

Evaluation of a rapid diagnostic test for detection of SARS-CoV-2 antigen in nasopharyngeal swabs

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Summary

Background and aims: Rapid and accurate diagnosis is essential to limit the spread of SARS-CoV-2 and for patient's management. Currently, real-time reverse transcription polymerase chain reaction (RT-PCR) is the recommended laboratory test to detect SARS-CoV-2. However, the requirement of special instruments and skilled personnel have limited the use of this technique. Recently, several rapid antigen detection tests have been devel-

oped and used as frontline. The aim of this work was to assess the performances of STANDARD F COVID-19 Ag FIA Kit, a rapid fluorescence immunoassay for the detection of SARS-CoV-2 nucleoprotein antigens, in comparison to RT-PCR.

Materials and methods: Twenty-three nasopharyngeal swabs were collected and tested.

Results: Among the 20 positive RT-PCR samples, 9 were detected by the immunofluorescence assay, reporting an overall sensitivity of 45%. The sensitivity increased to 64% in the case of a high viral load, where all three target genes, RdRp, N, and E, were detected by RT-PCR.

Conclusions: A better antigen detection rate is associated with low Cycle threshold values which are inversely related to the viral load. STANDARD F COVID-19 Ag test cannot be considered as the frontline assay for COVID-19 diagnosis, but it might be used in association with clinical signs of patients to reduce the number of RT-PCR testing.

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Introduction

The three epidemic outbreaks caused by human coronavirus (hCoV), *i.e.* SARS-CoV, MERS-CoV and SARS-CoV-2, emerging at the beginning of the 21st century, have highlighted the need for fast and accurate diagnostic assays. Initially reported in China, the last human coronavirus disease (COVID-19) caused by SARS-CoV-2 has spread rapidly around the world and it has become a major public health (1,2). Early diagnosis is crucial for the detection of COVID-19 infected subjects in order to control and limit the outbreak (3). The current gold-standard assay for the detection of SARS-CoV-2 is based on viral RNA amplification by using reverse transcription PCR (RT-PCR) (4). This test requires an average execution time of 2/3 hours, except for GeneXpert system (Cepheid 904, USA) which can provide a result in 50 minutes, but it needs skilled personnel and it is expensive. Therefore, the evaluation of immunological diagnostic assays which can detect SARS-CoV-2 antigens at lower costs compared to molecular tests, might be helpful for a rapid and accurate diagnosis of COVID-19. Currently, there are different immunological tests that can detect SARS-CoV-2 nucleoprotein antigens. These assays are performed by using different methodologies, as enzyme-linked immunosorbent assay (ELISA), chemiluminescence immunoassay (CLIA), colloidal gold immunochromatography and fluorescent immunoassays (FIA) (3,5,6). The aim of this study was to assess the performances of commercial STANDARD F COVID-19 Ag FIA Kit in comparison to molecular test for laboratory detection of SARS-CoV-2.

Materials and Methods

Clinical specimens

We collected 23 nasopharyngeal (NP) swabs from symptomatic subjects admitted to the Monzino IRCCS Cardiological Centre, Milan, Italy, between March and July, 2020. All samples were obtained using flocked swabs with Universal Transport Medium (UTM) (Copan Diagnostics, Murrieta, CA, USA).

One-step RT-PCR

NP swabs were processed using ELITE InGenius® instrumentation (Elitech Group, France). Briefly: the first step required the extraction of the viral RNA with an ELITE InGenius® SP200 kit starting from a UTM aliquot of 200 µL, and the second step involved the detection of the viral genome. The amplification was performed using GeneFinder™ COVID-19 Plus RealAMP Kit (OSANGHealthcare, Korea) which included probes that amplify three target genes of SARS-CoV-2, RNA-dependant RNA polymerase (RdRp) gene, envelope (E) gene and nucleocapsid (N) gene. Ribonuclease P (RNase P) was used as internal control (IC). Samples with SARS-CoV-2 RT-PCR Cycle threshold value (Ct) under 43 were considered positive, according to the manufacturer's instructions. The expected time of execution is about 3 hours.

Rapid SARS-CoV-2 antigen detection test

STANDARD F COVID-19 Ag FIA Kit (SD BIOSENSOR, Inc., Republic of Korea) is a ready-to-use test which allows rapid and qualitative detection of SARS-CoV-2 nucleoproteins in nasopharyngeal swab specimens. This test, based on immunofluorescence technology, uses europium conjugated monoclonal antibody to detect SARS-CoV-2 nucleoprotein antigens. The test was carried out according to manufacturer's instruction: i) the nasopharyngeal swab specimen was inserted in an extraction tube buffer and then swirled at least five times; ii) after removing the swab, 4 drops (approximately 100 µL) of mixed sample were added in the test device. When the sample come into contact with the strip, passive diffusion allows the sample to migrate and react with the anti-SARS-CoV-2 antibodies immobilized onto the membrane and make fluorescence signal. A control line is included in the strip to assess the correct migration

of the sample. The interpretation of the result is performed after 30 min by the STANDARD F200 Analyzer (SD BIOSENSOR, Inc., Republic of Korea), which provided a COI value (Negative <1).

