
NEWGENE
Bioengineering
Place pour la dérogation CE
1 Test
Kit autotest antigénique COVID-19
COVID-19 Antigen Detection Kit
Réservé au Diagnostic In Vitro
Veuillez lire scrupuleusement la notice à l'intérieur de la boîte avant utilisation
IVD
2°C
30°C
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Certificate of Registration

QUALITY MANAGEMENT SYSTEM - ISO 13485:2016

This is to certify that:

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and operates a Quality Management System which complies with the requirements of ISO 13485:2016 for the following scope:

Design and Development, Manufacture and Distribution of In-vitro Diagnostic Rapid Test Kit of Drug Abuse, Manufacture and Distribution of In-vitro Diagnostic Rapid Test Kit of Infectious Diseases.
药物滥用体外诊断快速检测试剂盒的设计，开发，制造和销售，传染病体外诊断快速检测试剂盒的制造和销售。

For and on behalf of BSI:

Gary E Slack, Senior Vice President - Medical Devices

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Performance Validation

of

a Rapid COVID-19 Antigen Test

as

Supervised Self-Testing Tool

19/01/2021- 05/03/2021

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Summary

The COVID-19 Antigen Detection Kit, hereafter called the Test Reagent in this document, developed by New Gene Bioengineering, is intended for rapid self-test of SARS-CoV-2 antigen in human nasal swab samples. Performance of this rapid self-testing tool is validated in this study.

Method: As planned in the study design, a nasopharyngeal swab sample collected from COVID-19 suspect by physicians, and a nasal swab sample collected by the suspects themselves were tested by the Reference Reagent and the Test Reagent, respectively. The sensitivity, and specificity of Test Reagent were calculated to evaluate its feasibility in assisting COVID-19 diagnosis under a supervised self-test environment.

Results: Compared to the Reference Reagent, The sensitivity, and specificity was 90.2% (95% CI: 84.3% - 94.4%), and 99.1% (95% CI: 96.7% - 99.9%), respectively, when using the Test Reagent as a rapid antigen self-test. The sensitivity of Test Reagent was 100.0% (95% CI: 88.4% - 100.0%) in samples from high viral load patients (Ct≤25), 97.7% (95% CI: 87.7% - 99.9%) in samples from moderate viral load patients (25<Ct≤30), 90.2% (95% CI: 76.9% - 97.3%) in samples from low viral load patients (30<Ct≤35), and 74.4% (95% CI: 57.9% - 87.0%) in samples from very low viral load patients (Ct>35).. Kappa consistency test returned kappa value 0.90 (95% CI: 0.86 - 0.95), suggesting good consistency between Test Reagent and Reference Reagent.

Conclusion: The results of the Test Reagent and the Reference Reagent had a high agreement rate, indicating that the Test Reagent can be used to assist the diagnosis of COVID-19 cases in self-test settings.

Acronyms

1. Test Reagents: COVID-19 Antigen Detection Kit developed by New Gene (Hangzhou) Bioengineering Co., Ltd.
2. Reference Reagent: The Novel Coronavirus (SARS-CoV-2) Real Time Multiplex RT-PCR Kit by Shanghai ZJ Bio-Tech Co., Ltd.
3. 2019-nCoV: Novel Coronavirus 2019
4. COVID-19: Corona Virus Disease 2019
5. SARS: Severe Acute Respiratory Syndrome coronavirus
6. MERS: Middle East Respiratory Syndrome
7. NMPA: National Medical Products Administration
8. IRB: Institutional Review Board
9. ORF1a/b: Open Reading Frame 1a and Open Reading Frame 1b of 2019-nCoV
10. N gene: Nucleocapsid gene of 2019-nCoV
11. E gene: Envelop gene of 2019-nCoV
12. UTM: universal transport media

Main contents

1. Introduction

Coronavirus disease 2019 (COVID-19) is caused by the SARS-CoV-2 virus. This virus belongs to the beta-coronavirus class, along with the Severe Acute Respiratory Syndrome coronavirus (SARS), and Middle East Respiratory Syndrome (MERS) coronavirus[1]. By the end of January 2021, more than 98.2 million cases, and a death toll of more than 2.1 million have been reported in nearly all countries[2]. The most common symptoms of COVID-19 are fever, dry cough, and fatigue. Other symptoms that are less common and may affect some patients include: loss of taste or smell, nasal congestion, conjunctivitis (also known as red eyes), sore throat, headache, muscle or joint pain, different types of skin rash, nausea or vomiting, diarrhea, chills or dizziness[3, 4].

