COVID-19 Antigen Rapid Test Kit (Saliva/Swabs)

Clinical Trial Report

Contents

Research Summary

[Background] COVID-19 belongs to β genus and is an enveloped virus, the particles are round or oval, and it is often polymorphic and 60-140nm in diameter. Its genetic characteristics are significantly different from SARS-CoV and MERS-CoV. Current research shows that its homology with bat SARS-like coronavirus (bat-SL-CoVZC45) is more than 85%. When isolated and cultured in vitro, COVID-19 can be found in human respiratory epithelial cells in about 96h, while it takes about 6 days to isolate and culture it in Vero E6 and Huh-7 cell lines.

Most of the understanding of the physicochemical properties of coronavirus comes from the researches on SARS-CoV and MERS-CoV. The virus is sensitive to ultraviolet rays and heat. Lipid solvents such as ether, 75% ethanol, chlorine-containing disinfectant, peracetic acid and chloroform can effectively inactivate the virus at 56°C for 30min. Chlorhexidine cannot effectively inactivate the virus.

Based on current epidemiological investigations, the incubation period of COVID-19 is 1-14 days, mostly 3-7 days. Clinically, fever, dry cough and fatigue are the main manifestations; and a few patients have symptoms such as nasal obstruction, running nose, sore throat, myalgia and diarrhea.Severe patients often suffer from dyspnea and/or hypoxemia one week after onset. In severe cases, it may quickly progress to ARDS, septic shock, metabolic acidosis and coagulation dysfunction difficult to correct, as well as multiple organ failure.

[Purpose] Use the "COVID-19 Antigen Rapid Test Kit (Saliva/Swabs) " (hereinafter referred to as "**evaluation reagent**") produced by Wuhan EasyDiagnosis Biomedicine Co., Ltd. for clinical test, and verify its safety and effectiveness.

[Method] Through a comparative study with clinically confirmed/excluded results, make a comparison by using the clinical sensitivity, clinical specificity and clinical accuracy for statistical analysis of the results, and evaluate the clinical performance of the evaluation reagent.

[Results]

(1) There are 534 specimens (267 oropharyngeal swabs and 267 nasopharyngeal swabs) completed in contrast research between evaluation reagent and clinical confirmed/ excluded results. All of them are included in the statistics, covering 130 confirmed specimens (65 oropharyngeal swabs and 65nasopharyngeal swabs) and 404 excluded specimens (202 oropharyngeal swabs and 202 nasopharyngeal swabs). The contrast between evaluation reagent results and clinically confirmed/excluded results meets the standard: a) clinical sensitivity 96.15%; b) clinical specificity 99.26%; c) clinical accuracy 98.50%.

(2) For the research of test results of oropharyngeal swabs and nasopharyngeal swabs, the clinical sensitivity of oropharyngeal swabs is 95.38%, clinical specificity is99.50% and clinical accuracy is 98.50%; the clinical sensitivity of nasopharyngeal swabs is 96.92%, clinical specificity is99.01% and clinical accuracy is 98.50%.

(3) In the stratified statistics of different stages of the disease, 52 specimens from 0-3 days, and the positive detection rate is 98.08%; 52 specimens from 4-7 days, and the positive detection rate is 96.15%; 26 specimens older than 7 days, and the positive detection rate is 92.31%.

(4) There are 555 saliva specimens completed in contrast research between evaluation reagent and clinical confirmed/ excluded results. For the research of test results of saliva specimens, the clinical sensitivity of saliva specimens is 96.09%, clinical specificity is 99.01% and clinical accuracy is 98.38%.

(5) In the stratified statistics of different stages of the disease, 51specimens from 0-3 days, and the positive detection rate is 98.04% ; 52 specimens from 4-7 days, and the positive detection rate is 96.15%; 25 specimens older than 7 days, and the positive detection rate is 92.00%.

[Conclusion] The comparison between the evaluation reagent test results and the clinically confirmed /excluded results shows high consistency, indicating that the evaluation reagents have good clinical applicability.

1. Instruction

COVID-19 belongs to β genus and is an enveloped virus, the particles are round or oval, and it is often polymorphic and 60-140nm in diameter. Its genetic characteristics are significantly different from SARS-CoV and MERS-CoV. Current research shows that its homology with bat SARS-like coronavirus (bat-SL-CoVZC45) is more than 85%. When isolated and cultured in vitro, COVID-19 can be found in human respiratory epithelial cells in about 96h, while it takes about 6 days to isolate and culture it in Vero E6 and Huh-7 cell lines.

