Novel Coronavirus (COVID-19) Antigen Detection Kit (Latex Immunochromatography)

Clinical Performance Evaluation Report

Abstract

The clinical test of Antigen Detection kit was carried out; a comparative study was conducted, and clinical diagnostic criteria of pneumonia caused by novel coronavirus and the result of disease progression were used as the comparison method. This study is a retrospective study.

1 General

1.1 The source, biology and physicochemical properties of the measured object

A novel coronavirus is a β genus coronavirus. It is enveloped and has round or elliptic particles, usually pleomorphic, with a diameter of 60-140nm. Its genetic characteristics were significantly different from sars-cov and mers-cov. At present, its homology with of bat sars-like coronavirus (bat-sl-covzc45) is more than 85%.

At present, the infection source is mainly from novel coronavirus infected patients. Asymptomatic infections may also be a source of infection. Respiratory droplets and close contact is the main route of transmission. It is possible to spread by aerosol in a relatively closed environment when exposed to a high concentration of aerosol for a long time. Since novel coronavirus can be isolated from feces and urine, note that feces and urine cause aerosol or contact transmission to the environment.

The crowd is generally susceptible. Based on the current epidemiological investigation, the incubation period is 1-14 days, most of which are 3-7 days. With fever, dry cough, fatigue as the main performance. A few patients were accompanied by nasal congestion, runny nose, sore throat, myalgia, and diarrhea. Severe patients developed dyspnea and/or hypoxemia one week after the onset of the disease, and in severe cases, they rapidly progressed to acute respiratory distress syndrome, septic shock, refractory metabolic acidosis, coagulation dysfunction and multi-organ failure. It is important to note that the course of severe and critical patients can be moderate or low fever, or even no obvious fever.

1.2 Clinical intended use

Antigen Detection Kit is an immunochromatographic assay for rapid, qualitative detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antigen N protein from the nasopharyngeal swab, oropharyngeal swab and saliva specimen, but not suitable for the detection of synthetic spike protein or British genetic variants . The test is to be used as an aid in the diagnosis of coronavirus infectious disease (COVID-19), which is caused by SARS-CoV-2.

The test provides preliminary results. Negative results cannot exclude SARS-CoV-2 infection and they cannot be used as the sole basis for treatment or other management decision.

For in vitro diagnostic use only. For professional use only.

1.3 Test Principle

This product uses highly specific antibody-antigen reaction and latex immunochromatographic technology. The reagent contains anti- SARS-CoV-2 monoclonal antibodies pre-fixed on the test area (T) on the membrane and anti SARS-CoV-2 monoclonal antibody latex-labeled conjugate labeled on the latex label pad.

During the test, the processed sample to be tested is dropped into the reagent loading place. When the sample contains SARS-CoV-2 antigen, the SARS-CoV-2 antigen in the sample is first combined with the anti- SARS-CoV-2 antibody labeled with latex microspheres, and then the conjugate is chromatographed on the membrane, and it is combined with another anti- SARS-CoV-2 antibody pre-immobilized on the membrane. When the anti- SARS-CoV-2 monoclonal antibody binds, a purple-red band will appear in the test area (T). If there is no SARS-CoV-2 antigen in the sample,

there will be no purple-red band in the test area (T). Regardless of whether the SARS-CoV-2 antigen is present in the sample, a purple-red band will appear in the quality control area (C). The purple-red band in the quality control area (C) is the standard for judging whether there are enough samples and whether the chromatography process is normal, and it also serves as an internal control standard for reagents.

2 Research purposes

Qualitative detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antigen was performed on nasopharyngeal swab, oropharyngeal swab and saliva specimen from confirmed/excluded SARS-CoV-2 cases with assessment reagent. The test results were compared with the clinical diagnostic criteria of covid-19 and the determination results of disease progression, and verify the clinical performance and effectiveness of the product.

3 Experiment management

3.1 Laboratory quality control

Formulated and strictly implemented the standard operating procedures for clinical testing projects, established laboratory quality control procedures, and regularly trained laboratory staff on quality control, statistics and various clinical testing techniques.

3.2 Statistics/data management

(1) The original data generated from the clinical trial shall be recorded and reviewed by the operator in a complete and accurate manner to ensure the reliability of the data and ensure that the conclusions in the clinical trial are derived from the original data.

(2) The process of statistical analysis of clinical trial data and the expression of its results adopt standard statistical methods, and the special person who is familiar with medical statistics is responsible for it.

4 Experiment design

4.1 Description of the experiment

The clinical diagnosis criteria of novel coronavirus pneumonia and the judgment of disease progression were selected as comparative methods for comparative study to verify the clinical performance of this product. This study is a clinically validated study using blind methods.

