Research Article

iMedPub Journals
www.imedpub.com

Health Science Journal
ISSN 1791-809X

2021
Vol. 15 No. 11: 903

DOI: 10.36648/1791-809X.15.11.903

Evaluation of a Rapid in Vitro Diagnostic Test Device for Detection of *SARS-CoV-2* Antigen in Nasal Swabs

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Citation: Prost M (2021) Evaluation of a Rapid in Vitro Diagnostic Test Device for Detection of *SARS-CoV-2* Antigen in Nasal Swabs. Health Sci J. 15 No. 10: 903.

Abstract

Objective: The aim of this work was to explore reliability and performance of an antigen rapid test device on random clinical specimens routinely collected for *SARS-CoV-2* diagnosis.

Method: Run a rapid in vitro diagnostic test device for detection of *SARS-CoV-2* antigen in nasal specimens taken among those routinely analyzed by RT-PCR in two different sites.

Results:

Site 1: Concordance for *SARS-CoV-2* negative results was 100% between Ag rapid test and RT-PCR.

Concordance for *SARS-CoV-2* positive results was 91% in our series (Ct range from 11 to 32). Concordance for positive results was 97,8% if we consider only specimens with Ct<25.

Site 2: The test results of RT-PCR reagent and the COVID-19 Antigen Detection Kit are summarized as follows: The sensitivity of the positive sample was 97.3%, and the specificity of the negative sample is 99.5%. The total accuracy achieved 98.3%.

Conclusion: Rapid Ag test devices are convenient devices to aid in the rapid diagnosis of *SARS-CoV-2* infections. Molecular diagnosis based upon viral RNA amplification is better because of its lower limit of detection. Specimens should be positive by rapid Ag testing if viral burden corresponds to Ct of around 28 or less by RT-PCR. This is very frequent with virus-producing patients. As this device targets N Ag, molecular variations within S gene did not influence performance of the test.

Keywords: Respiratory infection; *SARS-CoV-2*; Ag rapid test; COVID-19

Received with Revision on: November 26, 2021, **Accepted:** December 10, 2021, **Published:** December 17, 2021

Introduction

There is currently a worldwide pandemic caused by the "*SARS-CoV-2*" virus. The severe acute respiratory disease it causes has been named "Coronavirus Disease 2019" (COVID-19).

The reference method for the biological diagnosis of severe acute respiratory syndrome coronavirus 2 (*SARS-CoV-2*) is based on the detection of nucleic acids of the potential virus in respiratory specimens by a molecular biology method called RT-qPCR (real-time reverse transcription polymerase chain reaction). RT-qPCR, commonly used worldwide remains the gold standard for detecting *SARS-CoV-2* infection. However, this method requires specialized laboratory equipment and training and often takes a few hours [1].

SARS-CoV-2 antigens are generally detectable in upper respiratory specimens during the acute phase of infection. Rapid antigen tests have been developed for *SARS-CoV-2* antigens detection in the clinical specimens. These tests are easy to use, not expensive, can be used as near-patient tests and give rapid results (10-30 min) [2,3].

Material and Method

Site 1

Specimens were taken among those routinely analyzed by RT-PCR for *SARS-CoV-2* diagnosis during September and October 2021 at the Virology Laboratory of "Dijon Bourgogne University Hospital" (France) [4].

The specimen was added in the extraction buffers contained in the antigen-test kit. Extracted samples were then added to

the test cassettes and migrated according to manufacturer's instructions. Results were read and registered before the limit of timing (15 min).

Specimens were as follows:

- 100 PCR negative for *SARS-CoV-2*
- 100 PCR positive for *SARS-CoV-2*.

The routine diagnosis has been conducted by RT-PCR (Thermo Fisher Scientific®/Applied Biosystems® TaqPath™ COVID-19 CE-IVD RT-PCR Kit catalog ref. A48067) targeting S (spike glycoprotein), N (nucleocapsid) and Orf 1ab (Open reading frame) genes [5].

