COVID-19 Antigen Detection Kit

Clinical Study Report

Name of in vitro diagnostic reagents used in the test: COVID-19 Antigen Detection Kit

Specifications: 25 Tests/Box

Start and end time of the test: August 24th, 2020- September 25th, 2020

December 17th, 2020 - December 29th, 2020

Applicant: New Gene (Hangzhou) Bioengineering Co., Ltd.

Address: Room 1606, Floor 16, Building 5, 688 Bin'an Road, Changhe Street, Binjiang District, Hangzhou City, Zhejiang Province, P. R. China

Report Date: January 4th, 2021

Summary

The COVID-19 Antigen Detection Kit developed by New Gene (Hangzhou) Bioengineering Co., Ltd. can quickly and qualitatively detect the spike glycoprotein and nucleocapsid protein of novel coronavirus (SARS-CoV-2) in human nasopharyngeal swab, oropharyngeal swab, and sputum samples. It can be used as a supplementary test for COVID-19 diagnosis.

According to the clinical trial plan, the COVID-19 Antigen Detection Kit or "test reagent", is to test sputum and swab samples from COVID-19 suspects. Test results are compared with another commercial SARS-CoV-2 nucleic acid detection kit with NMPA approval, which is defined as the "gold standard". The sensitivity, specificity, and total accuracy are used to evaluate the reliability of the test reagent in clinical applications.

Method: A collection of clinical samples are examined by the test reagent and the gold standard in parallel, to calculate the clinical sensitivity, clinical specificity, and total accuracy of the test reagent.

Standard of criteria for a qualified test reagent: Clinical sensitivity ≥80%, clinical specificity ≥90%, and total accuracy ≥90%.

Results: Compared to the gold standard, the clinical sensitivity of test reagent is 97.3% (95% CI: 95.4% - 98.5%), the clinical specificity is 99.0% (95% CI: 97.2% - 99.8%), and the total accuracy is 98.0% (95% CI: 96.7% - 98.8%). For different sample types, the sensitivity, specificity, and total accuracy are 98.0% (95% CI: 95.3% - 99.3%), 99.1% (95% CI: 95.2% - 100.0%), and 98.3% (95% CI: 96.4% - 99.4%) in nasopharyngeal swab samples, 95.7% (95% CI: 90.3% - 98.6%), 99.0% (95% CI: 94.6% - 100.0%), and 97.2% (95% CI: 94.1% - 99.0%) in oropharyngeal swab samples, and 97.3% (95% CI: 92.4% - 99.4%), 99.0% (95% CI: 94.4% - 100.0%), and 98.1% (95% CI: 95.2% - 99.5%) in sputum samples, respectively.

Conclusion: Compared to the gold standard reagent, the test reagent has reliable performance in diagnosing COVID-19 cases.

Acronyms

Test reagent: The COVID-19 Antigen Detection Kit developed by New Gene (Hangzhou) Bioengineering Co., Ltd.

SARS-CoV-2: Novel Corona Virus 2019

Main contents

Introduction

The novel coronavirus SARS-CoV-2 is the causative pathogen for the global pandemic of COVID-19. It is contagious in humans, either symptomatically or asymptomatically.

Based on current epidemic knowledge, the asymptomatic infection may last for 1 day to 14 days, mainly 3 days to 7 days. Symptoms of COVID-19 include fever, fatigue, and cough. Some patients also complains about nasal obstruction, runny nose, score throat, muscle aches, and diarrhea.

In response to the emergent market needs, New Gene (Hangzhou) Bioengineering Co., Ltd. has developed the COVID-19 Antigen Detection Kit. Since studies report that nucleocapsid (N protein) is the most abundant viral protein during infection, N protein is chosen as the detection target of this product to achieve its best sensitivity in clinical applications.

Production of the COVID-19 Antigen Detection Kit is implemented in Class 100,000 cleanrooms, by proficient operators. Multiple quality control processes are included in the manufacture procedures to examine the quality of raw materials, semi-finished products, and finished products. The construction of cleanrooms, personnel training, and manufacture practices are implemented under relevant laws and regulations.

To evaluate the clinical performance of the COVID-19 Antigen Detection Kit, the current clinical trial is jointly carried out by the applicant and multiple clinical sites. The applicant is responsible for providing reagents and training relevant personnel with the operating procedures and technical principles to minimize operational bias. The clinical sites are responsible for the collection and storage of clinical trial samples, the implementation of clinical trials, the compilation of clinical trial records, and sharing test results with the applicant.

Trial objective

The objective of current trial is to evaluate the performance of test reagent in clinical applications, using a NMPA approved commercial SARS-CoV-2 nucleic acid detection reagentas the “gold standard” reagent.

Trial design

Clinical samples for the current trial are collected by the clinical sites. Each sample is tested by both the test reagent and gold standard reagent. The clinical sensitivity, clinical specificity, and total accuracy of test reagent are calculated based on the test results.

Results and analysis

Determining the sample size.