SARS-CoV-2 is thought to spread mainly through close contact among people. Air borne aerosols carrying the virus spread from one person to another through speaking, laughing, coughing, sneezing, and etc[5, 6]. Asymptomatically infected people can also spread their virus to the others[7]. This virus appears to spread more efficiently than influenza but not as efficiently as measles, which is among the most contagious viruses known to affect people.

Breaking off the disease transmission chain has been a vital key to successful disease control. To achieve this goal, people have to follow social distancing strictly and quarantine virus carriers as early as possible[8, 9]. Although hundreds of diagnostic tests have been invented, these tested are mainly restricted to processional use in medical laboratories[10]. In resource limited areas, the test capacity of medical labs can be easily overwhelmed by the tremendous need for viral testing, which in turn hinder the early detection of infected people. Therefore, a rapid, accurate, and easy-to-use screening tool that allowed for self-test could be a total game changer in combating COVID-19.

In the present study, non-professional users (untrained laypeople) were encouraged to perform the entire

testing procedures from sample collection to result interpretation under the observation of a trained professional health-care provider. Test results from non-professional users were compared to those from reference testing method in professional labs. Sensitivity and specificity were calculated to evaluate the feasibility to use the rapid antigen test as a supervised self-test tool in non-professional users.

2. Objective of Clinical Trial

The objective of current trial was to evaluate the performance of Test Reagent as a supervised rapid antigen self-test in early diagnosis of COVID-19.

3. Trial management

This clinical trial was jointly implemented by the applicant (New Gene (Hangzhou) Bioengineering Co., Ltd.), and the clinical institution (Haerbin Center for Disease Control and Prevention). The applicant was responsible for providing Test Reagents that manufactured under standard procedures, as well as supplying other consumables required for the trial. The clinical institutions were responsible for the collection and storage of clinical samples, the implementation of sample testing, data collection, statistical analysis, and the writing of clinical trial reports.

3.1 Quality Control of the Laboratory

To minimize operational biases in the trial, a kick-off meeting was hold before the trial was started. In the kick-off meeting, the objective, study process (participant inclusion, sample collection, sample testing, data analysis, and report writing), and responsibility assignation were introduced to relevant members in the clinical institution.

The professional sample testing was implemented in standard medical laboratory with isolated areas for sample processing, reagent preparation, and nucleic acid amplification. Besides, positive controls and negative controls were also included in each test run, in case of aerosol contamination or invalid test results.

3.2 Management of Data/Statistics

Sample testing results of both Test Reagent and Reference Reagent were recorded by the Data Manager from Haerbin CDC, in a daily updated brief report. This report was further reviewed by the Principle Investigator from the Haerbin CDC.

Statistical analysis was also performed by the Data Manager under the supervision of Principle Investigator. With the approval of Principle Investigator, analytical results were also shared with the Clinical Research Coordinator from the applicant.

4. Design of Clinical Trial

4.1 Description of the Overall Design of the Trial

This trial was to evaluate the product performance of Test Reagent as self-test. Indexes of product performance included sensitivity and specificity. To get an accurate estimation of these indexes, a

Reference Reagent was adopted as the diagnostic criteria. In this trial, a nasal swab sample and a nasopharyngeal swab sample from the same COVID-19 suspect were tested by the Test Reagent and the Reference Reagent, respectively. Testing results were summarized in a 2×2 table to calculate the performance indexes of Test Reagent.

4.2 Trial Design and Analytical Methods

4.2.1 Expected product performance

Based on the product performance data in R&D records, we speculated that when the product was operated by non-professional users as self-test, the sensitivity of Test Reagent may reach 80%, ranging between 70% and 90%, and the specificity may reach 95%, ranging between 90%-100%.

4.2.2 Sample Size Estimation.

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

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

where N stands for the sample size, $Z_{\alpha/2}^2$ 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, $\alpha=0.05$, so $Z_{\alpha/2}=1.96$. SN=0.8, and W=(90%-70%)/2=0.1.

Therefore positive sample size N_P=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 N_N= 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, 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.

4.2.3 Criteria for Sample Inclusion.

As the early period of viral infection in this trial was defined as the first week after symptom onset, COVID-19 patients who had symptom onset in the last 7 days, and quarantined COVID-19 suspects who had close contact with at least one confirmed patient in the last month but without any symptoms were included in this study.

