. Detailed assay procedure.[6]

RESULTS

Out of 113 asymptomatic participants, 24 were females and 89 males. Participants' age ranged from 13 to 74 years. We found out, among 47 RT-PCR positive cases tested, 7 were negative, 2 weak positive and 40 were Strong positive in Antigen test (Table 1). Those negative cases found had CT value of more than 33, whereas two weak positive cases had CT at the range of 31.

Table 1. Sensitivity, specificity, true positive, false positive, true negative and false negative results of the diagnostic test.

	PCR (Positive)	PCR (Negative)	Total
Antigen (Positive)	40 (TP)	0 (FP)	40
Antigen (Negative)	7 (FN)	66 (TN)	73
Total	47	66	113

Our result showed 85% sensitivity, 100% specificity, 100% positive predicted value, 100% Negative predicted value, and 93.80% accuracy.

DISCUSSION

Sensitivity ranged greatly throughout research studies (from 0 to 94%): 56.2% was the average sensitivity (95% CI 29.5 to 79.8%) and 99.5% was the average specificity (95% CI 98.1% to 99.9%; based on 8 assessments on 943 samples in 5 studies). However, our study shows sensitivity of 85% and specificity of 100% with BIOCREDIT.[7]

Hardly any test gives a 100% accurate result; tests need to be tested, preferably by comparison with a "gold standard," to assess their accuracy. The absence of such a consistent "gold standard" for COVID-19 testing makes it difficult to evaluate the accuracy of the test. Nevertheless, in comparison with RT-PCR as standard test, accuracy of the given antigen test came out to be 92.80%.[8]

In this study we took RT-PCR of the asymptomatic COVID-19 cases as the gold standard test to compare with but in real absence of a clear-cut "gold standard" is a difficult task for assessing COVID-19 tests; idealistically, clinical judgment could be the best available "gold standard" based on repeat swabs, history, and contact cases known to have SARS-CoV2, chest X-rays, and chest CT.[9]

The performance of the tests depends on a number of factors, such as the time since the onset of the disease, the concentration of the virus in the sample, the quality of the sample collected from the individual and how it is analyzed, and the exact specification of the reagents in the test kits. The sensitivity of these tests could be expected to vary from 34% to 80% depending on knowledge with antigen-based RDTs for other respiratory diseases such as influenza, in which affected patients have comparable influenza virus concentrations in respiratory samples as seen in COVID-19.[10]

Study done by Wang et al found out sensitivity of RT-PCR in 205 patients varied, at 93% for broncho-alveolar lavage, 72% for sputum, 63% for nasal swabs, and only 32% for throat swabs. Here in our study we took nasopharyngeal swab which shows sensitivity of 85% and specificity of 100% in comparison to that of RT-PCR.[11]

A study from China (with the manufacturer's participation) found an overall sensitivity of 68%; however, the sensitivity increased to 98% for samples with Ct values less than or equal to 30.[12] This excellent rate of identification in high viral load specimens (CT < 25) was confirmed in this report and in our recently published study.[13]

With high viral load samples, the RapiGen test also demonstrated an acceptable sensitivity (84.9%), but was much less sensitive (15.4%) when the viral load was low. This test had a visual readout that could have led to lower sensitivity. In two European studies, another visual band assay (Respi-Strip CORIS) was recently evaluated. Overall sensitivity ranged from 50% to 57.6%; however, detection rates improved for samples with high viral loads (CT < 25) reaching sensitivities of 73.9% to 82.2%.[14,15]

Depending on which gene targets are used, and whether multiple gene tests are used in combination, higher sensitivities are reported. 'E' and 'ORF' were the target genes for PCR in this study.[16,17]

Reported accuracies are much higher in one vitro study conducted by Corman VM, which measures the performance of primers using the coronavirus cell culture. However, in this study we have taken RT-PCR as the standard test that could have reduced the accuracy which is one of the limitations of this study.[18]

The accuracy may also vary depending on the disease stage and the extent of viral multiplication, in this study all participants were asymptomatic which can be correlate as low viral load condition.[19,20]

Huge knowledge gaps identified in a study highlight the immediate need for commercialized an-tigen test comparative studies.[21] A possible explanation of the variations in performance could be linked to differences in protein targets. However, only a minority of manufacturers report details regarding the detection system.[22]

In the procurement of simpler, scalable diagnostic tests, quality information is valuable for local decision-making. Although these tests are less sensitive than RT-PCR, when timely results are important but access to molecular testing is limited, they could be useful during pandemic situations.[23]

For the implementation of novel RDT-based algorithms, which are particularly important in poorer health systems and low-resource settings such as Nepal, the potential to detect early infections could be crucial. Possible frontline applications include testing based on the community.[24]

However, large-scale strategies need to be well designed to avoid negative effects in light of the imperfection of tests. Another application could be as an adjunct to RT-PCR to achieve quick preliminary results, e.g. for healthcare professionals, recruit police officers, mass screening, institutional management etc.[25] We have used only one antigen RDT company. Comparison with another company would have definitely shed more light on antigen testing. But due to unavailability and lack of financial resources we were not able to do so. But if we can choose brand according to the availability and resource for research study, it is important to note that the CE licensing process if it is based on manufacturers' self-reporting, which does not provide high performance, and may be misused.[26,27]

CONCLUSION

The antigen-based RDT evaluated here showed a high sensitivity and specificity in nasopharyngeal samples obtained from study subjects who were close contacts of confirmed cases COVID-19 staying in quarantine. The assay was easy to use and provided results in a timely manner which can be utilized in hospital for triaging asymptomatic patients who needs emergency surgery along with mass screening purpose and institutional system COVID-19 management. Hence, it has the potential to become an important tool for the early diagnosis of SARS-CoV-2, particularly in situations with limited access to molecular method.

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