Sample size for this trial was determined by the Buderer’s Formula as described in literature[11]. The Buderer’s Formula is described below

$N=Z\alpha/22\times SN\times(1-SN)/W2$,

where N stands for the sample size, Za/22 stands for the value from a standard normal table, with α being the type one error rate. SN stands for the sensitivity of “Test Reagent”. W stands for the width of sensitivity range.

In this trial, α=0.05, so Zα/2=1.96. SN=0.8, and W=(90%-70%)/2=0.1.

Therefore positive sample size NP=1.96^2×80%×(1-80%)/[(90%-70%)/2]^2 = 61.5, and the number of positive samples for this trial should be no less than 62.

Similarly, the minimal number of negative samples for 95% product specificity, ranging from 90% to 100%, is estimated as Negative Sample Size NN= 1.96^2×95%×(1-95%)/[(100%-90%)/2]^2 = 73.0.

In summary, the number of samples for this trial shall not be less than 135 for each sample type, of which the number of positive samples shall not be less than 62, and the number of negative samples shall not be less than 73.

Sample collection, storage, and transportation.

Clinical samples are collected from COVID-19 suspects, and kept frozen at -15°C~-25°C until used. Clinical samples included for this trial are randomly selected from COVID-19 suspects with symptoms. As the early period of viral infection in this trial is defined as the first week after symptom onset, samples collected more than one week after symptom onset are not included.

Each nasopharyngeal swab sample and oropharyngeal swab sample is eluted in 1 ml universal transport media (UTM). Each sputum sample is spiked into universal transport media (UTM) at the ratio of 1:3. Samples in UTM are heated at 56°C for 30 minutes to inactivate virus, and kept frozen at -70°C.

The “gold standard” reagent

Nucleic acid testing is currently the "gold standard" for COVID-19 diagnosis. A NMPA approved nucleic acid test reagent, namely the Novel Coronavirus (SARS-CoV-2) Real Time Multiplex RT-PCR Kit produced by Shanghai ZJ Bio-Tech Co., Ltd. is chosen as the “gold standard” reagent. It targets the ORF1ab gene, N gene, and E gene of the SARS-CoV-2, and is used as an auxiliary diagnosis and emergency reserve reagent for COVID-19.

Information of test reagent and the "gold standard" reagent.

Test reagent	COVID-19 Antigen Detection Kit		
Specification	25 Tests/Box	Lot No.	20200721-01 20200722-01
Period of Validity	1 year	Storage	2°C~30°C
Manufacturer	New Gene (Hangzhou) Bioengineering Co., Ltd.		

Gold Standard reagent	Novel Coronavirus (SARS-CoV-2) Real Time Multiplex RT-PCR Kit		
Approval Number	NMPA NO:20203400057		
Specification	50 Tests/Box		
Period of Validity	Six month	Storage:	Store at -20±5°C, keep away from light
Manufacturer	Shanghai ZJ Bio-Tech Co., Ltd.		

Operating Procedures.

As a retrospective study, clinical samples had been tested with the Gold Standard Reagent during the COVID-19 outbreak in China. Therefore, the operating procedures in this trial were mainly implemented on the test reagent. A brief description of the operating procedures are presented below.

1.Take sample solutions out of the -70°C refrigerator, put them still for 30 minutes to restore to room temperature.

2.Pipette a sample solution several times to mix the sediment with supernatant, transfer 50μL of the sample solution into 100μL of Sample Extraction Solution provided in the test reagent.

3.Three drops of sample mixture described above is loaded onto a test card. Keep the test card still for 15 minutes, and record the test result.

4.If the test result is invalid, the sample should be retested with another test card.

Statistical analysis method of clinical trial data

		Gold standard reagent		Total
		Positive	Negative	
Test reagent	Positive	a	b	a + b
	Negative	c	d	c + d
Total		a + c	b + d	a + b + c + d

Clinical sensitivity (%) = [a / (a + c)] ×100%

Clinical specificity (%) = [d / (b + d)] ×100%

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

The 95% Confidence Intervals are calculated following the binomial distribution.

Clinical trial results and analysis

Sample characterization

A collection of 788 sputum samples, including 361 nasopharyngeal swab samples, 218

oropharyngeal swab samples, and 209 sputum samples have been tested. These samples are taken from 788 suspected patients, of which 373 (47.3%) are female, and 415 (52.7%) are male. Their ages range from 17 to 88 years old, and are 46 years old on average. Cough (68.7%) and fever (56.9%) are the most common complained symptoms. Their sampling time is between Day 1 to Day 6 post onset, mainly on Day 2 (30.5%).

Result analysis

Product performance in different sample types

In 361 nasopharyngeal swab samples, the test reagent finds out 243 positive results, of which 242 samples are also reported positive by the gold standard reagent. One sample is reported positive only in test reagent, and another 5 samples are reported positive only in gold standard reagent. The other 113 samples are reported negative by both reagents. Testing results are presented in table below.