4.2 Experiment design and test method selection

(1) Sample size

According to the diagnostic criteria for novel coronavirus pneumonia; the sample nasopharyngeal swab, oropharyngeal swab and saliva specimen confirmed cases no less than 60 and excluded cases no less than 200.

(2) Sample selection basis, selection criteria, exclusion criteria and exclusion criteria

1) Selection criteria

The enrolled population of the clinical trial should be the applicable population of the product, which should be suspected cases of novel coronavirus pneumonia. For the definition of "suspected cases", refer to the "Novel Coronavirus Pneumonia Diagnosis and Treatment Program (Trial Version 7)".

The enrolled population of clinical trials should be able to represent various types of populations that suitable for the product, including confirmed cases of pneumonia (including partial recovery cases) and excluded cases of novel coronavirus infection.

a. According to the clinical diagnosis, refer to the sample storage period of the product manual, and select nasopharyngeal swab, oropharyngeal swab and saliva specimen samples that meet the requirements.

b. There is no limitation on gender and age. Select nasopharyngeal swab, oropharyngeal swab and saliva specimen samples of confirmed and excluded cases of novel coronavirus pneumonia.

2) Exclusion criteria

a. Those that did not meet the selection criteria and were selected by mistake;

b. Those that do not meet the sample storage requirements;

c. The sample information records are confusing or ambiguous.

3) Elimination criteria

a. Those that fail to meet the selection criteria and were selected by mistake and those who meet the exclusion criteria but not excluded;

b. Incorrect results due to operational errors and quality control failures.

(3) Sample collection, storage method, and transfer method

1) Sample collection

All samples come from the remaining samples of clinical testing. According to sample selection basis and selection criteria, swab samples are collected in clean, dry, waterproof containers that free of transmission media, preservatives, and detergents. Perform blind coding according to the blind coding and solve blindness procedures, and complete the sample selection.

2) Sample storage

The sample should be used as soon as possible after collection. If it cannot be used immediately, it must be stored at 2-8°C within 3 days. For long-term storage, it must be stored frozen below -70°C.

(4) Establishment of comparison method

The clinical diagnosis of novel coronavirus-infected pneumonia and the judgment results of disease progression (Reference PCR results) were selected as comparative method for comparative study.

(5) All products used in clinical trials

Reagent	Test reagents
Name	Novel Coronavirus (COVID-19) Antigen Detection Kit (Latex Immunochromatography)
Specification / Model	25 tests
Manufacturer	Zhejiang GENE SCIENCE Co., Ltd
LOT	20200401
Expiration date	Oct 31, 2021

Storage Condition	2℃~30℃

(6) Quality control method

1) Operation training

Before the start of this clinical trial, the manufacturer conducts training and pre-experiment on the trial researchers to enable the trial researchers to achieve the following goals after training:

a. Familiar and understand the clinical trial plan, familiar with the "Instruction" of the reagents used, and master the operation steps.

b. Be familiar with the safety instructions, precautions and relevant warnings in the "Instruction " of the reagents used.

c. Confirm all the functions of the reagents used. If there are any abnormal conditions that do not conform to the "Instruction", the sponsor should be notified in time, and the test can be carried out after the abnormalities are eliminated.

d. Implement unified recording methods and judgment standards.

2) Process control

a. The researcher should follow the requirements of the clinical trial record form and record the contents of the trial truthfully, in detail, and carefully to ensure that the content of the clinical trial record form is complete, true and reliable.

b. All observations and findings during this trial should be verified to ensure the reliability of the data and ensure that the conclusions in the clinical trial are derived from the original data.

c. In order to eliminate the influence of subjective deviations and personal preferences that may appear in the consciousness of the operator on the test results, this test uses a blind design.

3) Test control

The test is carried out in strict accordance with the instruction.

4) Security control

a. All human materials in this product have been determined to be free of HBsAg, HIV antibody, Treponema pallidum antibody and HCV antibody.

b. The clinical samples tested by this product are, nasopharyngeal swab, oropharyngeal swab and saliva specimen. The test researchers should strictly follow the requirements of the "National Clinical Inspection Operation Rules" and the instructions for use, wear masks and latex gloves to prevent biological contamination.

c. The product is not in direct contact with the patient during use. The samples tested are the remaining waste samples routinely tested by the test institute, and the data measured by this product are only used for clinical trials, not as a clinical test report issued by the hospital. There is no risk.

(7) Clinical performance evaluation method

Using clinical diagnostic criteria as controls, the clinical sensitivity, clinical specificity, and confidence interval of the detection reagent test results were calculated.

(8) Evaluation criteria of clinical performance statistical method

1) Statistical method

The results are summarized in the form of a 2 × 2 table, and the clinical sensitivity, clinical specificity, and confidence interval are calculated accordingly. A two-sided test was used, and the statistically significant test level α was set at 0.05, and the parameter confidence interval was estimated to use 95% confidence interval.

