Evaluation of COVID-19 Antigen Fluorescence Immunoassay Test for Rapid Detection of SARS-CoV-2

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Abstract

Introduction: Tests detecting SARS-CoV-2-specific antigen have recently been developed, and many of them are now commercially available. However, the real-world performance of these assays is uncertain; therefore, their validation is important. In this study, we have evaluated the performance of STANDARD F COVID-19 antigen fluorescence immunoassay (FIA) kit. **Methods:** Nasopharyngeal samples collected from patients were subjected to the test as per manufacturer's instructions. The performance of the kit was compared with the gold standard real-time polymerase chain reaction. **Results:** A total of 354 patients were tested with STANDARD F COVID-19 antigen FIA test kit. The overall sensitivity, specificity, positive predictive value, and negative predictive value of this test were found to be 38%, 99%, 96.2%, and 72%, respectively, with a diagnostic accuracy of 75.7%. **Conclusion:** STANDARD F COVID-19 antigen FIA showed high specificity and positive predictive value but low sensitivity and negative predictive value.

Keywords: Analyzer, COVID-19, fluorescence immunoassay, rapid antigen test, SARS-CoV-2

Introduction

The coronavirus pandemic has infected more than 13.8 million people and killed more than 590,000 worldwide since late January 2020, when this disease was first reported.[1] The enormous gap between the large number of patients or contacts and the laboratory capacities to perform real-time polymerase chain reaction (RT-PCR) in a timely manner is a major challenge to the current public health containment strategies.[2] Antibody tests in the market that could potentially indicate a person's immunity have been unreliable so far.[1] Therefore, there is a need for alternative assays such as antigen detection tests, which, in contrast to antibody tests, can detect the presence of the virus.

SARS-CoV-2 are single-stranded RNA viruses belonging to genera betacoronavirus.[3,4] Antigen tests for SARS-CoV-2 detect or can quantify the nucleocapsid (N) or spike (S1 and S2) proteins of the virus. S1 seems to be the most variable antigen, making it a good candidate to differentiate between other coronaviruses.[5,6] However, the S2 subunit shares similarity in antibody epitopes (region of an antigen recognized by an antibody) with S2 from the original SARS-CoV. Nucleoprotein (N) is the most abundant viral protein produced and shed during infection with SARS-CoV-2.

In this study, we evaluated STANDARD F COVID-19 Ag fluorescence immunoassay (FIA) kit which is based on fluorescent immunoassay for the qualitative detection of specific nucleoprotein antigens to SARS-CoV-2 present in human nasopharynx.

Methods

This cross-sectional study was conducted at a tertiary care centre in North India. The study was conducted from June 2020

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to August 2020 for validation of the kit. Samples of all patients suspected of COVID-19 were tested. A total of 354 patients were included in the study, of which 259 (73.1%) were from the Employee Health COVID Screening (EHS) Outpatient Department (OPD) and 95 (26.8%) were from the COVID wards who were admitted for treatment.

Principle

The SARS-CoV-2 antigen in an infected patient's specimen reacts with europium-conjugated monoclonal anti-SARS-CoV-2 antibody in the conjugation pad of test device and forms antigen-antibody fluorescence antibody particle complex. This complex moves along the membrane to be captured by the anti-SARS-CoV-2 antibody on the test line and make fluorescence signal. The intensity of the fluorescence light is scanned by the STANDARD F Analyzer which analyses the specimen by processing the results using preprogrammed algorithms and displays the test result on the screen.[7]

Procedure

Under all precautions mentioned in the guidelines,[7] sample was collected from the nasopharyngeal area and well squeezed into the extraction buffer. The nozzle provided was used to apply 4 drops of the extracted specimen to the well in the test device. The device was then incubated for 30 minutes at normal temperature. Thereafter, the test device was inserted to the test slot of the analyzer. The analyzer automatically scanned the device and displayed the result within two to five seconds. The result was interpreted as positive when the cutoff index (COI) value was greater than equal to1.0 and negative when it was less than 1.0. The COI is the numerical representation of the measured fluorescence signal.