The included positive samples should also reflect the distribution of different viral load in real samples. Specifically, the proportion of high viral load samples ($Ct\leq25$) should be ≤25%, the proportion of moderate viral load samples ($25<Ct\leq30$) should be ≥25%, the proportion of low viral load samples ($30<Ct\leq35$) should be ≥25%, and the proportion of very low viral load samples ($Ct>35$) should be ≥25%

4.2.4 Sample Collection, Storage, etc.

The first nasopharyngeal swab samples were collected by physicians from suspected COVID-19 patients, and preserved at 2°C~8°C for no more than 4 hours before RT-PCR testing. Testing results with the

Reference Reagent were reported within 8 hours post sample collection.

The second nasal swab were collected by the suspected COVID-19 patients themselves within 24 hours after the first sample collection. The sample was immediately tested by the suspected COVID-19 patients themselves following the instructions for use.

For children under the age of 14, the sample was collected and tested by their parents or legal guardians.

For users above 14 years old, the sample was collected and tested by themselves. If the users were not able to collect samples or perform tests, their legal guardians would help them with the tests. A Data Manager was also present at the testing site, he/she would not interfere with the operations, but only to prevent any potential injuries due to incorrect operations.

4.2.5 Operating Procedures.

1. The first nasopharyngeal swab sample was eluted in 0.6mL Universal Transport Media (UTM). 0.3mL of the UTM was used for nucleic acid extraction and tested by the Reference Reagent.
2. The second nasal swab sample was eluted in 0.3mL Sample Extraction Solution. Three drops of sample solution was loaded onto a test card. Keep the test card still for 15 - 30 minutes, and record the test result.
3. If the test result of a nasal swab sample was invalid, the sample should be retested with another test card.

4.2.6 Establishment of the Reference 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 by Shanghai ZJ Bio-Tech Co., Ltd., is chosen as the Reference reagent. It targets the ORF1a/b, E, and N gene of the SARS-CoV-2, and was used as an auxiliary diagnosis and emergency reserve reagent for COVID-19.

4.2.7 Information about the Test Reagent and the Reference Reagent

The basic information of clinical trial reagents and the registration status of the Reference Reagent.

Test Reagent	COVID-19 Antigen Detection Kit		
Specification	25 Tests/Box	Lot:	20201115-01
Period of Validity	1 year	Storage:	2°C~30°C
Manufacturer	New Gene (Hangzhou) Bioengineering Co., Ltd.		

Reference Reagent	Novel Coronavirus (SARS-CoV-2) Real Time Multiplex RT-PCR Kit		
Approval Number	NMPA NO:20203400057		
Specification	50 Tests/Box		
Period of Validity	6 months	Storage:	Store at -20±5°C, keep away from light

Manufacturer	Shanghai ZJ Bio-Tech Co., Ltd.

4.2.8 Time Table

15th January, 2021	Kick off meeting
16th January, 2021 ~ 17th January, 2021	Transportation of reagents and consumables
19th January, 2021 ~ 5th March, 2021	Sample testing, data collection and analysis

4.2.9 Statistical Analysis Methods

Test results were summarized in a 2×2 table to calculate the product performance indexes, including sensitivity, and specificity. Table format and formulas for product performance indexes were presented below.

		Reference 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

Sensitivity (%) = [a / (a + c)] ×100%

Specificity (%) = [d / (b + d)] ×100%

95% confidence intervals are calculated following the binomial distribution.

For positive samples reported by the Reference Reagent, the sensitivity of Test Reagent was also analyzed in groups of different viral load (high viral load: Ct≤25; moderate viral load: 25<Ct≤30; low viral load: 30<Ct≤35; very low viral load: Ct>35.)

Reference Reagent	Test Reagent	
	Positive	Negative
High: Ct≤25	H1	H2
Moderate: 25<Ct≤30	M1	M2
Low: 30<Ct≤35	L1	L2
Very Low: Ct>35	VL1	VL2
Total	H1+M1+L1+VL1	H2+M2+L2+VL2

Sensitivity - High Viral Load (%) = [H1 / (H1 + H2)] ×100%

Sensitivity - Moderate Viral Load (%) = [M1 / (M1 + M2)] ×100%

Sensitivity - Low Viral Load (%) = [L1 / (L1 + L2)] ×100%

Sensitivity - Very Low Viral Load (%) = [VL1 / (VL1 + VL2)] ×100%

95% confidence intervals are calculated following the binomial distribution.

The kappa consistency test was also used to evaluate the consistency of results between the Test Reagent

and the Reference Reagent. Statistical analysis was performed in software IBM SPSS 20.0.

