Clinical Evaluation Report of the In Vitro Diagnostic Reagents

Product Name	StrongStep® SARS-CoV-2 Antigen Rapid Test（Swab）
Packaging Specification	20 Tests/Kit
Evaluation Time	October. 2020
Clinical Evaluation Organization	Site 1: Jiangsu Provincial Center For Disease Control and Prevention (Address: No. 172 Jiaugnsu Road, Nanjing, China) Site 2: Hubei Provincial Center For Disease Control and Prevention (Address: No. 6 North Daoquan Road, Wuhan, China)
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CONTENT

[Introduction]

The novel corona viruses belong to the β genus. COVID-19 is an acute respiratory infectious disease. People are generally susceptible. Currently, the patients infected by the novel corona virus 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.

The current method for detecting the 2019-nCoV is to detect viral RNA by fluorescent PCR method for qualitative detection of 2019-nCoV nucleic acid. Routine viral nucleic acid detection includes a series of steps needed to do such as nucleic acid extraction and purification, reagent preparation, specimen loading and instrument testing. So as same to the new corona virus nucleic acid detection reagents which have been used to detect and monitor the disease. Each step requires careful operation by the inspector. It often takes 2-3 hours to get the test results. At the same time, there is a risk of contamination and infection to the experimental operation at each step, which places high requirements on the inspection process and the inspectors.

The StrongStep®SARS-CoV-2 Antigen Test employs chromatographic lateral flow test. Latex conjugated antibody (Latex-Ab) corresponding to SARS-CoV-2 are dry-immobilized at the end of nitrocellulose membrane strip.SARS-CoV-2 antibodies are bond at the Test Zone (T) and Biotin-BSA are bond at the Control Zone (C). When the sample is added, it migrates by capillary diffusion rehydrating the latex conjugate. If present in sample, SARS-CoV-2 antigens will bind with the least conjugated antibodies forming particles. These particles will continue to migrate along the strip until the Test Zone (T) where they are captured by SARS-CoV-2 antibodies generating a visible red line. If there are no SARS-CoV-2 antigens in sample, no red line is formed in the Test Zone(T). The streptavidin conjugate will continue to migrate alone until it is captured in the Control Zone(C) by theBiotin-BSA aggregating in a line, which indicates the validity of the test.

The StrongStep® SARS-CoV-2 Antigen Rapid Test is a rapid immunochromatographic assay for the detection of COVID-19 antigen to SARS-CoV-2 virus in human Nasal/Oropharyngeal swab. The assay is used as an aid in the diagnosis of COVID-19.

[Purpose]

To evaluate the clinical performance of the StrongStep® SARS-CoV-2 Antigen Rapid Test.

[Study Design]

The StrongStep® SARS-CoV-2 Antigen Rapid Test is supposed to test the specimen (human Nasal/Oropharyngeal swab) from suspected and confirmed COVID-19 infection patients.
And the results are supposed to be compared to PCR test (Sansure BioTech Inc). Adopting 2X2 tabulation and Kappa value, the consistency of StrongStep® SARS-CoV-2 Antigen Rapid Test with reference reagent is evaluated. The PCR results is the confirmation result

[Evaluation Method]

Specimen selection

To enroll subjects according to *COVID-19 Treatment Plan* (trial implementation 8th edition) by National Health Commission

Specimen Selection Criteria

Complete specimen information, including subjects' age, gender, specimen collection date and clinical diagnosis etc.

Positive specimen from subjects confirmed with COVID-19 infection

Negative specimen from subjects without infection

Negative specimen from subjects cured from COVID-19 infection

Specimens from patients infected with influenza virus or lower respiratory infection

Specimen Exclusion Criteria

Specimen volume is inadequate to support the test

Specimens collected not as required or that expired or deteriorated

Specimen Elimination Criteria

Specimen tested by device with quality deficiency

Specimen mistakenly enrolled by operator or/that with unconvincing results or/that can not be traced

Specimen Collection

Nasal Swab Sample:

Insert one swab into one nostril of the patient. The swab tip should be inserted up to 2.5 cm (1 inch) from the edge of the nostril. Roll the swab 5 times along the mucosa inside the nostril to ensure that both mucus and cells are collected.

Use the same swab, repeat this process for the other nostril to ensure that an adequate sample is collected from both nasal cavities.

