COVID-19 Neutralizing Antibody Detection Kit

Clinical Study Report

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

Specifications: 25 Tests/Box

Start and end time of the test: November 6th, 2020 - November 27th, 2020

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

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Summary

The COVID-19 Neutralizing Antibody Detection Kit developed by New Gene (Hangzhou) Bioengineering Co., Ltd. can quickly and qualitatively detect the neutralizing antibodies blocking the RBD region of SARS-CoV-2 in human blood/serum/plasma samples. It can be used as a supplementary test to detect the neutralizing antibodies in patients recovered from COVID-19, or healthy people who have received SARS-CoV-2 vaccination.

According to the clinical trial plan, the COVID-19 Neutralizing Antibody Detection Kit or "test reagent", is to test plasma samples from confirmed COVID-19 patients and healthy people. The sensitivity, specificity, and total accuracy are used to evaluate the reliability of test reagent in clinical applications.

Method: A collection of plasma samples from confirmed COVID-19 patients, and healthy donors are examined by the test reagent, to calculate its sensitivity, specificity, and total accuracy in clinical applications.

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

Results: In 137 plasma samples from recovered COVID-19 patients, the sensitivity of Test Reagent is 97.8% (95% CI: 93.7% - 99.5%). In 231 plasma samples from healthy donors, the specificity of Test Reagent is 99.1% (95% CI: 96.9% - 99.9%). The total accuracy of Test Reagent is 98.6% (95% CI: 96.9% - 99.6%).

Conclusion: The test reagent has reliable performance in detecting COVID-19 neutralizing antibodies in human samples.

Acronyms

Test reagent: The COVID-19 Neutralizing Antibody 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 needs, New Gene (Hangzhou) Bioengineering Co., Ltd. has developed the COVID-19 Neutralizing Antibody Detection Kit. It detects neutralizing antibodies in patients recovered from COVID-19, or healthy people who have received SARS-CoV-2

vaccination.

Production of the COVID-19 Neutralizing Antibody 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 Neutralizing Antibody Detection Kit, the current clinical trial is jointly carried out by the applicant and a clinical site. The applicant is responsible for providing test reagents and training courses to relevant personnel to minimize operational bias. The clinical site is responsible for the collection and storage of test samples, the implementation of testing procedures, and the compilation of test records.

Trial objective

The objective of current trial is to evaluate the performance of test reagent in clinical applications.

Trial design

Clinical samples for the current trial are collected by the clinical site, from confirmed COVID-19 patients and healthy donors. The sensitivity, specificity, and total accuracy of test reagent are calculated from the test results.

Results and analysis

Determining the sample size.

Based on the product performance data in R&D records, we speculate that the sensitivity of Test Reagent may reach 95%, ranging between 90%-100%, and the specificity may reach 95%, ranging between 90%-100%.

Sample size for this trial is determined by the Buderer's Formula as explained in literature. The Buderer's Formula is described below

$N=Z_{\alpha/2}^2 \times SN \times (1-SN)/W^2$,

where N stands for the sample size, $Z_{\alpha/2}$ 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 $Z_{\alpha/2}$=1.96. SN=0.95, and W=(100%-90%)/2=0.05.

Therefore positive sample size N_P=1.96^2×95%×(1-95%)/[(100%-90%)/2]^2=73, and the number of positive samples for this trial should be no less than 73.

Similarly, the minimal number of negative samples for 95% product specificity, ranging from 90% to 100%, is estimated as Negative Sample Size N_N= 1.96^2×95%×(1-95%)/[(100%-90%)/2]^2=73.

In summary, the number of samples for this trial shall not be less than 146, of which the number of positive samples and negative samples shall not be less than 73.

Sample collection, storage, and transportation.

Sample type included in this trial is plasma. Positive plasma samples are collected from confirmed COVID-19 patients (patients with positive RT-PCR results and symptoms of COVID-19), from whom plasma samples are collected at least 4 weeks after symptom onset. The negative plasma samples are selected from the blood bank, where samples are donated by healthy donors, and verified by a commercial RT-PCR kit to exclude plasma from any potentially SARS-CoV-2 infected donors.

