Clinical Study Report

Product Name: Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Antigen Detection Kit (Colloidal Gold-Based)

Specification: 1 test/kit, 5 tests/kit, 20 tests/kit

Proposed Research Time: February 2021 to June 2021

Site 1

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Site 2

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Institution and Researchers involved in Clinical Study

Institution	Researchers	Title	Responsibility
Alabiso Lab – Salus, Life Brain Group	Graziella Calugi	Principal Investigator	Prepare and review the protocol Report writing
	Marco Compagnoni	Statistician	Statistic analysis
	Maria Loredana Frassanito	Monitor	Quality Control
	+ 50	Sample Collector	Sample collection, blinding
	+ 50	Operator	Sample detection

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Locus Medicus Lab	Dr. Georgoulias Georgios	Principal Investigator	Prepare and review the protocol Report writing
	Nikolaos Manias	Statistician	Statistic analysis
	Papadimitropoulos Miltos	Monitor	Quality Control
	Nikolopoulos Dimitris	Sample Collector	Sample collection, blinding
	Draka Calliope	Operator	Sample detection

CONTENT

Abstract

（1）Overall distribution of samples

Sample	Positive	Negative
Number of cases	269	1327

（2） The sensitivity or specificity of this kit is as follows:

Vazyme SARS-CoV-2 Ag Test Kit	RT-PCR		
	Positive	Negative	Total
Positive	266	0	266
Negative	3	1327	1330
Total	269	1327	1596
Sensitivity	98.88 % (95% Cl: 96.77 % to 99.62 %)		
Specificity	100.00 % (95% Cl: 99.71 % to 100.00 %)		
Total coincidence rate	99.81 % (95% Cl: 99.45 % to 99.94 %)		

Clinical Study Report on Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Antigen Detection Kit (Colloidal Gold-Based)

I. Product information

1. Product Name

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Antigen Detection Kit (Colloidal Gold-Based)

3. Intended Use

This kit is applicable to clinical qualitative detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antigen in human nasal swab, nasopharyngeal swab, oropharyngeal swab and saliva samples in vitro for professional use. For self-testing, it is applicable in nasal swab samples only.

4. Specification

The kit has three specifications, 1 test/kit, 5 tests/kit, 20 tests/kit. For this clinical study, we use 20 tests/kit.

5. Storage Conditions and Validity Period

The kit is stored in a sealed state at 4° C to 30° C away from light for a validity period of 18 months. Once the package of the Test Cassette is opened (4° C~30° C, humidity < 65 %), it must be used within 1 hour.

6. PRINCIPLE OF INSPECTION

The double antibody sandwich method is adopted for this product to implement determination in the form of solid phase immunochromatography. The sample to be tested diffuses upward by capillary force at the sampling end, and when passing by the marker pad, the SARS-CoV-2 antigen in the sample is combined with the antibody on the marker pad to form a colloidal gold antibody-antigen complex. The complex continues to spread with the sample to reach the nitrocellulose membrane and is intercepted by the T-line (test line) coated with antibody, and the complex is captured to form an immune complex of colloidal gold antibody conjugates-antigen-coating antibody. The remaining colloidal gold conjugates continue to

ascend and are combined with C-line (quality control line), indicating completion of the reaction.

7. Operation Steps

1) The reagent card shall be fully returned to the room temperature before use.

2) The detection card shall be placed on a horizontal, dry surface after being taken out from the aluminum foil package.

3) The collected sample shall be added to sample eluent for eluting and mixing evenly.

4) Mix the sample by gently turning the tube upside down, squeeze the tube to add 4 drops (about 80 μL) to the sample well of the Test Cassette, and start counting.

5) The test cassette shall be strictly observed 10 minutes after the start of the detection, and the results shall be determined. The results observed after 15 minutes will be invalid.