Statistical analysis

Analyses were performed using SAS software package (Version 9.4 SAS Institute Inc., Cary, NC). Continuous variables are presented as mean ± standard deviation (SD) and categorical variables as absolute numbers and percentages. Correlations between the results obtained by STANDARD F COVID-19 Ag FIA and those using GeneFinder™ COVID-19 Plus RealAMP were determined by Spearman's rank test. Receiver Operating Characteristic (ROC) curves were calculated and the area under the ROC curve (AUC) with 95% confidence interval (CI) was used to compare the ability of the STANDARD F COVID-19 Ag FIA test to confirm GeneFinder™ COVID-19 Plus RealAMP results. Sensitivity, specificity, positive and negative predictive values (PPV, PPN) were calculated to assess the performance of STANDARD F COVID-19 Ag test with cut-off = 1 (COI, cut-off index).

Results

We collected 23 nasopharyngeal swab specimens. The median age of the study population was 56 years, with a sex ratio of 3.6 (men to females). According to the molecular assay results, 20 samples were positive and 3 negative, with median Ct values of 38 (range: 24-46), 33 (range: 21-46), and 35 (range: 20-46) for RdRp, N, and E genes, respectively. The results obtained by molecular assay were compared with those obtained by STANDARD F COVID-19 Ag antigenic test. Amongst the 23 samples, 12 specimens had concordant data with nine positive results and three negative results obtained with both detection methods. Discordant results with positive RT-PCR and negative STANDARD F COVID-19 Ag FIA assay were observed for 11 samples (55%) (Table 1). Sensitivity and specificity of STANDARD F COVID-19 Ag FIA detection test were 45% (95% CI, 23 to 67) and 100 %, respectively; positive predictive value (PPV) was 1 and negative predictive value (NPV) was 0.21 (95% CI, 0 to 0.43). The area

Table 1. Results of STANDARD F COVID-19 Ag FIA Kit compared to RT-PCR in the samples tested.

STANDARD F COVID-19 Ag FIA Kit	GeneFinder™ COVID-19 Plus RealAMP Kit		Total (n)
	Positive (n)	Negative (n)	
Positive	9 (45%)	0 (0%)	9
Negative	11 (55%)	3 (100%)	14
Total	20	3	23

Table 2. Results of the STANDARD F COVID-19 Ag FIA Kit compared to RT-PCR in the samples tested according to target genes detection.

STANDARD F COVID-19 Ag FIA Kit	N (%)	GeneFinder™ COVID-19 Plus RealAMP Kit: target genes (n)			Total
		N + E (%)	RdRp + N + E (%)	Negative (%)	
Negative	5 (100)	2 (50)	4 (36)	3 (100)	14
Positive	0 (0)	2 (50)	7 (64)	0 (0)	9
Total	5	4	11	3	23

RNA-dependant RNA polymerase (*RdRp*) gene, envelope (*E*) gene and nucleocapsid (*N*) gene.

under the curve was 0.85 (95% CI 0.76-0.94, $p<0.0001$). Out of the eleven discordant samples resulted positive using RT-PCR, four were positive for the detection of all three target genes RdRp, N, and E, two were positive for the detection of target genes E and N, and five for only the target gene N (Table 2). On the other hand, among the nine concordant specimens, two samples were positive for the detection of target genes N and E (median Ct of 33 and 35, respectively), and seven for the detection of all three target genes RdRp, N, and E (median Ct of 25, 23 and 22, respectively). Comparing the results between the two assays, we noted that a positive rapid test result was obtained if more target genes were detected by RT-PCR. Particularly, if the RdRp target gene was detected, the concordance increased up to 64% (Table 2).

We performed a correlation analysis between rapid test results and cycle thresholds of target genes RdRp, E, and N. The samples detected as positive by the rapid antigenic assay fully correlated with the molecular results, which presented Ct RdRp, N and E values less than 30 (Figure 1). No negative results were obtained by the molecular test with Ct target gene values less than 30. Eleven samples resulted negative using the rapid antigenic assay but positive using the molecular test presented median Ct values of 42, 35, and 40 for RdRp, N, and E genes, respectively.

