Appendix H
Clinical Report

Rapid COVID-19 (Antigen) Self-Test

[i] Jefferson, T et al: Viral cultures for COVID-19 infectivity assessment. Systematic review. doi: https://doi.org/10.1101/2020.08.04.20167932

[ii] Woelfel, R et al: Virological assessment of hospitalized patients with COVID-2019. Nature volume 581, pages 465–469 (2020)

Product Name
Rapid COVID-19 Antigen Self-Test

Sponsor
Zhejiang Orient Gene Biotech Co., Ltd.

Clinical Site
Clinical Performance of the Rapid COVID-19 Antigen Self-Test was evaluated by being involved in Point of Care site within the US, where patients were enrolled and tested. Testing was performed by Health Care Workers.

Test Interval

September, 2020-December, 2020

Introduction
Coronaviruses are enveloped RNA viruses that are distributed broadly among humans, other mammals, and birds that cause respiratory, enteric, hepatic, and neurologic diseases. Seven coronavirus species are known to cause human disease. Four viruses (229E, OC43, NL63, and HKU1) are prevalent and typically cause common cold symptoms in immunocompetent individuals. The three other strains (SARS-CoV, MERS-CoV, SARS-CoV-2) are zoonotic in origin and have sometimes been linked to fatalities.

The Rapid COVID-19 Antigen Self-Test is an in vitro immunochromatographic assay for the qualitative detection of nucleocapsid protein antigen from SARS-CoV-2 in direct swab specimens directly from individuals who are suspected of COVID-19 by their healthcare provider. It is intended to aid in the rapid diagnosis of SARS-CoV-2 infections. The Rapid COVID-19 Antigen Test does not differentiate between SARS-CoV and SARS-CoV-2.

The novel coronaviruses belong to the β genus. COVID-19 is an acute respiratory infectious disease. People are generally susceptible. Currently, the patients infected by the novel coronavirus are the main source of infection; asymptomatic infected people can also be an infectious source. Based on the current epidemiological investigation, the incubation period is 1 to 14 days, mostly 3 to 7 days. The main manifestations include fever, fatigue, and dry cough. Nasal congestion, runny nose, sore throat, myalgia, and diarrhea are found in a few cases.

This test is for detection of SARS-CoV-2 nucleocapsid protein antigen. Antigen is generally detectable in upper respiratory specimens during the acute phase of infection. Rapid diagnosis of SARS-CoV-2 infection will help healthcare professionals to treat patients and control the disease more efficiently and effectively.

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Principle

The Rapid COVID-19 Antigen Self-Test is an immunochromatographic membrane assay that uses highly sensitive monoclonal antibodies to detect nucleocapsid protein from SARS-CoV-2 in swab. The test strip is composed of the following parts: namely sample pad, reagent pad, reaction membrane, and absorbing pad. The reagent pad contains the colloidal-gold conjugated with the monoclonal antibodies against the nucleocapsid protein of SARS-CoV-2; the reaction membrane contains the secondary antibodies for nucleocapsid protein of SARS-CoV-2. The whole strip is fixed inside a plastic device. When the sample is added into the sample well, conjugates dried in the reagent pad are dissolved and migrate along with the sample. If SARS-CoV-2 antigen presents in the sample, a complex formed between the anti-SARS-2 conjugate and the virus will be captured by the specific anti-SARS-2 monoclonal antibodies coated on the test line region (T). Absence of the T line suggests a negative result. To serve as a procedural control, a red line will always appear in the control line region (C) indicating that proper volume of sample has been added and membrane wicking has occurred.

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CONTENT

Figure

Table

[i] Jefferson, T et al: Viral cultures for COVID-19 infectivity assessment. Systematic review. doi: https://doi.org/10.1101/2020.08.04.20167932

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1. Purpose

The primary objective is to determine the sensitivity and specificity of the Rapid COVID-19 Antigen Self-Test when testing intended use populations who meet the criteria of having COVID-19 infection by Centers for Disease Control and Prevention (CDC). The test is to be performed by healthcare professionals at clinical settings.

2. Study Design

2.1 Materials and Methods

The primary objective of this study is to determine the sensitivity and specificity of the Rapid COVID- 19 Antigen Test when testing intended use populations who meet the criteria of having COVID-19 infection by Centers for Disease Control and Prevention (CDC). The test is to be performed by healthcare professionals at clinical settings.