Withdraw the swab from the nasal cavity and put the swab front end into extraction tube , against the edge of the tube and break off the swab at the break point, let the swab tip fall into the tube.

Oropharyngeal Swab Sample:

Ask patient to open mouth and press tongue with tongue depressor if necessary. Use another swab into the oropharynx and scrap left and right side pharynx mucous membrane 2 times.

Withdraw the swab from the mouth and put the swab front end into extraction tube , against the edge of the tube and break off the swab at the break point, let the swab tip fall into the tube.

In order to get enough virus, it is suggest to use two or more swabs to collect different sites of sample and extract all the sampled swab in the same tube. It is recommend to use sputum or BALF specimen to get higher sensitivity when the specimen are available. To use these types of specimen, dip the swab into these specimen and then treat the swab as following procedure.

Reference Reagent Selection

According to *COVID-19 Treatment Plan* (trial implementation 8th edition) and *Technical Guidance of In-vitro Diagnostic Clinical Trial,* the test results are supposed be compared to SARS-CoV-2 PCR Test (Sansure BioTech Inc).

Inconsistent Result Confirmation

If StrongStep® SARS-CoV-2 Antigen Rapid Test result is different from SARS-CoV-2 PCR Test result (Sansure BioTech Inc), another company's SARS-CoV-2 PCR (bioPerfectus technologies) results is supposed to be the confirmation

Test Reagent

StrongStep® SARS-CoV-2 Antigen Rapid Test

Test procedure

Perform the test according to the test procedure in the package insert

Quality Control

NEGATIVE: The colored line in the control line region (C). No line appears in the test line regions. The result is SARS-CoV-2 Antigen negative.

POSITIVE:

The colored line in the control line region (C) and a colored line appears in test line region. The result is SARS-CoV-2 Antigen positive.

INVALID:

Control line is absence. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure.

Operator training

4 operators were included in this clinical trial, all the operators were trained to familiar with the clinical trial protocol and how to use this device.

Operator：Cui lunbiao and Wentian (Jiangsu Provincial Center For Disease Control and Prevention)
Caikun and Liguoming (Hubei Provincial Center For Disease Control and Prevention)

Data Statistics

The agreement rates and kappa values to the reference reagent are supposed be analyzed.

Table 2 x 2

Test Reagent	**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％

Kappa value should be carried out for the above-mentioned clinical data and the confidence interval (CI) is 95%.

The Kappa value is from 0 to 1. the closer to 1 the Kappa value, the more consistent the two tests. Averagely, if the

Kappa value is over 0.75, the test reagent and reference reagent are highly consistent.

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

2. [Results and Analysis]

2.1 Test Information

Test material: Sars-CoV-2 Antigen Rapid Test, Lot: 201002

Clinical sample: N= 104 positive specimens

405 negative specimens

Results

- **Table 1. CLINICAL PERFORMANCE**

	PCR Comparator			Total
		Positive	Negative	
StrongStep® SARS-CoV-2 Antigen Rapid Test	Positive	101	3	104
	Negative	4	402	406
	Total	105	405	510

Positive Percent Agreement: (PPA)= 96.19% (90.53%～98.95%)*
Negative Percent Agreement: (NPA)= 99.26% (97.85%～99.85%)*
*95% Confidence Interval
Kappa=0.9579 (0.9269～0.9889, highly consistent)

2.2 Analysis of Inconsistent Results

In this study, there were 7 specimens with test reagent results and reference reagent results that differed, including 4 false negative and 3 false positive.

These factors may cause false negative

- The concentration of antigen in human body is lower than the limit of detection
- Improper operation, like add inadequate specimen
- Improper reading time, like reading the results earlier than the designed time.

These factors may cause false positive

- Some substances in human swab specimen may cause false positive
- Improper operation, like adding too much specimen
- Improper reading time, like reading the results later than the designed time.

The repeated test was not carried out due to objective reasons.

2.3 Conclusion

The results show that the testing reagent and reference reagent have equivalent effectiveness in detecting COVID-19 when tested in the same clinical specimens. Compared with the reference reagent, the Positive agreement for is 96.19%; the Negative agreement is 99.26%; The kappa value of the consistency analysis was 0.9579 (0.9269～0.9889,). The results of the clinical evaluation show that the two reagents (methods) have a high degree of consistency and equivalent sensitivity and specificity in detecting COVID-19.