Clinical Evaluation Report of the In Vitro Diagnostic Reagents

Product Name	StrongStep® SARS-CoV-2 Antigen Rapid Test（saliva）
Packaging Specification	20 Tests/Kit
Evaluation Time	December. 2020
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[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 the Biotin-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 Saliva. 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 Saliva) 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

Saliva Sample：

Install the saliva collection funnel on the tube,then spit out the saliva into the tube through the saliva collection funnel until the saliva reaches the position of the saliva collection and filling line, then remove the saliva collection funnel, then screw the cover of the device.

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

3 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.

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.

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: 2012012

Clinical sample: N= 106 positive specimens

175 negative specimens

Results

- **Table 1. CLINICAL PERFORMANCE**

		PCR Comparator		Total
StrongStep® SARS-CoV-2 Antigen Rapid Test		Positive	Negative	
	Positive	102	2	104
	Negative	4	173	177
	Total	106	175	181

Positive Percent Agreement: (PPA)= 96.23% (90.62%～98.96%)*

Negative Percent Agreement: (NPA)= 98.86% (95.93%～99.86%)*

*95% Confidence Interval

Kappa= 0.9544 (0.9183～0.9905, highly consistent)

2.2 Analysis of Inconsistent Results

In this study, there were 6 specimens with test reagent results and reference reagent results that differed, including 4 false negative and 2 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 96.23%, the Negative agreement is 98.86%, The kappa value of the consistency analysis was 0.9544 (0.9183～0.9905). 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.