LYHER® Novel Coronavirus (COVID-19) Antigen Test Kit(Colloidal Gold)

Clinical Evaluation Report

Product Name: Novel Coronavirus (COVID-19) Antigen Test Kit(Colloidal Gold)

Start Date: April,2020

Finish Date: June,2020

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Contents

Clinical Study Summary

According to the *Guidelines for Clinical Trials of In vitro diagnostic reagents(No.16 of 2014)*, We entrustedMedical institutions in Liaoning, Heilongjiang, Beijing and Xinjiang to perform the clinical evaluation of Novel Coronavirus (COVID-19) Antigen Test Kit(Colloidal Gold)(hereinafter referred to as "Lyher Kit") developed by Hangzhou Laihe Biotech Co.,Ltd.

The clinical trial was compared with the clinical PCR test results and clinical diagnosis results to investigate the consistency of the products of Hangzhou Laihe Biotech Co.,Ltd with the clinical diagnosis results.

The total number of cases in this clinical study is 585, and the results are summarized as follows:

A total of 585 patients were collected in this clinical trial, including 203 confirmed cases and 382 excluded cases. Compared with the results of clinical diagnosis, the clinical sensitivity of Lyher Kit was 95.07%, the clinical specificity of Lyher Kit was 99.74% , the total coincidence rate was 98.12%. There was no statistically significant difference between the test results of Lyher Kit and the clinical diagnosis results, the results of Lyher Kit were highly consistent with the results of clinical diagnosis.

Conclusion: Novel Coronavirus (COVID-19) Antigen Test Kit(Colloidal Gold) produced by Hangzhou Laihe Biotech Co., Ltd showed no statistically significant difference between Lyher Kit test results and the clinical diagnosis results, which were highly consistent.

Abbreviation

Ⅰ. Suspected case

Conduct a comprehensive analysis combining the following epidemiological history and clinical manifestations:

1) Epidemiological history

①Travel or residence history in Wuhan and surrounding areas or other communities with reported cases within 14 days prior to onset of the disease;

②Have contact with novel Coronavirus infected person (with a positive NAT(nucleic acid test) of SARS-CoV-2) within 14 days before onset of illness

③Patients with fever or respiratory symptoms who had come into contact with patients from Wuhan and surrounding areas, or from communities with case reports, within 14 days before onset of illness;

④Cluster incidence (2 or more cases of fever and/or respiratory symptoms within 2 weeks in small areas such as homes, offices, school classes, etc.).

2) Clinical manifestation

①Fever and/or respiratory symptoms;

②Have the above COVID-19 imaging characteristics;

③In the early stage of the disease, the total number of white blood cells was normal or decreased, and the lymphocyte count was normal or decreased.

The patient who has any one of the epidemiological history and be conformed to any one of the clinical manifestations. Or who has no clear epidemiological history, but be conformed to have any 2 of the clinical manifestations.

Ⅱ. Confirmed cases (positive cases)

Suspected cases with one of the following etiological or serological evidence:

1) A positive NAT of SARS-CoV-2;

2) Viral gene sequencing is highly homologous with the novel coronavirus known;

3) Specific IgM and IgG antibodies to SARS-CoV-2 were positive in serum;specific IgG antibody to SARS-CoV-2 changed from negative to positive; or the amount of IgG antibodies increased by 4 times or more in the recovery stage than in the symptom onset stage.

Define the early stage as the time of onset of symptoms or confirmed infection is among 1-7 days, the middle stage as the time of confirmed infection is among 8-14 days,the late stage as the time of confirmed infection is over 14 days,

Ⅲ. The reagent to be evaluated

Novel Coronavirus (COVID-19) Antigen Test Kit(Colloidal Gold) manufactured by Hangzhou Laihe Biotech Co.,Ltd

Ⅳ. Comparator Reagent

Novel Coronavirus 2019-nCoV Nucleic Acid Detection Kit (Fluorescence PCR) manufactured by Shanghai BioGerm Medical Technology Co., Ltd.

1. Introduction

The incubation period of novel coronavirus infection is 1-14 days, with an average of about 5 days.Some of those infected may have no symptoms at all but still be contagious; Most of the patients will rapidly deteriorate into severe pneumonia, respiratory distress, asphyxia, etc. A small proportion of patients infected with the novel coronavirus may die.

Novel coronavirusIgM antibody can be detected approximately in the appearance of symptoms 3-5 days, asymptomatic patients with infection 5 days or so can generally be detected. IgG antibodies cannot be detected for about 10-14 days.

2. Objective

The objective of this clinical trial is to compare a certain number of clinical samples with the clinical diagnosis results for clinical single-blind detection, to evaluate the sensitivity, specificity and other indicators of the product, and to verify the accuracy of the product in clinical determination, so as to determine whether it meets the requirements of safety and effectiveness.