II. Background of clinical study

Common signs of people infected with COVID-19 include respiratory symptoms, fever, cough, shortness of breath, and wheezing. In severe cases, the infection can lead to pneumonia, severe acute respiratory syndrome, kidney failure, and even death. Although nucleic acid amplification testing is currently recommended by WHO as the only approach to clinical diagnostic testing for disease confirmation, its low speed limitations, complicated operating procedures, and the need for specific tools limit its flexibility in several application scenarios. Thus, the simpler, faster and cheaper immunodiagnostic method has become an effective alternative to nucleic acid detection. Antigen detection is a type of immunodiagnostic method that targets viral antigens using highly specific, high affinity antibodies. A positive antigen test can directly indicate COVID-19 infection.

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Antigen Detection Kit (Colloidal Gold-Based) is a product independently developed by Nanjing Vazyme Medical Technology Co., Ltd., which has passed the registration test of China Institute of Food and Drug Control, with qualified results, and currently, the clinical study needs to be conducted according to *ISO 20916:2019 In vitro diagnostic medical devices — Clinical performance studies using specimens from human subjects — Good study practice; Current Performance Of COVID-19 Test Methods and Devices and Proposed Performance Criteria.（16 April 2020）* Comparative research needs to be carried out between Vazyme reagent and COVID-19 nucleic acid detection reagents which have already been marketed. The clinical performance of

the reagent produced by Nanjing Vazyme Medical Technology Co., Ltd. needs to be confirmed as well, to formulate the clinical protocol.

III. Purpose of clinical study

The purpose is to evaluate the performance of the Nanjing Vazyme Medical Technology Co., Ltd. Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Antigen Detection Kit (Colloidal Gold-Based) (hereinafter referred to as "assessing reagent") produced by the Nanjing Vazyme Medical Technology Co., Ltd., compared with the listed SARS-CoV-2 nucleic acid detection reagent (Comparator).

IV. Research Design

This research follows *ISO 20916:2019 In vitro diagnostic medical devices — Clinical performance studies using specimens from human subjects — Good study practice; Current Performance Of COVID-19 Test Methods and Devices and Proposed Performance Criteria.（16 April 2020）*, comparing and analyzing the data results, to evaluate the clinical applicability of the test reagent. Statistics on the test results are made, and the statistical results are given to complete the clinical report.

This kit is applicable to clinical qualitative detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antigen in human nasal swab, nasopharyngeal swab, oropharyngeal swab and saliva samples in vitro for professional use. For self-testing, it is applicable in nasal swab samples only. Nasal swab samples were only used in this clinical study.

Aimed population: within the early stage of respiratory symptoms onset or other reasons to suspect COVID-19 infection.

All samples shall have the corresponding basic clinical information, including : sample number, gender, age and clinical diagnosis information.

1. Sample size

According to *In vitro diagnostics detecting SARS-CoV-2 nucleic acid and rapid diagnostics tests detecting SARS-CoV-2 antigens (WHO); Antigen Template for Test Developers (US-FDA) ;Current Performance Of COVID-19 Test Methods and Devices and Proposed Performance Criteria.（16 April 2020）*, the minimum amount of this study shall be: Positive samples shall not be less than 100, and negatives shall not be less than 400.

In the case of meeting the statistical requirements after the test starts, the sample size of each institution shall be adjusted to make the total sample size to meet the requirements if there are difficulties in

sample enrollment.

2. Sample selection basis and inclusion criteria

Inclusion criteria:

1) sufficient sample quantity, clear traceability records. Sample collection and processing is in accordance with reagent instructions or relevant legislation.

2) The sample should be correctly registered, all samples should have corresponding basic clinical information, including: sample number, gender, age and clinical diagnosis information.

3) Unlimited gender, But the age should over 2.

Exclusion criteria:

1) Collection time and information of the sample are unclear.

2) Samples that were stored without meeting the requirements of the evaluation reagent product manual, or the storage period exceeded.

3) Researchers judge samples that do not meet testing requirements.

