Novel Coronavirus Spike Glycoprotein Detection Kit

(Ligand-receptor Competitive Chromatography)

Clinical Study Report

Name of in vitro diagnostic reagents used in the test: Novel Coronavirus Spike Glycoprotein Detection Kit (Ligand-receptor Competitive Chromatography)

Specifications: 25 Tests/Box

Start and end time of the test: August 11th, 2020- September 2nd, 2020

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

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Summary

The Novel Coronavirus Spike Glycoprotein Detection Kit (Ligand-receptor Competitive Chromatography) developed by New Gene (Hangzhou) Bioengineering Co., Ltd. can quickly and qualitatively detect the spike glycoprotein and nucleocaspid protein of novel coronavirus (SARS-COV-2) in human sputum/stool samples. It can be used as a supplementary test for COVID-19 diagnosis.

According to the clinical trial plan, the Novel Coronavirus Spike Glycoprotein Detection kit (Ligand-receptor Competitive Chromatography) or "test reagent", is to test sputum/stool 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 agreement rate 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 agreement rate of the test reagent.

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

Results: Compared to the gold standard, the clinical sensitivity of test reagent is 96.3%, the clinical specificity is 98.8%, and the total agreement rate is 97.7%. For different sample types, the sensitivity, specificity, and total agreement rate are 96.4%, 99.5%, and 98.5% in sputum samples, 96.3%, 97.4%, and 96.8% in stool samples, respectively.

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

Acronyms

Test reagent: The Novel Coronavirus Spike Glycoprotein Detection kit (Ligand-receptor Competitive Chromatography) 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 Novel Coronavirus Spike Glycoprotein Detection kit (Ligand-receptor Competitive Chromatography). This product utilizes *in vitro* expressed human angiotensin I converting enzyme 2 (ACE2) protein to capture and visualize viral particles in test samples. Since ACE2 is the cellular receptor of SARS-COV-2 spike glycoprotein, it plays an indispensable role in disease infection and progression. Although various virus sub-types have emerged during the global pandemic, this product can reliably detect all contagious SARS-COV-2 sub-types, as long as they bind to ACE2 as the invasion target. Another system that detects the nucleocaspid protein (N protein) is also incorporated into the test line. As studies report that nucleocaspid is the most highly expressed viral protein *in vivo*, the incorporation of N protein detection system will enhance product sensitivity in clinical applications.

Production of the Novel Coronavirus Spike Glycoprotein Detection Kit (Ligand-receptor Competitive Chromatography) 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 Novel Coronavirus Spike Glycoprotein Detection kit (Ligand-receptor Competitive Chromatography), the current clinical trial is jointly carried out by the applicant and multiple clinical sites. The applicant is responsible for providing test reagents and training relevant personnel with the operating procedures and technical principles to minimize operational bias. The clinical site is responsible for the collection and storage of clinical trial samples, the implementation of clinical trials, the compilation of clinical trial records, and the composing of clinical trial reports.

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 reagent as the “gold standard”.

Trial design

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

Results and analysis

Determining the sample size.

Considering the uncertainty of obtaining positive samples, the number of samples for this clinical trial shall be no less than 140, of which the number of positive samples for each sample type

shall not be less than 35.

Sample collection, storage, and transportation.

Clinical samples are collected from COVID-19 suspects, and preserved in virus preservation solution. Keep the solution frozen at -15°C~-25°C until used.

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	Novel Coronavirus Spike Glycoprotein Detection kit (Ligand-receptor Competitive Chromatography)		
Specification	25 Tests/Box	Lot No.	20200715-01
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.		

Quality control methods

The clinical trial is strictly implemented in accordance with the corresponding instruction manual.

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

Clinical trial results and analysis

Sample characterization

A collection of 574 samples were tested with test reagents; including 325 sputum samples, and 249 stool samples. These samples are taken from 563 suspected patients, of which 272 (48.3%) are female, and 291 (51.7%) are male. Their ages range from 17 to 88 years old, and are 42 years old on average. Fever (57.0%) and Cough (53.1%) are the most common complained symptoms. Their first sampling time is between Day 1 to Day 7 post onset, mainly on Day 2 (22.0%). Eleven suspected patients have their second samples collected on Day 5 or Day 6.

Result analysis

Product performance in different sample types

In 325 sputum samples, the test reagent finds out 108 positive results, of which 107 samples are reported positive by both reagents. One sample is reported positive only in test reagent, and another 4 samples are reported positive only in gold standard reagent. The other 213 samples are reported negative by both reagents. Testing results are presented in table below.

Sputum		Gold standard reagent		Total
		Positive	Negative	
Test reagent	Positive	107	1	108
	Negative	4	213	217
Total		111	214	325

Clinical sensitivity (%) = [107 / (107 + 4)] ×100% = 96.4%

Clinical specificity (%) = [213 / (1 + 213)] ×100% = 99.5%

Total agreement rate (%) = [(107 + 213) / (107 + 1 + 4 + 213)] ×100% = 98.5%

In 249 stool samples, the test reagent finds out 132 positive results, of which 129 samples are reported positive by both reagents. Three samples are reported positive only in test reagent, and another 5 samples are reported positive only in gold standard reagent. The other 112 samples are reported negative by both reagents. Testing results are presented in table below.

Stool		Gold standard reagent		Total
		Positive	Negative	
Test	Positive	129	3	132

reagent	Negative	5	112	117
Total		134	115	249

Clinical sensitivity (%) = [129 / (129 + 5)] ×100% = 96.3%

Clinical specificity (%) = [112 / (3 + 112)] ×100% = 97.4%

Total agreement rate (%) = [(129 + 112) / (129 + 3 + 5 + 112)] ×100% = 96.8%

Product performance in all sample types

The test reagent finds out 240 positive results, of which 236 samples are reported positive by both reagents. Four samples are reported positive only in test reagent, and another 9 samples are reported positive only in gold standard reagent. The other 325 samples are reported negative by both reagents. Testing results are presented in table below.

Sputum/Stool		Gold standard reagent		Total
		Positive	Negative	
Test reagent	Positive	236	4	240
	Negative	9	325	334
Total		245	329	574

Clinical sensitivity (%) = [236 / (236 + 9)] ×100% = 96.3%

Clinical specificity (%) = [325 / (4 + 325)] ×100% = 98.8%

Total agreement rate (%) = [(236 + 325) / (236 + 4 + 9 + 325)] ×100% = 97.7%

Discussion and conclusion

In this clinic trial, performance of the test reagent “Novel Coronavirus Spike Glycoprotein Detection Kit (Ligand-receptor Competitive Chromatography)” is evaluated on a collection of 574 clinical samples. Compared to a commercial Real Time Multiplex RT-PCR, the test reagent have shown sensitivity, specificity, and agreement rate of 96.3%, 98.8%, and 97.7%. For different sample types, the sensitivity, specificity, and total agreement rate are 96.4%, 99.5%, and 98.5% in sputum samples, 96.3%, 97.4%, and 96.8% in stool 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 Novel Coronavirus Spike Glycoprotein Detection Kit (Ligand-receptor Competitive Chromatography). This product is promising to assist the diagnosis of COVID-19 cases in large scales.