Most of the understanding of the physicochemical properties of coronavirus comes from the researches on SARS-CoV and MERS-CoV. The virus is sensitive to ultraviolet rays and heat. Lipid solvents such as ether, 75% ethanol, chlorine-containing disinfectant, peracetic acid and chloroform can effectively inactivate the virus at 56°C for 30min. Chlorhexidine cannot effectively inactivate the virus.

Based on current epidemiological investigations, the incubation period of COVID-19 is 1-14 days, mostly 3-7 days. Clinically, fever, dry cough and fatigue are the main manifestations; and a few patients have symptoms such as nasal obstruction, running nose, sore throat, myalgia and diarrhea. Severe patients often suffer from dyspnea and/or hypoxemia one week after onset. In severe cases, it may quickly progress to ARDS, septic shock, metabolic acidosis and coagulation dysfunction difficult to correct, as well as multiple organ failure.

1.1 Source and Biological, Physicochemical Characteristics of Tested Substance

Tested substances are human oropharyngeal swabs and nasopharyngeal swabs from COVID-19 (SARS-CoV-2) residual specimen of clinical test organization.

1.2 Intended Use

This product is intended for in vitro qualitative detection of the nucleocapsid protein antigen from SARS-CoV-2 in saliva and oropharyngeal (throat)/nasopharyngeal swabs from individuals suspected of COVID-19 by their healthcare provider. The kit is intended for use by laboratory trained personnel

1.3 Test Principle

This kit is used for qualitative detection of COVID-19 (SARS-CoV-2) antigen in human saliva and oropharyngeal (throat)/nasopharyngeal swabs by Colloidal Gold Immunochromatography Assay. The to-be-tested liquid containing the specimen will move forward along the test card by capillary action. If the specimen contains COVID-19 (SARS-CoV-2) antigen, the antigen will be bound with the monoclonal antibody of colloidal gold labeled COVID-19 (SARS-CoV-2), and the immune complex will be captured by COVID-19 (SARS-CoV-2) monoclonal antibody fixed on the membrane to form a purple red test line, which shows positive COVID-19 (SARS-CoV-2) antigen; if the test line does not show color, a negative result will be displayed. The test card also contained one C line, no matter whether test line occurs or not, the purplish red C line shall occur, otherwise the test result will be invalid.

This product consists of the test strips and antigen extract R1. The test strips of test card include: nitrocellulose membrane coated with COVID-19 (SARS-CoV-2)

monoclonal antibody and C line antibodies, conjugate pad with colloidal gold labeled COVID-19 (SARS-CoV-2) monoclonal antibody, absorbent paper and PVC sheet.

2. Research Purpose

Use the "COVID-19 Antigen Rapid Test Kit (Saliva/Swabs) " (hereinafter referred to as "**evaluation reagent**") produced by Wuhan EasyDiagnosis Biomedicine Co., Ltd. for clinical test, and verify its safety and effectiveness.

3. Test Design

3.1 General Design and Plan Description of Test

The test will use the random and contrast test design.

(1) Randomly purpose a laboratory number for each enrolled specimen, corresponding to original specimen number.