2) Evaluation criteria

a. Clinical sensitivity

The results are summarized in the form of a 2 × 2 table, and the clinical sensitivity of the detection results is ≥94%.

b. Clinical specificity

The results are summarized in the form of a 2 × 2 table, and the clinical specificity of the detection results is ≥98%.

5 Analysis of clinical trial results

5.1Oropharyngeal swab samples and nasopharyngeal swabs

Using clinical diagnostic criteria as controls, the clinical sensitivity, clinical specificity, and confidence interval of the detection results were calculated.

Results		RT-PCR		Subtotal
		Positive	Negative	
Antigen Detection Kit	Positive	192	3	195
	Negative	11	233	244
Subtotal		203	236	439

Sensitivity: 192/203=94.58% (95%CI: 90.51% - 97.26%)

Specificity: 233/236=98.73% (95%CI: 96.33% - 99.74%)

Total Coincidence Rate: 425/439=96.81% (95%CI: 94.71% - 98.25%)

Total N (valid PCR results)	439
Age [mean (min-max), N]	35.18(7-73), 439
Gender [%F, (n/N)]	47.84%, (210/439)
Days from symptom onset [median (Q1-Q3), N]	4(3-5), 204
Days ≤ 3 (n, %)	88, 43.14%
4 ≤ Days ≤7 (n, %)	116, 56.86%
Positivity [%, (n/N)]	46.47%, (204/439)

PCR Ct [median (Q1-Q3), N	29 (27-31), 204
31≤Ct ≤38 (n, %)	60, 29.41%
25≤Ct ≤30 (n, %)	134, 65.69%
Ct < 25 (n, %)	10, 4.90%
Clinical Sensitivity (95% CI), N	94.12% (90.00~96.60), 204
Sensitivity days ≤ 7, N	94.12% (90.00~96.60), 204
Sensitivity Ct≤ 30, N	93.75% (88.55~96.68%), 144
Sensitivity Ct < 25, N	100.00% (72.25～100.00) ,10
Clinical Specificity (95% CI), N	99.15% (96.95~99.77), 439
Invalid rate (%, n/N)	0%, 0/439

5.2 Statistical results of saliva samples

236 saliva samples which include 115 confirmed as COVID-19 positive and 121confirmed as COVID-19 negative by RT-PCR assay, were obtained for testing, and then compared the test results of Antigen Test Kit with RT-PCR results. The results are shown below.

Results		RT-PCR		Subtotal
		Positive	Negative	
Antigen Detection Kit	Positive	110	1	111
	Negative	5	120	125
Subtotal		115	121	236

Sensitivity: 110/115=95.65% (95%CI: 90.14%~98.57%)

Specificity: 120/121=99.17% (95%CI: 95.48%~99.98%)

Total Coincidence Rate: 230/236=97.46% (95%CI: 94.55%~99.06%)

Total N (valid PCR results)	236
Age [mean (min-max), N]	34.57 (9-76), 236
Gender [%F, (n/N)]	57.20%, (135/236)
Days from symptom onset [median (Q1-Q3), N]	4 (3-5), 115
Days ≤ 3 (n, %)	51, 44.35%
4 ≤ Days ≤7 (n, %)	64, 55.65%
Positivity [%, (n/N)]	48.73%, (115/236)
PCR Ct [median (Q1-Q3), N	27(26-39), 115

31≤Ct ≤38 (n, %)	12, 10.43%
25≤Ct ≤30 (n, %)	94, 81.74%
Ct < 25 (n, %)	9, 7.83%
Clinical Sensitivity (95% CI), N	95.65% (90.22～98.13), 115
Sensitivity days ≤ 7, N	95.65% (90.22～98.13), 115
Sensitivity Ct≤ 30, N	95.15% (89.14～97.91) , 103
Sensitivity Ct < 25, N	100.00% (70.08～100.00) ,9
Clinical Specificity (95% CI), N	99.17% (95.47 - 99.85), 236
Invalid rate (%, n/N)	0%, 0/236

6 Discussion and conclusion

To conduct clinical trials on the SARS-CoV-2 Antigen Test Kit produced by Zhejiang GENE SCIENCE Co., Ltd, the clinical diagnosis of novel coronavirus pneumonia and the judgment results of disease progression were used as comparative method for comparative study.

Statistical analysis results:

Results of statistical analysis of samples of oropharyngeal swab and nasopharyngeal swab：

Sensitivity: 192/203=94.58% (95%CI: 90.51% - 97.26%)

Specificity: 233/236=98.73% (95%CI: 96.33% - 99.74%)

Total Coincidence Rate: 425/439=96.81% (95%CI: 94.71% - 98.25%)

Statistical analysis results of saliva samples：

Sensitivity: 110/115=95.65% (95%CI: 90.14%~98.57%)

Specificity: 120/121=99.17% (95%CI: 95.48%~99.98%)

Total Coincidence Rate: 230/236=97.46% (95%CI: 94.55%~99.06%)

Statistical analysis results show that the Novel Coronavirus (COVID-19) Antigen Detection Kit produced by Zhejiang GENE SCIENCE Co., Ltd. meets the clinical needs.