Nasopharyngeal Swab		Gold standard reagent		Total
		Positive	Negative	
Test reagent	Positive	242	1	243
	Negative	5	113	118
Total		247	114	361

Clinical sensitivity (%) = [242 / (242 + 5)] ×100% = 98.0%

95% confidence interval: 95.3% - 99.3%

Clinical specificity (%) = [113 / (1 + 113)] ×100% = 99.1%

95% confidence interval: 95.2% - 100.0%

Total accuracy (%) = [(242 + 113) / (242 + 1 + 5 + 113)] ×100% = 98.3%

95% confidence interval: 96.4% - 99.4%

In 218 oropharyngeal swab samples, the test reagent finds out 113 positive results, of which 112 samples are also reported positive by the gold standard reagent. One sample is reported positive only in test reagent, and another 5 samples are reported positive only in gold standard reagent. The other 100 samples are reported negative by both reagents. Testing results are presented in table below.

Oropharyngeal Swab		Gold standard reagent		Total
		Positive	Negative	
Test reagent	Positive	112	1	113
	Negative	5	100	105
Total		117	101	218

Clinical sensitivity (%) = [112 / (112 + 5)] ×100% = 95.7%
95% confidence interval: 90.3% - 98.6%
Clinical specificity (%) = [100 / (1 + 100)] ×100% = 99.0%
95% confidence interval: 94.6% - 100.0%
Total accuracy (%) = [(112 + 100) / (112 + 1 + 5 + 100)] ×100% = 97.2%
95% confidence interval: 94.1% - 99.0%

In 209 sputum samples, the test reagent finds out 110 positive results, of which 109 samples are reported positive by both reagents. One sample is reported positive only in test reagent, and another 3 samples are reported positive only in gold standard reagent. The other 96 samples are reported negative by both reagents. Testing results are presented in table below.

Sputum		Gold standard reagent		Total
		Positive	Negative	
Test reagent	Positive	109	1	110
	Negative	3	96	99
Total		112	97	209

Clinical sensitivity (%) = [109 / (109 + 3)] ×100% = 97.3%
95% confidence interval: 92.4% - 99.4%
Clinical specificity (%) = [96 / (1 + 96)] ×100% = 99.0%
95% confidence interval: 94.4% - 100.0%
Total accuracy (%) = [(109 + 96) / (109 + 1 + 3 + 96)] ×100% = 98.1%
95% confidence interval: 95.2% - 99.5%

Product performance in all sample types

The test reagent finds out 466 positive results, of which 463 samples are reported positive by both reagents. Three samples are reported positive only in test reagent, and another 13 samples are reported positive only in gold standard reagent. The other 309 samples are reported negative by both reagents. Testing results are presented in table below.

Nasopharyngeal Swab/Oropharyngeal Swab/Sputum		Gold standard reagent		Total
		Positive	Negative	
Test	Positive	463	3	466

reagent	Negative	13	309	322
Total		476	312	788

Clinical sensitivity (%) = [463 / (463 + 13)] ×100% = 97.3%
95% confidence interval: 95.4% - 98.5%
Clinical specificity (%) = [309 / (3 + 309)] ×100% = 99.0%
95% confidence interval: 97.2% - 99.8%
Total accuracy (%) = [(463 + 309) / (463 + 3 + 13 + 309)] ×100% = 98.0%
95% confidence interval: 96.7% - 98.8%

Discussion and conclusion

In this clinic trial, performance of the test reagent "COVID-19 Antigen Detection Kit" is evaluated on a collection of 788 clinical samples. Compared to a commercial Real Time Multiplex RT-PCR, the test reagent have shown sensitivity, specificity, and accuracy of 97.3% (95% CI: 95.4% - 98.5%), 99.0% (95% CI: 97.2% - 99.8%), and 98.0% (95% CI: 96.7% - 98.8%). For different sample types, the sensitivity, specificity, and total accuracy are 98.0% (95% CI: 95.3% - 99.3%), 99.1% (95% CI: 95.2% - 100.0%), and 98.3% (95% CI: 96.4% - 99.4%) in nasopharyngeal swab samples, 95.7% (95% CI: 90.3% - 98.6%), 99.0% (95% CI: 94.6% - 100.0%), and 97.2% (95% CI: 94.1% - 99.0%) in oropharyngeal swab samples, and 97.3% (95% CI: 92.4% - 99.4%), 99.0% (95% CI: 94.4% - 100.0%), and 98.1% (95% CI: 95.2% - 99.5%) in sputum samples, respectively. These results suggest a promising future of test reagent in clinical applications.

Although the antigen test directly detect viral proteins without amplification process, which makes it less sensitive than conventional nucleic acid tests, the antigen tests have two inherent advantages for clinical applications. The first advantage is short turn around time. Antigen tests usually take 20 to 30 minutes, making it possible for point-of-care testing (POCT). However, nucleic acid tests take 2 to 3 hours. In some countries, it may even take days to report a nucleic acid test result to suspects. Such a delay will absolutely hinder the control and prevention of disease transmission. The second advantage of antigen tests is easy-to-use. Antigen tests don't require large investment in laboratory construction, or complicated procedures like RNA extraction, and reagent preparation. The operators will be able to run a antigen test independently, with a one-hour simple training. Therefore, antigen tests are most suitable for large applications in resource limited areas.

In summary, the current clinical trial has proven the reliable performance of COVID-19 Antigen Detection Kit. This product is promising to assist the diagnosis of COVID-19 cases in large scales.