RT-PCR was taken as the gold standard and was simultaneously conducted in the Virology Laboratory at the same centre

Statistical analysis

The collected data were analyzed with IBM SPSS statistics for Windows, Version 23.0 Armonk, NY: IBM Corp.

Results

A total of 354 patients were tested with this antigen kit. Fifty-four (15.2%) were positive and 300 (84.7%) were negative with this test. Table 1 depicts the table for evaluation.

With RT-PCR, 136 (38.4%) were positive and 218 (61.5%) were negative. Out of 259 patients tested in EHS OPD, 30 were antigen positive while 41 were positive by PCR, the percentage positivity being 73.1%. From COVID ward, 24 out of 95 were antigen positive, percentage positivity being 25.2%. The overall sensitivity, specificity, PPV, and NPV of this antigen test when compared to the RT-PCR was found to be 38%, 99%, 96.2%, and 72%, respectively, with a diagnostic accuracy of 75.7%. Table 2 shows the performance of the COVID-19 antigen FIA test against the RT-PCR.

Figure 1 depicts the receiver operator curve, showing low sensitivity of the test.[8]

Table 1: Result of antigen fluorescence immunoassay test against real-time polymerase chain reaction

COVID-19 antigen FIA with RT-PCR			
Antigen FIA	RT-PCR		Total
	Positive	Negative	
Positive	52	2	54
Negative	84	216	300
Total	136	218	354

FIA: Fluorescence immunoassay, RT-PCR: Real-time polymerase chain reaction

Table 2: Performance of COVID-19 Ag fluorescence immunoassay against real-time polymerase chain reaction

COVID-19 Ag FIA	Values (%)	95% CI (%)	
		LB	UB
Sensitivity (%)	38	30	46.9
Specificity (%)	99	96.7	99.8
PPV (%)	96.2	86.5	99.0
NPV (%)	72	69.2	74.5
Accuracy (%)	75.7	70.8	80.0

CI: Confidence interval, UB: Upper bound, LB: Lower bound, PPV: Positive predictive value, NPV: Negative predictive value, FIA: Fluorescence immunoassay

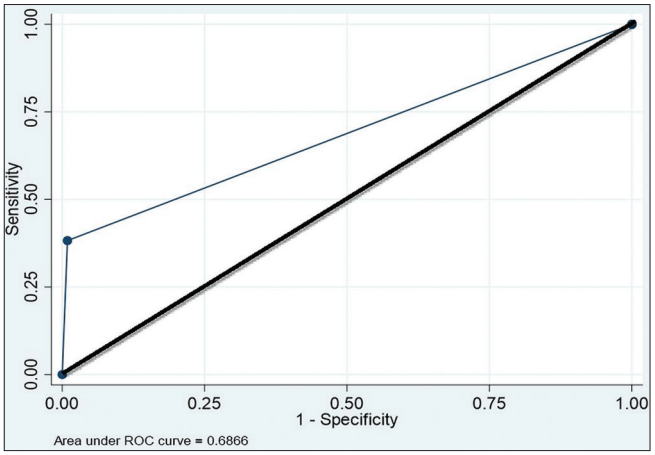

Figure 1: Receiver operator curve: Performance of antigen fluorescence immunoassay test

Discussion

At present, the standard and formative assessment of diagnosis of COVID-19 is high-throughput sequencing or an RT-PCR assay.[9] However, these methods require sophisticated laboratory infrastructure and are expensive.