2.2 Principle

The Healgen Rapid COVID-19 Antigen Self-Test is an immunochromatographic membrane assay that uses highly sensitive monoclonal antibodies to detect nucleocapsid protein from SARS-CoV-2 in nasopharyngeal (NP) swab and nasal swab. The test strip, shown in Figure 1 is composed of the following parts: Sample pad, reagent pad, reaction membrane, and absorbing pad. The reagent pad contains the colloidal-gold conjugated with the monoclonal antibodies against the nucleocapsid protein of SARS-CoV-2; the reaction membrane contains the secondary antibodies for nucleocapsid protein of SARS-CoV-2. The whole strip is fixed inside a plastic device**.**

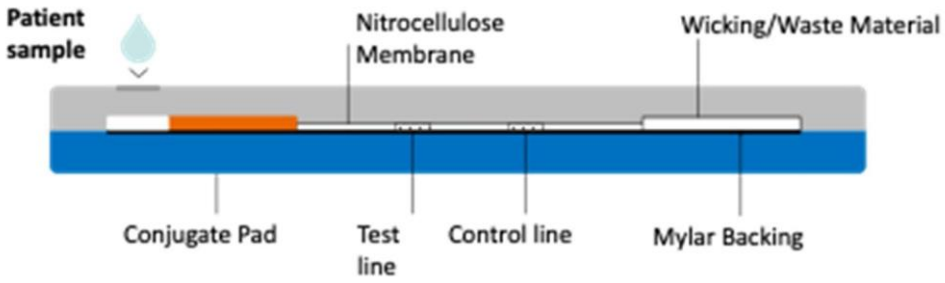

Figure 1:Test strip design

When the sample is added into the sample well, conjugates dried in the reagent pad are dissolved and migrate along with the sample. If SARS-CoV-2 antigen is present in the sample, a complex forms between the anti-SARS-CoV-2 conjugate and the virus will be captured by the specific anti-SARS-CoV-2 monoclonal antibodies coated on the test line region (T). Absence of the T line suggests a negative result. To serve as a procedural control, a red line will always appear in the control line region (C) indicating that proper volume of sample has been added and membrane wicking has occurred.

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2.3 Sample population and size

The clinical evaluation will be conducted at the actual user site and the study population will be "real-world" patients. To support the test performance, clinical specimens will be tested in a randomized, blinded fashion.
The testing to be conducted will include the following:
A. Enroll subjects known to be positive for COVID-19 by a RT-PCR assay within 14 days. These would be the patients that are already under the PI's care.
B. Enroll subjects where the healthcare provider suspects the individual may have COVID-19 infection based on the CDC description of COVID-19 symptoms.
C. All the subjects will agree to be simultaneously sampled for a COVID-19 RT-PCR test and sampled for an antigen test at the clinical site.
D. If a subject has a known RT-PCR result less than 14 days ago, the RT-PCR test can be waived.

2.4 Inclusion and Exclusion Criteria

2.4.1 Inclusion Criteria

1. Must be 21 years old or older.
2. Has symptoms that lead the healthcare provider to suspect the individual of possibly having SARS-CoV-2 infection.
3. Was exposed to a COVID-19 patient within 14 days that leads the healthcare provider to suspect the individual of possibly having SARS-CoV-2 infection
4. Has an immediate need to determine COVID-19 status for occupational purposes.
5. Must be willing to provide a sample for COVID-19 RT-PCR testing if the subject has not been previous tested for COVID-19 RT-PCR within 14 days.
6. Must be willing to provide a sample for additional tests required by the study site.
(antigen test or RT-PCR).
7. Must be able to sign a consent form.
8. Must be able to provide swab samples.

2.4.2 Exclusion Criteria

1. Is receiving treatment with infusion of convalescent plasma or other antibody therapy related to SARS-CoV-2 infections.
2. Is participating in a SARS-CoV-2 vaccine study.
3. Tested positive for COVID-19 positive more than 14 days ago.

2.5 Candidate Test

Rapid COVID-19 Antigen Self-Test
Lot: 2008139

2.6 Comparator Test

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The comparator tests included high sensitivity Emergency Use Authorized RT-PCR tests used at each testing site as the routine testing method for COVID-19 diagnostics. The EUA RT-PCR tests use a chemical lysis step followed by solid phase extraction of nucleic acid. The patient specimens were all prospective collected and immediately tested by operators.
FDA Emergency Use Authorized RT-PCR tests routinely are used as the testing method for COVID-19 diagnostics. Multiple RT-PCR tests were used as the comparator assay because Manufacturer had no control of which assay the test site used for patient testing. Sometimes, a testing site used multiple RT-PCR assays due to test supply constraints. In addition, it is very burdensome to collect multiple samples from one subject to accommodate an additional, separate RT-PCR test because the subject was already sampled twice (once for the clinical testing and once for the investigational testing).
The comparator method used in the study was Thermo Fisher TaqPath COVID-19 Combo Kit

2.7 Test Procedure

Perform the Test according to the Instructions for Use (IFU) package insert.
The technique is described and illustrated in the Quick Reference Instruction (QRI)
The test device and swab is provided with the test kit. The fresh specimens were tested immediately, and no transport media was used for shipping the samples to a different location for testing. All clinical specimens tested in this submission were tested and evaluated in accordance with the proposed diagnostic algorithm.

