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Evaluation of the Diagnostic Accuracy of All Test SARS-CoV-2 Rapid Antigen Test and Detection of Thirteen Variants

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

[1]*Department of Nursing Sciences, Zhejiang Gongshang University, Hangzhou, China*

[2]*Department of Nursing Sciences, Wenzhou Medical University, Wenzhou, China*

[*]**Corresponding author:** Dr. Zhang Lei, Department of Nursing Sciences, Zhejiang Gongshang University, Hangzhou, China, E-mail: zhanglei@zjgsu.edu.cn

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Abstract

Early and rapid detection remains vital to slow the community spread of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) infection. Reverse-Transcriptase Polymerase Chain Reaction (RT-PCR) is currently the diagnostic reference standard for the diagnosis of COVID-19. However, expensive and specialized laboratory instruments and clinical expertise are required to conduct RT-PCR assays. Moreover, many countries around the world have experienced supply shortages of reagents, long turnaround times and other challenges. To evaluate the sensitivity and specificity of the All Test SARS-CoV-2 Antigen Rapid Test (Nasal Swab), a total of 100 positive samples were subject to antigen and PCR tests. The sensitivity, specificity and accuracy of the antigen test were 91.0%, 100% and 95.5% respectively. Rapid antigen tests offer results more rapidly (15-20 minutes) and at a lower cost than RT-PCR. These tests are well suited for Point-Of-Care Testing (POCT), as they can easily be performed and interpreted without equipment, are inexpensive, and improve turnaround times.

Keywords: COVID-19; Rapid antigen tests; Nucleocapsid protein

Introduction

The US Food and Drug Administration (US FDA) approved the first COVID-19 antigen test, which is rapid, direct and cost-effective, near the end of August 2020. These tests detect specific proteins attached to the surface of SARS-CoV-2 in samples obtained from the upper airways using an immuno-chromatographic procedure [1-3].

General information

In this study, the All Test SARS-CoV-2 Antigen Rapid Test (Nasal Swab) was evaluated and compared to the PerkinElmer RT-PCR setup. The SARS-CoV-2 Antigen Rapid Test from All Test is a rapid chromatographic immunoassay for the qualitative detection of SARS-CoV-2 Nucleocapsid protein antigen in nasal swab specimens from persons with suspected SARS-CoV-2 infection. This test can aid in the diagnosis of SARS-CoV-2 infection. In this study, a total of 100 negative and 100 positive COVID-19 samples were included in this comparison. The sensitivity of the All Test antigen rapid test type was determined to be 91.0%. The specificity was determined to be 100% and accuracy was 95.5%. Additionally, the antigen rapid tests were able to detect all thirteen variants of SARS-CoV-2 variant (Supplement Table 1).

Evaluation

The Antigen Rapid Test was evaluated by Microbe and Lab BV in November 2021. The antigen rapid test results were compared against the PerkinElmer PCR setup results. The rapid test was conducted with fresh nasal swabs from unselected patients who were symptomatic participants within the first seven days after symptom onset until 100 positive samples were collected and tested. In addition, thirteen variants of COVID samples were tested to evaluate the antigen rapid test COVID variants detection capability.

Principle

The All Test SARS-CoV-2 Antigen Rapid Test is a qualitative membrane-based immunoassay for the detection of SARS-CoV-2 Nucleocapsid protein antigens in nasal swab specimen. SARS-CoV-2 Nucleocapsid protein antibody is coated in the test line region. During testing, the specimen reacts with SARS-CoV-2 Nucleocapsid protein antibody-coated particles in the test. The mixture then migrates upward on the membrane by capillary action and reacts with the SARS-CoV-2 Antigens. A colored line will appear in test line region as a result of this. If the specimen does not contain antigens to SARS-CoV-2, no colored line will appear in the test line region, indicating a negative result. To serve as a procedural control, a colored line will always appear in the Control (C) location on the cassette, indicating that the proper volume of nasal swab specimen has been added and membrane wicking has occurred.

Definitions

The SARS-CoV-2 Antigen Rapid Test by All Test was compared to the PerkinElmer rt-RT-qPCR and evaluated based on sensitivity, specificity, and accuracy. Sensitivity is defined as the proportion of true positives that are correctly identified (sensitivity=true positives/(true positives+false negatives)). Specificity is defined as the proportion of true negatives that are correctly identified (specificity=true negative/(true negatives+false positives)). Accuracy is defined as the percentage of correctly classified samples (accuracy=(true positives+true negatives)/(true positives+true negatives+false positives+false negatives)).

Test kit

All Test SARS-CoV-2 Antigen Rapid Test (Nasal Swab) (LOT: ATNCP21090008). Kit components are detailed in Table 1.

Component	Description
Buffer	Buffer to dissolve specimen in.
Cassette	Results will be visible on the cassette.
Test tube	Flexible tube for adding buffer and dissolving the specimen.
	Nozzle caps for the test tubes are provided.
Sterile swab	Flexible swabs for nasal and nasopharyngeal use.
Instructions for Use	Manual for storage and use of the kit components.