Various tests detecting SARS-CoV-2-specific antigen have been developed, and many of them are now commercially available.[10] In our study, we evaluated the STANDARD F COVID-19 Ag FIA against the gold standard RT-PCR for its validation. The FIA reader gave an actual reading as a printout, making the read-outs more objective as compared to the immunochromatographic tests where faint bands may be

difficult to read. The percentage positivity of tests conducted at EHS OPD (73.1%) was higher compared to the tests done on patients admitted due to COVID 19 in the ward (25.2%). The reason might be; that in the ward, recovering patients' antigen amount would have decreased and this would have lead to the low positivity rate. As this rapid antigen test provides immediate results at relatively low cost with less expertise and without any need of sophisticated infrastructure; hence it can be useful where congregate settings need to be screened such as containment zones, airports, railway stations, long-term care facilities, workplace, school, and remote places.

A similar study based on fluorescence immunochromatographic SARS-CoV-2 antigen test (Bioeasy 54 Biotechnology Co., Shenzhen, China) was done at Santiago, Chile, which exhibited an overall sensitivity and specificity of 93.9% (confidence interval [CI] 95%: 86.5–97.4) and 100% (CI 95%: 92.1–100), respectively, with a diagnostic accuracy of 96.1%.[11] This study also emphasized on the need of immediate test after the onset of symptoms.

Limitation

This test utilizes a small battery or electricity operated reading device, which might not be an ideal bedside emergency test. Samples will have to be brought to a central point where the reader is installed. Antigen test positivity is high only when tested in early stages of infection when viral load is high. Due to its low sensitivity, validation of this test kit is definitely questionable and the qualitative fluorescence method needs improvement for better accuracy in the coming days.

Research quality and ethics statement

This study was approved by the Institutional Review Board/ Ethics Committee approval number IEC 668/03.07.2020. The authors followed applicable EQUATOR Network (http://www.equator-network.org/) guidelines during the conduct of this research project.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Available from: https://www.bloomberg.com/graphics/2020-coronavirus-cases-world-map/Mapping the Coronavirus Outbreak across the World. [Last updated on 2020 Jul 18].
2. World Health Organisation. Co-ordinated Global Research Roadmap. Available from: https://www.who.int/who-documents-detail/a-coordinated-global-research-roadmap, 307 2020a (20). [Last accessed on 2020 May 15].
3. Chen Y, Liu Q, Guo D *et a/*.Emerging coronaviruses: Genome structure, replication, and pathogenesis. J Med Virol 2020;92:418-23.
4. Ou X, Liu Y, Lei X, Li P, Mi D, Ren L, *et al*. Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV. Nat Commun 2020;11:1620.
5. Lee CY, Lin RT, Renia L, Ng LF. Serological approaches for COVID-19: Epidemiologic perspective on surveillance and control. Front Immunol 2020;11:879.
6. Ahmed SF, Quadeer AA, McKay MR. Preliminary identification of potential vaccine targets for the COVID-19 coronavirus (SARS-CoV-2) based on SARS-CoV immunological studies. Viruses 2020;12:254. Published online 2020 Feb 25. doi: 10.3390/v12030254
7. Instructions for Using STANDARD F COVID-19 Ag FIA Test. Available from: http: sdbiosensor.com/xe/product/7662. [Last accessed on 2020 Nov 09].
8. Hoffman Julien IE. Basic Biostatistics for Medical and Biomedical Practitioners. 2nd ed. p. 330, ISBN 978-0-12-817084-7.
9. National Health Commission of the People's Republic of China. New Coronavirus Pneumonia Prevention and Control Program (Trial Version 7th ed.). Available from: http://www.nhc.gov.cn/yzygj/s7653p/202003/46c9294a7dfe4cef80dc7f5912eb1989.shtml. [Last accessed on 2020 Apr 04].
10. Foundation for Innovative New Diagnostics. FIND SARS-CoV-2 Diagnostics: Performance Data; 2020. Available from: https://www.finddx.org/covid19/dx-data. [Last accessed on 2020 May 21].
11. Porte L, Legarraga P, Vollrath V, Aguilera X, Munita JM, Araos R. Evaluation of a novel antigen-based rapid detection test for the diagnosis of SARS-CoV-2 in respiratory samples. Int J Infect Dis 2020;99:328-33.