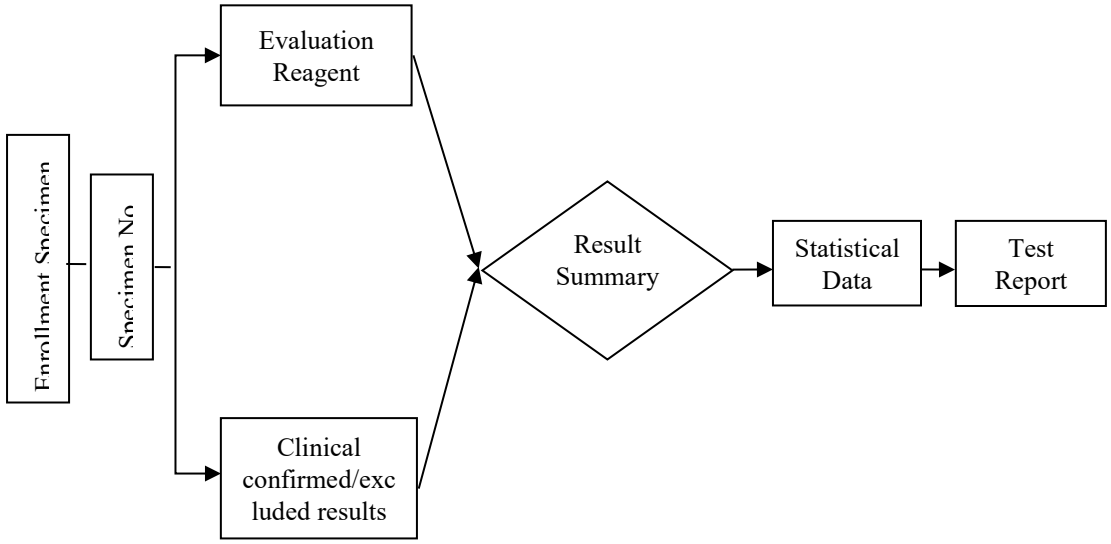
(2) According to the product instruction of evaluation reagent, all enrolled specimens are tested with evaluation reagent; according to the product instruction of contrast reagent, all enrolled specimens are tested with contrast reagent. The confirmation method of clinically confirmed/excluded results of all enrolled cases is based on confirmation procedure of first case, second case and subsequent infection cases of each province stipulated in Diagnosis Plan of Pneumonia Inflected by COVID-19 and Confirmation Procedure of First Pneumonia Case Inflected by COVID-19 in Each Province (District, City) issued by National Health Commission.

(3) Judge the test results according to product instruction.

(4) Calculate the clinical sensitivity, clinical specificity and clinical accuracy of contract research between test kit and clinical confirmed/ excluded results.

(5) Finally, make a summary of research result according to evaluation standard.

Test Flow Chart



For avoiding the deviation of test result, the main researcher shall purpose a specimen laboratory number for each enrolled specimen before testrandomly, and those numbers are corresponding to original clinical specimen ID; During test, inspectors

shall use the specimen laboratory number only and the corresponding clinical diagnostic information of each specimen cannot be obtained so as to prevent inspectors from being influenced by the clinical diagnosis when carry out result analysis; After test, the main researcher will disclose the clinical diagnosis and other information, and process the data.

3.2 Test Design and Test Method Selection

3.2.1 Specimen Enrollment/Exclusion Standard

3.2.1.1 Enrollment Standard

(1) The suspected pneumonia cases infected by COVID-19 from clinical test organization include confirmed pneumonia case infected by COVID-19 and excluded case.

(2) The specimen types are saliva and oropharyngeal (throat)/nasopharyngeal swabs. Use test reagents to test samples.

3.2.1.2 Exclusion Standard

(1) Specimen collection does not satisfy the requirement of evaluation reagent instruction.

(2) Specimen storage does not satisfy the requirement of evaluation reagent instruction.

(3) Insufficient sample allowance.

(4) Contaminated samples.

3.2.1.3 Exclusion Standard

(1) The specimen without specific confirmed/excluded results.

(2) The specimen with invalid results due to specimen collection.

3.2.2 Specimen Collection and Transportation Method

(1) Specimen collection: it shall carry out specimen enrollment/exclusion standard. Specimen collection meets the requirement of test kit instruction.

(2) Specimen transportation: COVID-19 specimen shall be transported by referring Technical Guidelines for Laboratory Test of Infection with COVID-19.

3.2.3 Contrast Test Establishment

Applicant can contrast and research two methods, namely IVD reagent for test and clinical confirmed/excluded results, and similar products which have been marketed in domestic, so as to verify the clinical performance of IVD reagent for test.

The research evaluated the clinical sensitivity, clinical specificity and clinical accuracy of evaluation reagent through the contrast research of clinical confirmed/excluded results.

3.2.4 All Products for Clinical Test

The information of products used for this clinical test is shown in table below.

Table 2 Information of Products Used for Clinical Test

Reagent/Device Name		Spec./Model	Manufacturer	Lot No.	Valid until	Storage conditions
Evaluation Reagent	COVID-19 Antigen Rapid Test Kit (Saliva/Swabs)	20 tests/kit	Wuhan EasyDiagnosis Biomedicine Co., Ltd.	20080101	2020.08.03	Store at 2℃~30℃ in dark place

3.2.5 Test Method

All reagent processing and use shall be in accordance with product instruction. Inspect the product lot number and expiry date. Specimen test result shall be judged according to result judgment standard of evaluation reagent and contrast reagent.

Result judgment standard of evaluation reagent:

(1) **Negative result:** if only C line appears, and T line does not show color development, it means that no COVID-19 (SARS-CoV-2) antigen is detected, and the result is determined to be negative.