5. Clinical Trial Results and Analysis

5.1 Sample characterization

A collection of 371 nasal swab samples and of 371 nasopharyngeal swab samples were examined by the Test Reagent and the Reference Reagent, respectively. These samples were taken from 371 suspected patients, of which 197 (53.1%) were female, and 174 (46.9%) were male. Their ages ranged from 7 to 85 years old, and were 42 years old on average. The sampling time was between Day 1 to Day 7 post symptom onset or quarantine, mainly on Day 3 (34.2%).

5.2 Product Performance Calculation

In 371 COVID-19 suspects, both the Test Reagent and the Reference Reagent found out 138 positive cases. Two cases were reported positive only in Test Reagent, and 15 cases were reported positive only in Reference Reagent. The other 216 cases were reported negative by both reagents. There were no invalid results reported in the study. Testing results were summarized in table below.

		Reference Reagent		Total
		Positive	Negative	
Test Reagent	Positive	138	2	140
	Negative	15	216	231
Total		153	218	371

Compared to the Reference Reagent, the performance indexes of Test Reagent were calculated as follows.

Sensitivity (%) = [138 / (138 + 15)] ×100% = 90.2%;

95% confidence interval: 84.3% - 94.4%;

Specificity (%) = [229 / (3 + 229)] ×100% = 99.1%;

95% confidence interval: 96.7% - 99.9%;

Proportions of samples from different viral load patients are: 19.6% from high viral load patients (Ct≤25), 28.1% from moderate viral load patients (25<Ct≤30), 26.8% from low viral load patients (30<Ct≤35), and 25.5% from very low viral load patients (Ct>35).

Reference Reagent	Test Reagent	
	Positive	Negative
High: Ct≤25	30	0
Moderate: 25<Ct≤30	42	1
Low: 30<Ct≤35	37	4
Very Low: Ct>35	29	10

Total	138	15

Sensitivity - High Viral Load (%) = [30 / (30 + 0)] ×100% = 100.0%

95% confidence interval: 88.4% - 100.0%

Sensitivity - Moderate Viral Load (%) = [42 / (42 + 1)] ×100% = 97.7%

95% confidence interval: 87.7% - 99.9%

Sensitivity - Low Viral Load (%) = [37 / (37 + 4)] ×100% = 90.2%

95% confidence interval: 76.9% - 97.3%

Sensitivity - Very Low Viral Load (%) = [29 / (29 + 10)] ×100% = 74.4%

95% confidence interval: 57.9% - 87.0%

Results of kappa consistency test showed kappa value=0.90, 95% confidence interval 0.86 - 0.95. Kappa value > 0.75, suggesting good consistency between Test Reagent and Reference Reagent.

6. Discussion and Conclusion

In this study, performance of the COVID-19 Antigen Detection Kit was evaluated on 371 COVID-19 suspects. The sensitivity, and specificity was 90.2% (95% CI: 84.3% - 94.4%), and 99.1% (95% CI: 96.7% - 99.9%), respectively, when using the Test Reagent as a rapid antigen self-test. The sensitivity of Test Reagent was 100.0% (95% CI: 88.4% - 100.0%) in samples from high viral load patients (Ct≤25), 97.7% (95% CI: 87.7% - 99.9%) in samples from moderate viral load patients (25<Ct≤30), 90.2% (95% CI: 76.9% - 97.3%) in samples from low viral load patients (30<Ct≤35), and 74.4% (95% CI: 57.9% - 87.0%) in samples from very low viral load patients (Ct>35). Kappa consistency test returned kappa value 0.90 (95% CI: 0.86 - 0.95), suggesting good consistency between Test Reagent and Reference Reagent.

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, however, nucleic acid tests take 2 to 3 hours. In some countries, it may even take days to report a nucleic acid test result. Such a delay will absolutely hinder the control and prevention of disease transmission. Products with short turn around time completely change the strategies to control COVID-19. When using as a self test, antigen tests can be implemented in a more frequent way than nucleic acid tests, like twice or three times a week, to identify the most contagious patients in community as early as possible. This strategy may help to stop the transmission of COVID-19 in the early period of infection.

The second advantage of antigen tests is easy-to-use. Antigen tests don't require large investments on laboratory construction, or complicated procedures like RNA extraction, and reagent preparation. With the instructions for use, non-professional users will be able to run the test independently. Therefore, antigen tests are most suitable for large scale applications in community setting rather than centralized laboratory settings.

In summary, The COVID-19 Antigen Detection Kit has shown satisfying sensitivity, and specificity in the present clinical trial. It can be used as a rapid self-test tool to assist the early diagnosis of COVID-19

cases for non-professional users.

Notes On Special Circumstances in Clinical Trials

No special circumstances reported.

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Data Manager: [signature] Date: 2021.03.09

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