7 Cross reactivity

7.1 Experimental scheme

The cross reactions in the test program to collect a set of potential cross reactants, including common viral and bacterial microorganisms, such as local human coronavirus (HKU1, OC43, NL63 and 229E), parainfluenza 1, 2, 3, 4, influenza A, influenza B, respiratory syncytial virus, a rhinovirus,

adenovirus type, enterovirus, haemophilus influenzae, streptococcus pneumoniae, streptococcus pyogenes, candida albicans, Bordetella pertussis, pneumonia mycoplasma, pneumonia chlamydia, eosinophilic lung legionella, staphylococcus epidermidis, staphylococcus aureus, mycobacterium tuberculosis, pneumocystis jirovecii (PJP)and pooled human nasal wash, etc.The basic information such as the categories and concentrations of the above-mentioned microorganisms is shown in Table 1.

When experimenting, other microbial samples known to have novel coronavirus negative were first diluted to a certain level of infection concentration (typically, concentration of 10^6 cfu/mL or higher for bacterial, and 10^5 pfu/mL or higher for virus), the test was carried out with reference to the operating instructions of the product, and each sample was repeated for 3 times, and then the specificity analysis was performed.

Table 1 Potential cross-reactant and test concentration

Potential Cross-Reactant	Test Concentration
Human coronavirus 229E (heat inactivated)	1.0×10^5 $TCID_{50}$/mL
Human coronavirus OC43	1.0×10^5 $TCID_{50}$/mL
Human coronavirus NL63	1.0×10^5 $TCID_{50}$/mL
Adenovirus	1.0×10^5 $TCID_{50}$/mL
Human Metapneumovirus	1.0×10^5 $TCID_{50}$/mL
Parainfluenza virus 1	1.0×10^5 $TCID_{50}$/mL
Parainfluenza virus 2	1.0×10^5 $TCID_{50}$/mL

Parainfluenza virus 3	1.0 x 10^5 $TCID_{50}$/mL
Parainfluenza virus 4	1.0 x 10^5 $TCID_{50}$/mL
Influenza A	1.0 x 10^5 $TCID_{50}$/mL
Influenza B	1.0 x 10^5 $TCID_{50}$/mL
Enterovirus	1.0 x 10^5 $TCID_{50}$/mL
Respiratory syncytial virus	1.0 x 10^5 $TCID_{50}$/mL
Rhinovirus	1.0 x 10^5 $TCID_{50}$/mL
HCoV-HKU1	10μg/mL
MERS-CoV Nucleoprotein	0.25ng/mL
Haemophilus influenza	1.5 x 10^6 CFU/mL
Streptococcus pneumoniae	1.5 x 10^6 CFU/mL
Streptococcus pyogenes	1.5 x 10^6 CFU/mL
Candida albicans	1.5 x 10^6 CFU/mL
Bordetella pertussis	1.5 x 10^6 CFU/mL
Mycoplasma pneumoniae	1.5 x 10^6 CFU/mL
Chlamydia pneumoniae	1.5 x 10^6 CFU/mL
Staphylococcus epidermidis	1.5 x 10^6 CFU/mL

Staphylococcus aureus	1.5 x 10^6 CFU/mL
Legionella pneumophila	1.5 x 10^6 CFU/mL
Mycobacterium tuberculosis	1.5 x 10^6 CFU/mL
Pneumocystis jirovecii (PJP)	1.5 x 10^6 CFU/mL
Pooled human nasal wash	100%

7.2 Test method

The samples with concentrations in the above table were prepared from the mixture of the novel coronavirus negative mixtures of other microorganisms and normal nasal lotion. Each sample was tested separately using the three batches of the SARS-CoV-2 Antigen Test Kit (Colloidal Gold), and each sample was repeated for three times.

7.3 Test result

After testing, Novel Coronavirus (COVID-19) Antigen Detection Kit (Latex Immunochromatography) has no cross-reactivity with the above cross-reactant.

8 Endogenous interfering substances

The analytical performance evaluation results of Novel Coronavirus (COVID-19) Antigen Detection Kit (Latex Immunochromatography) showed that mucin ≤10g/L, blood ≤10%, pus ≤5%, will not interfere with the test results. Oxymetazoline ≤0.375mg/mL, Dexamethasone ≤2.5mg/L, Sulfur ≤50mg/mL, Zanamivir ≤1.25mg/L, Mupirocin ≤5mg/mL, Tobramycin ≤0.8 mg/L, will not interfere with the test results.