The Antigen test device was *SARS-CoV-2* Antigen Rapid Test (Nasal Swab) (AllTest®, China). This test is a qualitative membrane-based immunoassay for the detection of SARS-CoV-2 nucleocapsid protein antigens in swab specimen. The test result is obtained in no more than 15 minutes.

Site 2

Total 420 Nasal swab samples (220 positive samples and 200 negative samples) collected from COVID-19 suspected participants are tested by a SARS-CoV-2 antigen rapid test, namely SA*RS-CoV-2* Antigen Rapid Test (Nasal Swab) by Hangzhou AllTest Biotech Co., Ltd, and Nasophaiyngeal swab specimens were tested by a COVID-19 RT-PCR detection reagent, namely the Vitassay qPCR *SARS-CoV-2* by Vitassay Healthcare S. L. U. (Spain) as the reference. The sensitivity, specificity, and total accuracy of the *SARS-CoV-2* Antigen Rapid Test (Nasal Swab) are calculated using COVID-19 RT-PCR detection reagent as the reference method.

Operating procedures for RT-PCR reagent and the COVID-19 Antigen Detection Kit are strictly followed to the instructions for use by manufactures. Positive controls and negative controls are included in every test run to monitor the potential risk of false positive and false negative results.

Results and Discussion

Site 1

The 100 samples negative for *SARS-CoV-2* by RT-PCR were all negative by the Alltest Ag test. Concordance for *SARS-CoV-2* negative results was then 100%.

RT-PCR Thermo gave positive results on 100 specimens. The highest the Cycle threshold (Ct), the highest the chance for an Ag rapid test to be negative, but also the lowest for the patient to be contagious [6]. Ct ranged from 11 to 31 for S target except for 2 specimens that did not respond to target S due to a mutation (deletion 69-70). Ct ranged from 11 to 32 for N target. Ct ranged from 11 to 31 for Orf1ab target. For 92 specimens, all the 3 Ct were <26.00.

A sample is considered positive for *SARS-CoV-2* if it is positive for at least two of the three RT-PCR targets studied. Among the 100 specimens *SARS-CoV-2* positive by RT-PCR, 9 gave negative results with the Alltest Ag test. All their Ct were ≥ 24.00, except for one wild type (S Ct=21, N Ct=23, Orf1ab Ct=23) and for one del69-70 variant (S negative by RT-PCR, N Ct=18, Orf1ab Ct=21).

For 70 of the RT-PCR positive samples, rapid Ag test positive results were obtained within 2 min for the device.

For 15 of the RT-PCR positive samples, rapid Ag positive results were obtained between 2 min and 7 min.

For 6 of the RT-PCR positive samples rapid Ag positive results were obtained between 10 and 15 min.

Taken together, positive results by AllTest rapid antigen test were as many as 91 among the 100 RT-PCR positive specimens. Concordance for SARS-CoV-2 positive results were 91%.

Most of the discrepancies between RT-PCR and rapid Ag tests results were due to a lower viral burden. Indeed, 7 of the 9 samples that gave negative result with rapid Ag test had Ct ≥ 25.00 for both N and ORF1ab targets. The other two specimens that gave negative results by Ag testing, despite Ct<25.00 by RT-PCR, are one del69-70 variant and another one with Ct=23 for targets N and ORF1ab. So, 88 samples among 90 RT-PCR positive specimens with Ct<25.00 for at least two targets gave concordant results with All test antigen test. Concordance for Ct<25 RT-PCR positive results was 97,8% for All Test device.

Among the 63 RT-PCR positive specimens for which spike mutations were determined, 61 variants had the 452R mutation including 6 of the 9 negative samples by Ag device. Two variants had the del69-70, one of the two gave a negative result by Ag device. Most mutations in N gene were 452R and gave most of the time strong and quick positive results with the Ag device. This result is explained by the fact that antigen targeted by All test rapid test is N and S antigen is therefore not involved in assay performance. These results are summarized in **Table 1.**

In this study, we also recorded the duration of symptoms before RT-PCR (in days). Among 100 RT-PCR positive specimens, 9 patients did not give an answer and data were available for 91 patients. Among these, 21 were asymptomatic and 70 had symptoms. 6 of the 9 patients with negative samples by Ag test were asymptomatic.