(2) **Positive result:** If both C line and T line show color development, it means that COVID-19 (SARS-CoV-2) antigen is detected, and the result is determined to be positive.

(3) **Invalid result:** If C line does not appear no matter whether T line shows color development, it means the test is invalid.

At this time, it indicates that the incorrect improper operation or deterioration or damage of the test card. Please read the instructions carefully again, and retest with a new test card. If the problem persists, immediately stop using that batch of products, and contact the supplier.

3.2.6 Quality Control Methods

3.2.6.1 Pre-test Quality Control

For carrying out clinical evaluation test better and ensuring researcher be familiar with experiment operation and test process meet the requirement of plan, pre-test quality control mainly include:

(1) The technician of Wuhan EasyDiagnosis Biomedicine Co., Ltd. shall train the researcher participating in test and conduct the unified judgment standard.

(2) Clinical test personnel shall be familiar with and understand the clinical test method of this product, be familiar with product instruction of evaluation reagent, master the operation procedures and precautions in product instruction.

(3) The reagents used for clinical test shall be put into use after passing the ex-factory inspection and being attached with certificate document of inspection.

3.2.6.2 Process Control

For ensuring the authenticity and compliance, test process was controlled as follow:

(1) The personnel who participated in clinical test were passed clinical test training.

(2) For eliminating the influence from possible subjective deviation and individual preference in operator consciousness on test result, this test uses blind design, namely that test operator does not know the specific specimen information while the specimen information was stored by other researchers. Specimen clinical information will be not disclosed until completing test.

(3) All specimen operation experiment shall eliminate possible safety accident caused by biological hazard and prevent specimen disclosure and pollution.

(4) All observed results and discoveries in clinical test shall be verified to ensure the data reliability and ensure that all conclusions from clinical test are from original data.

4. Clinical Test Results and Statistical Analysis

4.1 Oropharyngeal swabs and nasopharyngeal swabs specimens

In this research, there are 534 specimens (267 oropharyngeal swabs and 267 nasopharyngeal swabs) enrolled. All of them are included in the statistics, covering 130 confirmed specimens (65 oropharyngeal swabs and 65 nasopharyngeal swabs) and 404 excluded specimens (202 oropharyngeal swabs and 202 nasopharyngeal swabs). Carry out contrast analysis for 534 specimens.

Table 3 Contrast Results Statistics of Clinically Confirmed/Excluded Results (267 oropharyngeal swabs + 267 nasopharyngeal swabs)

Evaluation Reagent	Clinical Confirmed/Excluded Results		Total
	Confirmed	Excluded	
Positive	125	3	128
Negative	5	401	406
Total	130	404	534

Result calculation:

(1) Clinical sensitivity: $p_1 =$ 96.15%, 95% confidence interval: [91.31%,98.35%].

(2) Clinical specificity: $p_2 =$ 99.26%, 95% confidence interval: [97.84%, 99.75%].

(3) Clinical accuracy: $p_3 =$ 98.50%, 95% confidence interval: [97.07%, 99.24%].

There are267 oropharyngeal swabs enrolled in this research, including 65 confirmed throat swab specimens and 202 excluded throat swab specimens. Carry out contrast analysis for 267 throat swab specimens separately.

Table 4 Contrast Results Statistics of Clinically Confirmed/Excluded Results

Evaluation Reagent	Clinical Confirmed/Excluded Results		Total
	Confirmed	Excluded	
Positive	62	1	63
Negative	3	201	204

Total	65	202	267

Result calculation:

(1) Clinical sensitivity: p_1 =95.38%, 95% confidence interval: [89.29%, 98.42%].

(2) Clinical specificity: p_2 =99.50%, 95% confidence interval: [97.25%, 99.91%].

(3) Clinical accuracy: p_3 =98.50%, 95% confidence interval: [96.21%, 99.42%].

There are 267nasopharyngeal swabs enrolled in this research, including 65 confirmed nasopharyngeal swab specimens and202 excluded nasopharyngeal swab specimens. Carry out contrast analysis for 267 throat swab specimens separately.

Table 5 Contrast Results Statistics of Clinically Confirmed/Excluded Results

Evaluation Reagent	Clinical Confirmed/Excluded Results		Total
	Confirmed	Excluded	
Positive	63	2	65
Negative	2	200	202
Total	65	202	267

Result calculation:

(1) Clinical specificity: p_1 =96.92%, 95% confidence interval: [89.46%,99.15%].