Moreover, we analyzed the correlation between COI values of rapid test and cycle thresholds of target genes RdRp, E, and N. COI values greater than 1 considered as positive by antigenic test fully correlated with the molecular positive results, which presented Ct RdRp, N and E values less than 30. Eleven samples resulted negative using the rapid antigenic assay (median COI values of 0.43) but positive using the molecular test presented median Ct values of 42, 35, and 40 for RdRp, N, and E genes, respectively. The three samples resulted negative using the molecular test

(median Ct values of 46) presented a median COI value of 0.33 using the rapid antigenic test.

Discussion

In the current pandemic context of COVID-19, diagnostic testing for SARS-CoV-2 is necessary to identify positive subjects among the population and limit the spread of the virus. An accurate and rapid diagnosis is followed by a correct management of the infected subjects. Several companies have developed rapid tests for both antibodies and antigens detection of SARS-CoV-2 (3). The fluorescent immunoassay STANDARD F COVID-19 Ag FIA might represent one of the aforementioned assays for the laboratory detection of SARS-CoV-2 nucleoprotein antigens. Although this test presents several advantages, such as the ease and speed of execution, the reduced impact on instrumentation, the lower cost, the absence of skilled personnel, compared with molecular assays, data showed here suggested that this immunofluorescent assay is suffering from poor sensitivity. This rapid test detected SARS-CoV-2 nucleoprotein antigens in nasopharyngeal specimens with Ct less than 30, a result that is presumably related to a high viral load, but the sensitivity decreases in the case of the detection of target genes with Ct greater than 30, most probably due to a low viral load. In our study, the specificity was 100 %, while the overall sensitivity of STANDARD F COVID-19 Ag FIA was 64% if we considered the positive samples presenting the molecular detection of all target genes. This low sensitivity was already observed in other rapid diagnostic tests for SARS-CoV-2 and in those during Influenza A (H1N1) pandemic (7-9). A limit of the study is that negative samples

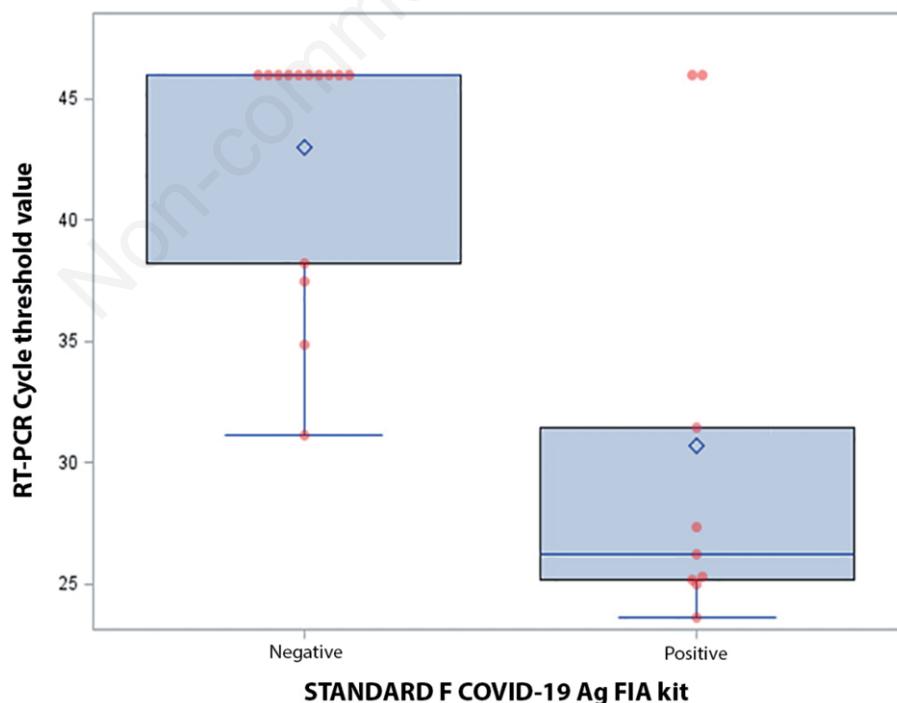


Figure 1. RT-PCR Cycle threshold of RdRp gene for STANDARD F COVID-19 Ag positive and negative tests in studied samples. Values shown are median (Blue line at the top of box in negative result, blue line within box in positive result), 25th and 75th percentiles (bottom and top of box, respectively), and mean (open diamond). Red circles represent the Ct values of samples. Similar results were obtained for N and E target genes (data not shown).

are only three. A larger number of subjects are needed in order to verify and improve our results.

Conclusions

Our data showed that the STANDARD F COVID-19 Ag test cannot be considered as a frontline assay to rule-out negative subjects, but it might be used for rapid SARS-CoV-2 detection in endemic area and following European Centre for Disease prevention and Control (ECDC) and Istituto Superiore Sanità (ISS) guidance's on the appropriate use of antigenic tests both for people with or without symptoms (10,11).

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