Information of test reagent and the other reagents used in this trial.

Test reagent	COVID-19 Neutralizing Antibody Detection Kit		
Specification	25 Tests/Box	Lot No.	20201028-01
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.

1. Take plasma samples out of the -20°C freezer, put them into 37°C water bath. Check the plasma samples every 3 minutes until they are fully thawed.
2. For each plasma sample, transfer 20μL of the plasma sample into the sample loading hole on a test card.
3. Immediately add 70μL of antibody detection buffer provided in the Test Reagent into the samples loading hole on the test card. Keep the test card still for 15 to 30 minutes, and record the test result.
4. If a test result is invalid, the corresponding sample should be retested with another test card.

Interpretation of Results

Positive(+): C line appears while no T line appears in 15 to 30 minutes after sample loading, or the T line appears with lowered color intensity compared to the C line. It indicates that there are

neutralizing antibodies in the specimen.

Negative(-): Both of T and C line appear in 15 to 30 minutes, and the color intensity of the T line is similar to or higher than that of C line. It indicates that there are no neutralizing antibodies in the specimen.

Invalid: As long as the C line does not appear, it indicates that the test result is invalid, and should retest with another test card.

Statistical analysis method of clinical trial data

		Plasma Samples		Total
		Patients	Healthy Donors	
Test reagent	Positive	a	b	a + b
	Negative	c	d	c + d
Total		a + c	b + d	a + b + c + d

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

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

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

95% confidence intervals are calculated following the binomial distribution.

Clinical trial results and analysis

Sample characterization

A collection of 368 plasma samples, which are from 137 confirmed COVID-19 patients and 231 healthy donors, have been examined with the test reagent. Among these donors, 205 (55.7%) are female, and 163 (44.3%) are male. Their ages range from 19 to 68 years old, and are 38 years old on average. Their sampling times for confirmed patients are between Day 31 to Day 98 post onset.

Result analysis

For neutralizing detection, the Test Reagent finds out 136 positive results, of which 134 samples are from COVID-19 patients. Two samples from healthy donors are reported positive by Test Reagent, and another 3 samples from COVID-19 patients are reported negative by Test Reagent. The other 229 samples from healthy donors are reported negative by Test Reagent.

Neutralizing Antibody		Plasma Samples		Total
		Patients	Healthy Donors	
Test reagent	Positive	134	2	136
	Negative	3	229	232
Total		137	231	368

Sensitivity (%) = [134 / (134 + 3)] × 100% = 97.8%

95% Confidence Intervals: 93.7% - 99.5%

Specificity (%) = [229 / (2 + 229)] × 100% = 99.1%

95% Confidence Intervals: 96.9% - 99.9%

Total accuracy (%) = [(134 + 229) / (134 + 2 + 3 + 229)] × 100% = 98.6%

95% Confidence Intervals: 96.9% - 99.6%

Discussion and conclusion

In this clinic trial, performance of the test reagent "COVID-19 Neutralizing Antibody Detection Kit" is evaluated on a collection of 368 plasma samples. In 137 plasma samples from recovered COVID-19 patients, the sensitivity of Test Reagent is 97.8% (95% CI: 93.7% - 99.5%). In 231 plasma samples from healthy donors, the specificity of Test Reagent is 99.1% (95% CI: 96.9% - 99.9%). The total accuracy of Test Reagent is 98.6% (95% CI: 96.9% - 99.6%). These results indicate a reliable performance of Test Reagent in clinical applications.

Unlike the other diagnostic reagents and kits, the COVID-19 Neutralizing Antibody Detection Kit is intended to detect the antibodies that protect recovered patients from reinfection with the SARS-CoV-2 virus, as well as the protective antibodies in people who received vaccinations. As the large scale vaccinations has started all over the world, monitoring the level of neutralizing antibodies in general populations will of great importance in disease control and decision making of government policies. As a rapid, easy-to-use immunoassay kit, this product will facilitate the procedures of neutralizing antibody detection, and enhance the test throughput to meet public needs.

In summary, the current clinical trial has proven the reliable performance of COVID-19 Neutralizing Antibody Detection Kit in detecting the protective neutralizing antibodies in human samples.