(2) Clinical sensitivity: p_2 =99.01%, 95% confidence interval: [96.46%, 99.73%].

(3) Clinical accuracy: p_3 =98.50%, 95% confidence interval: [96.21%, 99.42%].

In this research, there are 534 specimens enrolled, 52 specimens with an onset period of 0-3 days, 52 specimens with an onset period of 4-7 days, and 26 specimens with an onset period of more than 7 days.

Table 6 Stratified comparison results of samples at different stages of disease

Disease stage	total	Number of detected cases	Positive detection rate/sensitivity	95% confidence interval	
				Upper limit Lower limit	Upper limit Lower limit
0-3days	52	51	98.08%	89.88%	99.66%
4-7days	52	50	96.15%	87.02%	98.94%
＞7days	26	24	92.31%	75.86%	97.86%

4.2 Saliva specimens

The results are summarized in the following table.

Table 7 Contrast Results Statistics of Clinically Confirmed/Excluded Results

(555 Saliva specimens)

Evaluation Reagent	Clinical Confirmed/Excluded Results		Total
	Confirmed	Excluded	
Positive	123	4	127
Negative	5	423	428
Total	128	427	555

Result calculation:

(1) Clinical sensitivity: $p_1 =$96.09%, 95% confidence interval: [91.49%,98.01%].

(2) Clinical specificity: $p_2 =$99.06%, 95% confidence interval: [97.72%, 99.53%].

(3) Clinical accuracy: $p_3 =$98.38%, 95% confidence interval: [97.00%, 99.09%].

Statistics of stratified comparison results of saliva specimens at different stages of disease

In this research, there are 555 specimens enrolled, 51specimens with an onset period of 0-3 days, 52 specimens with an onset period of 4-7 days, and 25specimens with an onset period of more than 7 days..

Table 8 Stratified comparison results of saliva specimens at different stages of disease

Disease stage	total	Number of detected cases	Positive detection rate/sensitivity	95% confidence interval	
				Upper limit Lower limit	Upper limit Lower limit
0-3days	51	50	98.04%	91.10%	98.25%
4-7days	52	50	96.15%	88.09%	97.87%
＞7days	25	23	92.00%	77.09%	95.72%

5. Conclusions

5.1 Oropharyngeal swabs and nasopharyngeal swabs specimens

Experimental results and analysis show that: there are 534 specimens (267 oropharyngeal swabs and 267 nasopharyngeal swabs) completed in contrast research between evaluation reagent and clinical confirmed/ excluded results. All of them are included in the statistics, covering 130 confirmed specimens (65 oropharyngeal swabs and 65 nasopharyngeal swabs) and 404 excluded specimens (202 oropharyngeal swabs and 202 nasopharyngeal swabs). The contrast between evaluation reagent results and clinically confirmed/excluded results meets the standard: a) clinical sensitivity 96.15%; b) clinical specificity 99.26%; c) clinical accuracy 98.50%. Evaluation reagent was highly consistent with clinical diagnosis results.

Experimental results and analysis show that: for the research of test results of oropharyngeal swabs and nasopharyngeal swabs, the clinical sensitivity of oropharyngeal swabs is 95.38%, clinical specificity is 99.50% and clinical accuracy is 98.50%; the clinical sensitivity of nasopharyngeal swabs is 96.92%, clinical specificity is 99.01% and clinical accuracy is 98.50%; evaluation reagent was highly consistent with clinical diagnosis results.

Test results and analysis show that in the stratified statistics of different stages of the disease, 52 specimens from 0-3 days, and the positive detection rate is 98.08%; 52 specimens from 4-7 days, and the positive detection rate is 96.15%; 26 specimens older than 7 days, and the positive detection rate is 92.31%.

5.2 Saliva specimens

Experimental results and analysis show that: for the research of test results of 555 saliva specimens, the clinical sensitivity of saliva is 96.09%, clinical specificity is 99.01% and clinical accuracy is 98.38%, evaluation reagent was highly consistent with clinical diagnosis results.

Test results and analysis show that in the stratified statistics of different stages of the disease, 51specimens from 0-3 days, and the positive detection rate is 98.04% ; 52 specimens from 4-7 days, and the positive detection rate is 96.15%; 25 specimens older than 7 days, and the positive detection rate is 92.00%.

The above results indicate that the evaluation reagents can meet the requirements for safety and effectiveness in clinical use and have good clinical applicability.