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

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

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Summary

The COVID-19 Antigen Detection Kit developed by New Gene (Hangzhou) Bioengineering Co., Ltd. can quickly and qualitatively detect the nucleocaspid protein of novel coronavirus (SARS-COV-2) in human sputum/swab 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 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 96.1%, the clinical specificity is 99.0%, and the total agreement rate is 97.5%. For different sample types, the sensitivity, specificity, and total agreement rate are 97.3%, 99.0%, and 98.1% in sputum samples, 95.7%, 99.0%, and 97.2% in throat swab samples, 95.1%, 99.1%, and 97.2% in nasal swab 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 nucleocaspid (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 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 194, of which the number of positive samples shall not be less than 62 for each sample type.

Sample collection, storage, and transportation.

Clinical samples are collected from COVID-19 suspects, and kept 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	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.		

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 644 sputum samples, including 209 sputum samples, 218 throat swab samples, and 217 nasal swab samples have been tested. These samples are taken from 644 suspected patients, of which 274 (42.5%) are female, and 370 (57.5%) are male. Their ages range from 17 to 88 years old, and are 47 years old on average. Cough (73.6%) and fever (63.4%) are the most common complained symptoms. Their sampling time is between Day 1 to Day 6 post onset, mainly on Day 2 (32.0%).

Result analysis

Product performance in different sample types

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%

Clinical specificity (%) = [96 / (1 + 96)] ×100% = 99.0%

Total agreement rate (%) = [(109 + 96) / (109 + 1 + 3 + 96)] ×100% = 98.1%

In 218 throat 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.

Throat 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%

Clinical specificity (%) = [100 / (1 + 100)] ×100% = 99.0%

Total agreement rate (%) = [(112 + 100) / (112 + 1 + 5 + 100)] ×100% = 97.2%

In 217 nasal swab samples, the test reagent finds out 99 positive results, of which 98 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.

Nasal Swab		Gold standard reagent		Total
		Positive	Negative	
Test reagent	Positive	98	1	99
	Negative	5	113	118
Total		103	114	217

Clinical sensitivity (%) = [98 / (98 + 5)] ×100% = 95.1%

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

Total agreement rate (%) = [(98 + 113) / (98 + 1 + 5 + 113)] ×100% = 97.2%

Product performance in all sample types

The test reagent finds out 322 positive results, of which 319 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.

Sputum/Throat Swab/Nasal Swab		Gold standard reagent		Total
		Positive	Negative	
Test reagent	Positive	319	3	322
	Negative	13	309	322
Total		332	312	644

Clinical sensitivity (%) = [319 / (319 + 13)] ×100% = 96.1%

Clinical specificity (%) = [309 / (3 + 309)] ×100% = 99.0%

Total agreement rate (%) = [(319 + 309) / (319 + 3 + 13 + 309)] ×100% = 97.5%

Discussion and conclusion

In this clinic trial, performance of the test reagent “COVID-19 Antigen Detection Kit” is evaluated on a collection of 644 clinical samples. Compared to a commercial Real Time Multiplex RT-PCR, the test reagent have shown sensitivity, specificity, and agreement rate of 96.1%, 99.0%, and 97.5%. For different sample types, the sensitivity, specificity, and total agreement rate are 97.3%, 99.0%, and 98.1% in sputum samples, 95.7%, 99.0%, and 97.2% in throat swab samples, 95.1%, 99.1%, and 97.2% in nasal swab 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.