20 patients had symptoms one day before or the day of diagnosis. 20 patients were symptomatic between 2 and 4 days before the

Table 1 Samples included in the present evaluation grouped by Ct values.

Sample Groups	Ct Values of ORF Gene	Estimated Viral RNA Copy Numbers	Sample Size
High Positive	Ct ≤ 20	>10^7 copies/reaction	178
High/Medium Positive	21<Ct ≤ 25	I0^6~10^7 copies/reaction	12
Medium/Low Positive	25<Ct ≤ 30	I0^4~10^5 copies/reaction	10
Low Positive	Ct>30	<10^4 copies/reaction	20
Negative	Not detected	Not detected	200

Table 2 Test results of RT-PCR reagent.

	RT-PCR Reagent		Total
	Positive	Negative	
Antigen Positive	214	1	215
test Negative	6	199	205
Total	220	200	420
Sensitivity (%) = 214 + (214+6) x 100% = 97.3% (CI*: 94.2%〜99.0%) Specificity (%) = 199 + (199+1) x 100% = 99.5%; (CI*: 97.3%〜100%) Total accuracy (%) = (214+199) + (214+1+6+199) x 100% = 98.3% (CI*: 96.6%〜99.3%) Kappa=0.96			

day of diagnosis, 4 between 5 and 7 days, 7 between 8 and 14 days and 4 between 15 and 28 days. 15 patients did not precise the duration of symptoms.

In most cases, diagnosis was performed shortly after the first symptoms. Symptomatology could not be associated with Ct Value or result obtain with Ag device.

Site 2

Sample size: The expected sample size for this study is >420, including at least 220 nasal swab samples fi-om RT-PCR confirmed *SARS-CoV-2* positive cases, and at least 200 nasal swab samples from RT-PCR confirmed *SARS-CoV-2* negative cases.

The test results of RT-PCR reagent and the COVID-19 Antigen Detection Kit are summarized in 2x2 **table 2** below.

Discussion

In our series, the All test device provided most of the expected positive results and all negative RT-PCR results were concordant. The specificity of Ag tests is generally high and false positive test results are unlikely. The diagnostic performance of the All test rapid Ag test is in conformity with the Technical Working Group of EU Health Security Committee (HSC-TWG), i.e. ≥ 90% sensitivity and >98% specificity [7].

Rapid Ag test devices are convenient devices to aid in the rapid diagnosis of SARS-CoV-2 infections. Molecular diagnosis based upon viral RNA amplification is better because of its lower limit of detection. Specimens should be positive by rapid Ag testing if viral burden corresponds to Ct of around 28 or less by RT-PCR. This is very frequent with virus-producing patients. This threshold is very often reached in patients with viruses and cases with this burden level are likely to account for a significant proportion of transmissions [3].

In the present study, the *SARS-CoV-2* Antigen Rapid Test (Nasal Swab) by HANGZHOU ALLTEST BIOTECH CO., LTD has shown highly reliable performance in sample from suspected COVID-19 patients or asymptomatic patients. Compared to conventional RT-PCR tests, the rapid antigen tests can meet a wider range of test needs. Also, the rapid antigen tests doesn't require special instruments and training to use, they are also capable for resource limited scenarios like point-of-care testing and self-test by laypeople. Therefore, the implementation of rapid antigen test may totally change the strategies to control COVID-19.

In summary, the *SARS-CoV-2* Antigen Rapid Test (Nasal swab) has shown satisfying sensitivity, specificity, and total accuracy in the present evaluation. It can be used as a rapid tool to assist the early diagnosis of COVID-19 case.

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