Table 1: Rapid SARS-CoV-2 antigen test kit components.

Sample preparation

Study 1: For each unselected patient, one sterile nasal swab was collected and then tested with the All Test SARS-CoV-2 Rapid Antigen Test according to the instructions for use of the antigen rapid test. A second specimen, a Nasopharyngeal (NP) swab, was collected and stored in a Viral Transport Medium (VTM) and then sent to the laboratory for PCR testing (PerkinElmer PCR setup).

Study 2: Both SARS-CoV-2 variant panel 1 and 2 (RIVM; Supplementary Table 1) samples were used to investigate whether the antigen rapid test can detect all SARS-CoV-2 variants. Since the variant samples were not in viral transport media, the sample was taken with a sterile swab described in the instructions for use of the antigen rapid test.

Result reading

Results were scored 15 minutes after loading the specimen on the cassette. Only results with a visible control band were counted as valid. Test results with an absent control band could not be interpreted and were considered invalid. The test result was considered negative if a purplish-red band only appeared at the control band area (indicated by a “C”). The test result was considered positive if purplish-red bands appeared at both the control band area and at the test band area (indicated by a “T”). Even very faint test bands were considered positive if a control band was visible.

PerkinElmer rt-RT-qPCR (Lot: CMG-1033-S; EXP.:01 FEB 2022): RNA was isolated using Chemagic™ viral DNA/RNA isolation (Chemagic™ viral DNA/RNA Kit special H96; PerkinElmer, United States), and performed according to Microbe and Lab’s internal protocol. In short, a mixture of 300 µl containing lysis buffer, poly A, and proteinase K (75:1:2.5) was added to 300 µl of sample in VTM. Following the default Chemagic Viral300 360 H96 prefilling short VD200406 protocol on the Chemagic™ 360 2040-0020 (PerkinElmer), isolation was continued. An elution volume of 100 µl was chosen.

The PCR setup targets the N-gene (FAM, 465-510 nm), the ORF1ab-gene (VIC/HEX, 533-580 nm), and the Human Leukocyte Antigen (HLA-) gene (Internal Positive Control (IPC), Cy5, 618-660 nm). The PCR master mix contents per sample included 1 µL CoV-2 Reagent A and 5 µL CoV-2 Enzyme Mix (SARS-CoV-2 RT-qPCR Reagent kit, PerkinElmer). The QuantStudio™ 5 Real-Time PCR System (ThermoFisher Scientific Applied Biosystems) was used for running rt-RT-qPCRs (Table 2).

Temperature	Time	Number of Cycles
50°C	15 minutes	1
95°C	2 minutes	1
95°C	3 seconds	50
60°C	15 seconds	
75°C	3 seconds	

Table 2: PerkinElmer rt-qPCR temperature cycles.

A negative control (demi water) and an Independent Run Control (IRC, previously tested positive sample) were taken along for each isolation. IRCs were created by pooling positive sample until 5 ml was obtained and diluted in 45 ml Phosphate Buffer Saline (PBS). For each PCR run, a Positive Control (PC) was included. Samples with clear S-curves and a Ct-value for the IPC were considered valid. Of these valid samples, samples with a Ct-value for either one or both SARS-CoV-2 target genes (N and ORF1ab) were considered positive. The whole procedure was repeated for invalid specimens.

Variant detection

Both SARS-CoV-2 variant panel 1 and 2 (RIVM; Supplementary Table 1) samples were isolated and included in PCR. For the PCR, 100 µL samples were used for isolation. PCR was performed according to the protocol described earlier.

Out of the 100 positive patient samples, the All Test SARS-CoV-2 Antigen Rapid Test (Nasal Swab) detected 91 positive samples from the 100 (sensitivity 91% (95% CI: 86.2%-94.2%)). From the nine samples which were detected as false negatives, four had a CT value between 18 and 22 and five samples had a CT value between 26 and 31 (Supplementary Table 4). Since no false positive results were obtained, the specificity of the All Test SARS-CoV-2 Antigen Rapid Test was determined to be 100% (95% CI: 98, 1%-100%). The accuracy of the All Test rapid test was 95.5% (95% CI: 91.7%-97.6%). Wilson binomial method was used for CI-calculations. Furthermore, the All Test SARS-CoV-2 Antigen Rapid Test detected all thirteen variants included in SARS-CoV-2 variant panel 1 and 2 (Supplementary table 1) [4-10].

Conclusion

Rapid and accurate identification of patients with SARS-CoV-2 infections is critical to contain the spread of COVID-19 pandemic. This evaluation was done to investigate the performance of the All Test SARS CoV-2 Antigen Rapid Test (lot number ATNCP21090008). The Antigen Rapid Test yielded excellent specificity and accuracy, and very good overall sensitivity. In summary, we found the Antigen Rapid Test from All Test performs well as a POCT for early diagnosis of COVID-19.

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