Clinical Report

COVID-19 Ag Rapid Test

Sponsor: Intelos Inc and BioNano Health Guard Research Center
Address: 2F, 617-32, Jungang-daero, Buk-gu, Daegu, Republic of Korea；

Manufacturer: Zhejiang Anji Saianfu Biotech Co., LTD
Address: 2nd Floor,No.3 Factory, No.489 WenYun Road,TangPu Industrial Park,Dipu Subdistrict, Anji county, 313303 HuZhou City, ZheJiang Province， P. R. China

Product Name
COVID-19 Ag Rapid Test

Manufacturer
Zhejiang Anji Saianfu Biotech Co.,Ltd.

Clinical Site
Clinical Performance of the COVID-19 Ag Rapid Test was evaluated by Intelos Inc and BioNano Health Guard Research Center in Korea, where patients were enrolled and tested. Testing was performed by 3 Health Cuard Workers.

Test Interval

Oct 28th,2020-Nov 5th,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 COVID-19 Ag Rapid Test is an in vitro immunochromatographic assay for the qualitative detection of nucleocapsid protein antigen from SARS-CoV-2 in direct nasopharyngeal (NP) 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 coronavirus 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.

Principle

The COVID-19 Ag Rapid Test is an immunochromatographic membrane assay that uses highly sensitive monoclonal antibodies to detect nucleocapsid protein from SARS-CoV-2 in nasopharyngeal (NP) 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.

Purpose

The primary objective is to determine the sensitivity and specificity of COVID-19 Ag Rapid 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.

Design

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 with the goal of testing a minimum of 48 positive and 100 negative specimens which are confirmed by PCR met in a randomized, blinded fashion.

Specimen information

Inclusion and Exclusion Criteria

Inclusion Criteria

1. All specimen age must in random.
2. The specimen is confirmed with real-time PCR

Exclusion Criteria

1.Specimens with unclear PCR results
2.Specimen information is unclear.
3.Contaminated specimens
4.Insufficient specimens to test

Acceptance Criteria

Control line should be appeard.

Candidate Test

COVID-19 Ag Rapid Test

Comparator Test

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.

Results, Data process and Analysis

Candidate Test	Reference Reagent		Total
	Positive	Negative	
Positive	a	b	a+b(γ_1)
Negative	c	d	c+d(γ_2)
Total	a+c(C_1)	b+d(C_2)	a+b+c+d (N)

Positive agreement =[a/(a+c)]×100%

Negative agreement=[d/(b+d)]×100%

Total agreement=[(a+d)/(a+b+c+d)] ×100 %

The kappa value should be calculated for the clinical data above and the confidence interval (CI) set at 95%. The Kappa value ranges from 0 to 1. The closer the Kappa value is to 1, the more consistent the two tests. On average, if the Kappa value is over 0.75, the candidate test and reference reagent are highly consistent.

$$\text{Kappa}=\frac{N(a+d)-(\gamma_1 C_1+\gamma_2 C_2)}{N^2-(\gamma_1 C_1+\gamma_2 C_2)}$$

Results Analysis

		Real-time RT-PCR		Total
		+	-	
COVID-19 Ag Test	+	46	1	47
	-	2	99	101
Total		48	100	148

- **Sensitivity:** [a/(a+c)]×100 % = [46/(46+2)]×100 % = 95.8%, 95% CI 84.57% to 99.28%
- **Specificity:** [d/(b+d)]×100 % = [99/(99+1)]×100 % = 99%, 95% CI 93.75% to 99.95%
- **Accuracy:** Total Agreement = [(a+d)/(a+b+c+d)] ×100 % = [(46+99)/ (46+2+1+99)] ×100 % = 97.9%

$$\text{Kappa} = \frac{N(a+d)-(\gamma_1 C_1+\gamma_2 C_2)}{N^2-(\gamma_1 C_1+\gamma_2 C_2)} = 95.3\%$$

Reviewed by: Seontae Kim

--Comment： Colloidal gold rapid detection test is used as a preliminary screening tool. Sensitivity is particularly important in the detection of sars-cov-2 antigens. If the sensitivity is low, it will lead to missed detection, and it may cause a wider spread of the virus after the false positive patients go back. A lower false negative can be accepted, because if the initial screening is false positive for the sars-cov-2 antigen test, the hospital will further use PCR to confirm the diagnosis.

Conclusion

The clinical evaluation for the COVID-19 Ag Test for rapid detection of SARS-CoV-2 antigen was conducted at the Intelos Inc and BioNano Health Guard Research Center in Korea, 148 residual and selected specimens from Individal persons, who were hospitalized or visited, were tested with comparing to commercialized PCR molecular assays.

The COVID-19 Ag Test showed 95.8% of sensitivity and 99% of specificity. Among the positive specimens confirmed by real-time PCR, COVID-19 Ag was not detected when the CT values of each genes in the real-time PCR results were high(weak positive). It is expected that better results were obtained from fresh specimens than from residual specimens.

The limitation of our study was a small number of specimens (positive specimens;48, negative specimens; 100）