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Evaluation of a Self-testing Rapid Diagnostic Test Device for Detecting *SARS-CoV-2* in Oral Fluid

Zhang Lei[1]*, Yang Feng[2] and Zhu Junzhe[3]

1 Zhejiang Gongshang University, Hangzhou, China
2 Community Health Service Center, Yipeng street, Qiantang District, Hangzhou, China
3 Wenzhou Medical University, Wenzhou, China

***Corresponding author:**
Zhang Lei

zhanglei@zjgsu.edu.cn

Tel: 13588036333

Zhejiang Gongshang University, Hangzhou, China

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Abstract

Testing continues to be one of the World Health Organization's (WHO) key strategies to better understanding and monitoring the spread and trends of *SAR-CoV-2* transmission. Mounting an effective respond to the virus requires timely detection of cases. In many countries around the world, antigen-based rapid diagnostic tests have been a turning point to getting test results quickly. Antigen rapid tests are a key for point-of-care COVID-19 testing due to faster test results, as well as the tests do not need to be run on existing laboratory infrastructure or require skilled healthcare workers to process the test kits. Faster test results help improve the timeliness of diagnosis and lead to better decision making for managing COVID-19. Self-testing further improves access to testing which can contribute to early detection of COVID-19 and the isolation of confirmed cases.

Objective: The aim of this evaluation report was to explore the reliability and performance of the All Test COVID-19 Antigen Rapid Test for Self-Testing (Oral Fluid) on clinical specimens collected for *SARS-CoV-2* diagnosis and compared to a laboratory run RT-qPCR (real-time reverse transcription polymerase chain reaction) test.

Method: The method was to run the All Test Antigen Rapid Test device for detection of *SARS-CoV-2* antigen in oral fluid and compare the results to the RT-PCR system whose samples were collected by nasopharyngeal swabs for validation of the performance. The test kits were evaluated according to the procedures described in the manufacturers' instructions for use.

Results: Concordance for *SARS-Cov-2* negative results was 100% between the All Test Antigen Rapid Test and RT-PCR. Concordance for *SARS-CoV-2* positive results was also 100% in our series (Ct range from 31-35).

Conclusion: Rapid antigen rests are convenient and economical devices to aid in the rapid diagnosis of *SARS-CoV-2* infections. Specimens should be positive by rapid antigen testing if viral burden corresponds to Cycle Threshold (Ct value) of around 33 or less by RT-PCR. This is very frequent with virus-producing patients. In this evaluation, the All Test Rapid antigen test devices provided the expected positive results, and all negative RT-PCR results were concordant.

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

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Introduction

The World Health Organization declared COVID-19 a global pandemic on March 11, 2020 [1]. The severe acute respiratory syndrome coronavirus 2 (*SARS-CoV-2*) is responsible for coronavirus disease 2019, which emerged as a novel human pathogen at the end of 2019. Since its emergence nearly two years ago, the virus has caused more than 257 million confirmed cases and has led to more than 5 million deaths globally as of November 22, 2021 [2]. SAR-CoV-2 causes symptoms such as cough and fever, severe pneumonia, and death.

Currently, the gold standard for biological diagnosis of *SARS-CoV-2* is based on the detection of nucleic acids of the potential virus in respiratory specimens by the molecular biology method RT-qPCR. RT-qPCR uses nasopharyngeal (N) swabs, throat (T) swabs, or saliva to collect samples for testing [3]. RT-qPCR kits that do not require viral RNA extraction and high-throughput RT-qPCR systems have been developed. Although such tests

are widely utilized in public health laboratories and large well-equipped hospitals, they are unavailable in local clinics and community health centers where patients who suspect they have COVID-19 often go first [4]. Therefore, specimens need to be transported to and examined at sites that have RT-qPCR capability, which delays the test result and increases the anxiety of the suspected COVID-19 patients. To improve this situation, rapid antigen tests for COVID-19, which do not require specific and expensive lab equipment, have been approved for clinical use in many countries around the world.

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* antigen detection in clinical specimens. One of the main advantages of a rapid antigen test is the speed of the test. These tests are easy to use, inexpensive, can be used as a point-of-care test (POCT) and give rapid results in 15-30 minutes [4-6]. Antigen tests are also important in the overall response against COIVD-19 as they can generally be produced at a lower cost than PCR tests and help public health officials better identify infection rates closer to real time.

Material and Method

The AllTest COVID-19 Antigen Rapid Test (Oral Fluid) was evaluated. This test is a qualitative lateral flow immunoassay that allows for rapid detection of COIVD-19 nucleocapsid protein antigens from *SARS-CoV* and *SARS-CoV-2*. Oral fluid is collected by the patient using an oral fluid collection device. The patient adds the extraction buffer to the tube with oral fluid and mixes well. The mixture of extraction buffer and oral fluid is then dropped onto a lateral flow cassette. The liquid will move through the cassette and if *SARS-CoV-2* antigen is present, a colored line will develop at the Test (T) location on the cassette. A colored line will always develop at the Control (C) location on the cassette to indicate that the assay worked correctly. If *SARS-CoV-2* is not detected, only one line will be present at the Control (C) location on the cassette. The test result is obtained in no more than 20 minutes.