4 Sample preparation

The samples should be eluted with the sample eluent provided with this kit immediately after collection, and tested as soon as possible after elution. If the specimens cannot be processed immediately, preserve them as follows: one day at 2℃-8℃ and permanently at -70℃ and below.

5 Sample Blinding

After the samples were enrolled, the samples were coded by the blind editor authorized by the clinical evaluation, in which the blind editor was not involved in the test operation of the clinical evaluation.

After the end of the research, the blinding shall be uncovered, the statistics shall be summarized, and the clinical information shall be analyzed.

6. Preparation of Research Materials

6.1 Test reagent

Product Name: Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Antigen Detection Kit (Colloidal Gold-Based)

Lot: V2021030857A, V2021030957A, V2021031057A

Manufacturer: Nanjing Vazyme Medical Technology Co., Ltd.

6.2 Comparator

Site 1

Product Name: Bio-Rad Reliance SARS-CoV-2 RT-PCR Assay Kit (IVD)

Manufacturer: Bio-Rad Laboratories, Inc.

Site 2

Product Name:SARS-CoV-2 Real-TM

Lot: 28E21J774 F2912S-2-T

Manufacturer: Sacace Biotechnologies Srl

7 Sample Detection

7.1 Personnel training

Prior to the start of the evaluation, the lab operators were trained to correctly perform the tests and follow the protocol.

7.2 clinical study

According to the sample storage period and sample collection, the clinical researchers shall carry out the official clinical study by blind methods. Firstly, the Sample Collectors for clinical study shall collect samples and blind them, and then the operators conduct the detection. After all detection are completed, the detection personnel shall carry out recording and sorting out the test results of the day in accordance with the sample number. After all detections are completed, the sample collectors shall uncover the blind, the operators shall sort out and summarizes the data, and the statistician shall conduct the statistical analysis. The samples with inconsistent test results of test reagent and comparator shall be combined with the clinical diagnosis of patients to analyze the reasons for the difference and possible results.

8 Statistics, Analysis and Clinical Reports

The statisticians are responsible for conducting the statistics and analysis on the test results, and the clinical report shall be written by the principal investigator. The sponsor shall summarize the data of each clinical study site and complete the summary report.

9 Recording and Preservation of Original Data

The research operators shall fill in the test record, which shall use a unified form to truly, normatively and completely record the detection and data, and the test results shall be recorded in accordance with the result calculation method of the test reagent, and the original data shall be kept in the electronic version for filing. It shall not delete, modify, increase or decrease the test records at will. Without the unanimous permission of the applicant and the person in charge of the experimental unit, the detection records shall not be copied, reproduced, photographed or photocopied to other personnel and units in any form.

The original records of the entire clinical study process shall be kept in the clinical studylaboratories for 5 years or more.

The clinical evaluation report is in duplicate, laboratories and the sponsor keep one copy for each.

The articles related to the clinical evaluation data to be published shall have the consent of the sponsor.

V. Evaluation Methods of Clinical Performance

1. Statistical analysis and evaluation criteria

All the sample data meeting the requirements of the research plan are statistically analyzed, and the following table is used to organize the research data.

		Comparator results		Total
		Positive (+)	Negative (-)	
Assessing reagent results	Positive (+)	A	B	A+B
	Negative (-)	C	D	C+D
Total number		A+C	B+D	A+B+C+D

The following formula is used to calculate the test indicators:

Sensitivity =A/(A+C) *100%

Specificity =D/(B+D) *100%

Total coincidence rate = (A+D)/(A+B+C+D) * 100%

Kappa analysis is carried out with SPSS software, and consistency analysis is conducted between the test results of assessing reagent and the test results of comparator, and the test level is $\alpha = 0.05$. The reference evaluation principles for Kappa values are as follows: In case of $0.75 < \kappa \leqslant 1$, the consistency is good; In case of $0.4 < \kappa \leqslant 0.75$, the consistency is general; In case of $0 \leqslant \kappa \leqslant 0.4$, the consistency is poor;

The confidence interval and significance levels can quantify this statistical uncertainty in estimates due to the subject/sample selection process.