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3. Results, Data process and Analysis

3.1 Overall Results Nasal Swab

The study was conducted at a site located in the USA. A total of 237 fresh nasal swab samples was collected and tested, which includes 109 positive samples and 128 negative samples. The Healgen Rapid COVID-19 Antigen Self-Test results were compared to results of USFDA Emergency Use Authorized RT-PCR assay for SARS-CoV-2 in nasopharyngeal swab specimens. The comparator method was Thermo Fisher TaqPath COVID-19 Combo Kit. Overall study results are shown in **Table 1.**

Table 1: Rapid COVID-19 Antigen Self-Test (Nasal Swab) vs PCR (Nasopharyngeal Swab)

Method		**PCR**		**Total Results**
Rapid COVID-19 Antigen Self-Test (Nasal Swab)	Results	Positive	Negative	
	Positive	106	0	106
	Negative	3	128	131
Total		109	128	237

Relative Sensitivity: 97.25% (95% Cl*: 92.17% to 99.43%)
Relative Specificity: 100% (95% CI*: 97.69% to 100%)
Accuracy: 98.73% (95%CI*: 96.35% to 99.74%)

3.2 Days Post Symptom Onset Demographics

During enrollment patients were screened for evidence of symptoms and the onset of symptoms. Aggregated test performance as a function of the days post symptom onset is presented in **Table 2.** Of note is that the test demonstrates high sensitivity even in the case of asymptomatic patients. This is a critical requirement for population screening.
Analysis of positive patients experiencing symptom onset within 7 days were recorded. Correlation between the Rapid COVID-19 Antigen Test and the PCR comparator stratified by days post symptom onset are shown in **Table 2.**

Table 2:Days Post Symptom Onset Demographics

Site 1				
For PCR Positive Specimens		Candidate Device Results		
Days Post Symptom Onset	**Samples Tested**	**Antigen Positive Results**	**PPA**	**95% CI**

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Asymptomatic	53	50	**94.34%**	84.34%~98.82%
0	0	0	**N/A**	N/A
1	0	0	**N/A**	N/A
2	12	12	**100.00%**	73.54% - 100%
3	17	17	**100.00%**	80.49% - 100%
4	13	13	**100.00%**	75.29% - 100%
5	5	5	**100.00%**	47.82% - 100%
6	6	6	**100.00%**	54.07% - 100%
7	3	3	**100.00%**	29.24% - 100%
Total	**109**	**106**	**97.25%**	92.17% to 99.43%

3.3 Cycle Threshold (Ct) Analysis

For the positive test results, the cycle threshold counts were recorded and compared against the rapid antigen results, presented in **Table 3**. Literature suggests that Ct values greater than 30 are generally non-infectious, corresponding to a viral load of >1E6 copies per mL[i,ii]. The test performance in **Table 3** is separated for samples below Ct<30 and for those where Ct≥30. For Ct<30 the test is highly sensitive and false negatives were observed only in cases where Ct≥30. This suggests the rapid antigen test could be an effective method of screening for infectious, asymptomatic patients which is a key requirement in the high-volume population screening that is critical to limit the spread of the disease.

The performance of the Rapid COVID-19 Antigen Test with positive results stratified by the comparator PCR method cycle threshold (Ct) counts in **Table 3**.

Table 3: Positive Result Stratified by Cycle Threshold (Ct) Value

Rapid COVID-19 Antigen Self- Test	RT-PCR Comparator Positive Result Cycle Threshold (Ct)	
	Ct < 30	Ct ≥ 30
Positive	83	23
Negative	1	2
Total	84	25
Positive Agreement (95% CI)	98.81%(93.54%-99.97%)	92.00%(73.97%-99.02%)

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4. Conclusion

Performance of a Rapid Antigen test (Rapid COVID-19 Antigen Test) was evaluated in a clinical study at US site. The comparative method was a USFDA Emergency Use Authorized RT-PCR Assay (Thermo Fisher TaqPath) with samples taken from the Anterior Nares.
For Nasal sampling with the Rapid COVID-19 Antigen Test, the overall test accuracy over 237 samples was 98.73% with a sensitivity of 97.25% and a specificity of 100% relative to RT-PCR. For this sampling method, analysis relative to symptom onset was not performed. Performance relative to symptom onset days indicated that the test performs very well even in the case of asymptomatic patients and at Ct < 30), indicating its applicability in screening in patients that may or may not present with symptoms, but could be infectious.

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