The antigen results in this evaluation were compared to the RT-PCR system whose samples were collected by nasopharyngeal swabs for the validation of the performance.

Viral RNA extraction kit from Norgen Biotek (Canada) was used for the *SAR-CoV-2* viral RNA extraction according to the manufacturer's instructions for use, for which 250 µL of each sample was collected by nasopharyngeal swabs (NPS) into a viral transport medium (VTM). For each batch of samples to be tested, an extraction control (EC) was included. The samples and spiked EC were processed and extracted. The extracted RNA was eluted in 50 µL RNase-free water, 5 µL of which was used for the PCR reaction per test. Precautions were taken while handling extracted RNA samples to avoid RNA degradation. The detection was then performed by RT-PCR using the TaqPath 1-Step RT-qPCR Master Mix kit from Thermo Fisher on the Roche 480 Light Cycle II platform. The probe used in the test are Light-Mix Modular *SARS-CoV-2* (COVID-19) RdRp which targets the ORF1ab genes.

PCR brand: TaqPath 1-Step RT-qPRC Master Mix kit from Thermo Fisher

Lot number: 82293145**,**

Expiration date: 2021-12-30.

Predefined and publicly available 'prioritization' criteria to pass on to the lateral flow devices consisting of:

- an analytical Limit of Detection (LOD) corresponding to a RT-PCR Cycle threshold (Ct) of approximately 25 (~100,000 RNA copies/mL);
- an analytical specificity of ≥ 97%
- an analytical sensitivity of ≥ 85%
- a kit failure rate of <10%

Sample evaluation

Sample evaluation and collection was carried out from July 2021 to August 31, 2021. 148 samples were obtained from healthcare settings and stored in phosphate buffered saline (PBS) before processing. Among them, 50 tested *SARS-CoV-2* negative by the laboratory using RT-PCR, while the other 98 tested *SARS-CoV-2* positive. All samples were tested by both RT-PCR and the All Test antigen test on the same day. From the 148 samples, both the specimens were collected from the group within 7 days of the onset of symptoms or from asymptomatic patients. Both the antigen and RT-PCR kits were far from the packaging expiration date.

Results

The 50 samples that tested negative for *SARS-CoV-2* by RT-PCR also tested negative by the All Test antigen test. Concordance for *SARS-CoV-2* negative results were then 100%.

RT-PCR Thermo Fisher gave positive results on 98 specimens and also gave positive results using the All Test antigen test kits.

Study cohort

Sensitivity limits: For testing the device's detection limit, the following testing results of the All Test Antigen kits compared to the PCR confirmed results.

The Ct 33 is the limitation for the All Test Antigen kit and the virus dose was already very low. Most patients will reach Ct 31 after one week of reporting symptoms**.**

In this evaluation, 98 positive samples were randomly tested with the following results **(Table 1):**

Table 1 Preliminary check of positive samples.

	Positive (Ct<34)	Negative
PCR lot: 82293145	98	0
All Test Antigen Device	98	0

Table 2 Preliminary check of negative samples.

	Positive	Negative
PCR lot: 82293145	0	50
All Test Antigen Device	0	50

For the negative samples, a total 50 tests were carried out with the following results **(Table 2):**

Estimation of clinical performance

The summarized results are as follows in **Table 3**

Kit failure rate

The summarized results are as follows in **Table 4.**

Table 3 Estimation of Clinical Performance.

	Sensitivity	Specificity
All Test Antigen Device	>99.99%	>99.99%
The sensitivity was witnessed in the lab to be 100% and specificity was witnessed in the lab to be 100% for the samples tested		

Table 4 Kit Failure Rate.

	Failed	Succeed
All Test Antigen Device	0	148

Conclusion

The All Test COVID-19 Antigen Rapid Test (Oral Fluid) kits manufactured by Hangzhou All Test Biotech Co., Ltd tested in this evaluation performed as intended when compared to samples tested using the RT-PCR method. All 148 test results succeeded when compared to the PCR method results. Overall, each antigen test showed both excellent sensitivity and specificity. The accuracy achieved by the All Test antigen rapid test combined with the rapid turnaround time compared to RT-PCR suggests that these tests could have a significant impact on the pandemic if applied in thoughtful testing and screen strategies. We must note that the lab evaluation could differ from the performance outside the laboratory due to the limited sample range of the kits and close monitoring when used by clinical professionals.

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