The 95% confidence intervals were calculated in order to assess the level of uncertainty introduced by sample size, using the Wilson's score method.

Calculation formula: [100%*(Q1-Q2)/Q3, 100%*(Q1+Q2)/Q3]

Ql, Q2 and Q3 are calculated according to the formulas below.

Calculation formula of the relevant parameters for the confidence interval of sensitivity:

$Q1=2A+1.96^2=2A+3.84$

$Q2=1.96\sqrt{1.96^2+4AC/(A+C)}=1.96\sqrt{3.84+4AC/(A+C)}$

$Q3=2(A+C+1.96^2)=2(A+C)+7.68$

Calculation formula of the relevant parameters for the confidence interval of specificity:

$Q1=2D+1.96^2=2D+3.84$

$Q2=1.96\sqrt{1.96^2+4BD/(B+D)}=1.96\sqrt{3.84+4BD/(B+D)}$

$Q3=2(B+D+1.96^2)=2(B+D)+7.68$

Calculation formula of the relevant parameters for the confidence interval of total coincidence rate:

$Q1=2(A+D)+1.96^2=2(A+D)+3.84$

$Q2=1.96\sqrt{1.96^2+4(A+D)(B+C)/(A+B+C+D)}=1.96\sqrt{3.84+4(A+D)(B+C)/(A+B+C+D)}$

$Q3=2(A+B+C+D+1.96^2)=2(A+B+C+D)+7.68$

2. Validation of result difference samples

The samples with inconsistent test results of assessing reagent and comparator shall be combined with the clinical diagnosis of patients to analyze the reasons for the differences and possible results.

3. Acceptance criteria for results

Sensitivity: > 80 %

Specificity: > 97 %

Ⅵ. Clinical Study Results and Analysis

1. Sample Distribution Statistics

The clinical study was carried out in strict accordance with the clinical protocol. A total of 1569 nasal swab specimens were collected from the patients at Lab. The patients presenting the COVID-19 like symptoms within 7 days of symptom onset at the collection site are enrolled. The nasal swabs were randomized and blinded tested by operators following product package insert. A companion nasopharyngeal (NP) swab was also collected from the same patient and confirmed as positive or negative and validated with Ct counts by the RT-PCR as a comparator method.

Sample	Positive	Negative
Number of cases	269	1327

2. Sex and age distribution of samples

A total of 1000 samples, including 899 males and 697 females. The specific distribution of samples is shown in the following table:

Number of samples	Total	548
Sex	Male（N,%）	899（56.33%）
	Female（N,%）	697（43.67%）
Age（y）	X±SD	42±18
	Min-Max	2~95

3. Data Statistics and Analysis

Vazyme SARS-CoV-2 Ag Test Kit	RT-PCR		
	Positive	Negative	Total
Positive	266	0	266
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Total	269	1327	1596
Sensitivity	98.88 % (95% Cl: 96.77 % to 99.62 %)		
Specificity	100.00 % (95% Cl: 99.71 % to 100.00 %)		
Total coincidence rate	99.81 % (95% Cl: 99.45 % to 99.94 %)		

Ct Value Distribution	
Sensitivity (95% Cl) Ct＞30, N	93.62% (95%Cl: 82.84% to 97.81%), 44
Sensitivity(95% Cl)　25＜Ct ≤ 30, N	100% (95%Cl: 94.87 % to100.00%), 71
Sensitivity (95% Cl) Ct≤25, N	100% (95%Cl: 97.57 % to100.00%), 154

Ⅶ. Conclusion

This clinical evaluation has performed a full analysis of the experimental reagents through methodological comparisons, and the results all meet the criteria for clinical evaluation Results showed that Vazyme SARS-CoV-2 Antigen Rapid Test Kit meet the Requirements of clinical evaluation.

Ⅷ. Appendix 1. Evaluation data sheet of sample test results