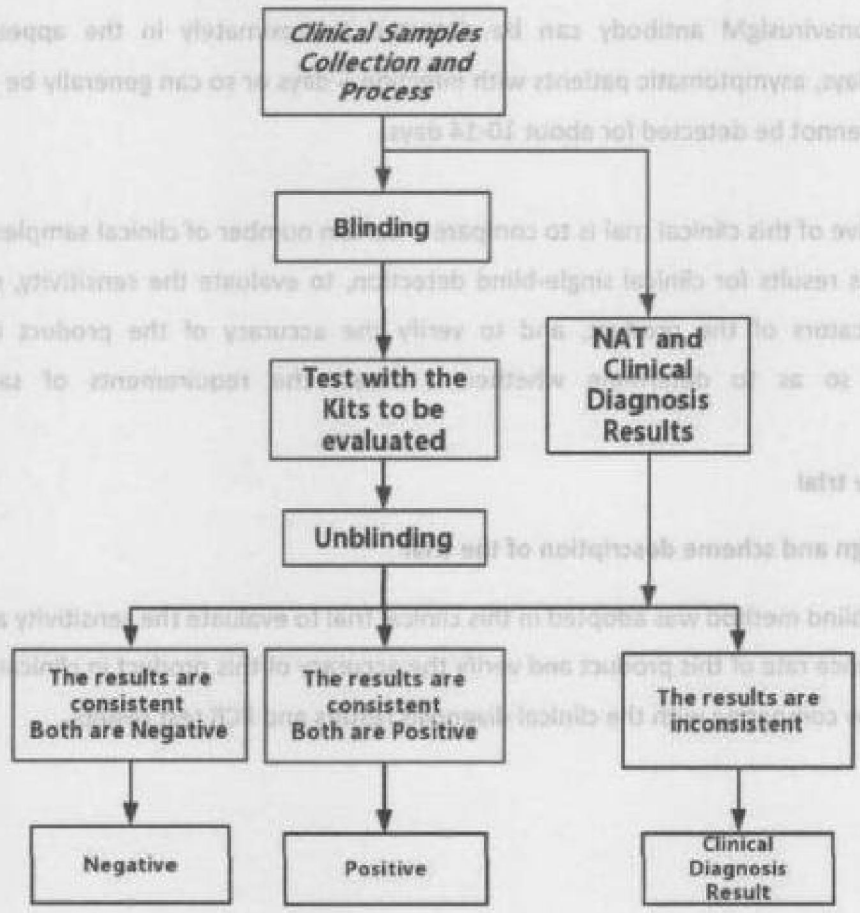
3. Design of the trial

3.1 Overall design and scheme description of the trial

The single blind method was adopted in this clinical trial to evaluate the sensitivity and specific coincidence rate of this product and verify the accuracy of this product in clinical determination by comparing with the clinical diagnosis results and PCR test results.

After the selected samples were coded sequentially, the test operator would test the samples according to the requirements of the respective specifications using the Novel Coronavirus (COVID-19) Antigen Test Kit(Colloidal Gold) manufactured by Hangzhou Laihe Biotech Co., Ltd. while the subject information remained blind.

The specific test procedure is as follows:



Note:①the samples of each subject should be tested blind according to the instructions of Lyher Kit.

② After all samples are tested, remove blindness and query the NAT results and clinical diagnosis information of all samples. The sample with inconsistent results is judged by the trial operator according to the clinical diagnosis.

The test results of Lyher Kit are interpreted according to the instructions.When the results are consistent with the NAT and clinical results, the test results are recorded; while they are inconsistent with the NAT and clinical results, they were judged according to the clinical diagnosis results. According to the test results,the clinical application performance of Lyher Kit was evaluated, and the equivalence and consistency of Lyher Kit with NAT results and clinical results were investigated.

3.2 Trial design and study method selection

(1) Sample size and sample size determination basis

The sample size was determined according to the relevant provisions of "Key Points of the Technical Review on Novel Coronavirus 2019 Antigen/Antibody Test Reagent Registration (Tentative)" and the clinical purpose of the product. The main sample size of this clinical trial of this product should be at least 500 cases, including at least 50 positive cases at early stage, at least 50 positive cases at middle stage, at least 50 positive cases at late stage. There shall be 100~200 cases of excluded and confirmed samples in each medical institution.

(2) Sample selection criteria, inclusion criteria, exclusion criteria

- **Selection basis**

This product is intended used for qualitatively testing novel coronavirus antigens in human specimens collected by nasopharyngeal/oropharyngeal swab.

Clinical study samples can be selected from outpatients' or inpatients' specimens collected by nasopharyngeal or oropharyngeal swab that meet the inclusion criteria.

- **Inclusion criteria:**

1. The samples were COVID-19 confirmed cases (including some cases in convalescence) and excluded cases
2. Clinical patients with different disease progression: samples of related cases including at the early stage, middle stage, late stage/convalescence, etc
3. All cases should be suspected or confirmed
4. There is no limitation on age and gender if sufficient samples can be taken as required

- **Exclusion criteria:**

1. The collection time of samples is not clear, no nucleic acid test results or clinical information is missing;
2. Insufficient sample size due to error during test operation;
3. Samples such as those contaminated during specimen preservation were found before the test operation;
4. Improperly preserved specimens should be excluded.

· Criteria for ruling out the abnormal samples from the selected samples

1. Samples that do not meet the requirements of the specimen collection method;
2. Samples that have been dried or contaminated;
3. The diagnostic information of the sample is found to be missing or untraceable before statistics.
4. Samples with incomplete information

(3) Collection, preservation and transportation of specimens:

- **Specimen collection**

The target specimens of Lyher kit can be collected by nasopharyngeal/oropharyngeal swab.

The collection of specimens: Sample and collect according to the requirements in the

instructions.

Before testing, the specimens must be brought to room temperature and thoroughly mixed. Do not freeze and thaw the samples repeatedly.

- **Specimens sent for test**

After a certain amount of specimens are collected, they are uniformly blinded, and then tested with Lyher Kit.

3.3 The determination of the comparative method

In order to fully evaluate the clinical performance of this product, NAT and clinical diagnosis results were used as the control.

（1）Product information used in clinical trials:

	The reagent to be evaluated	Reagents for clinical diagnosis
Product Name	Novel Coronavirus (COVID-19) Antigen Test Kit(Colloidal Gold)	Novel Coronavirus 2019-nCoV Nucleic Acid Test Kit (Fluorescence PCR)
Specifications	25 Pcs/Box	/
Validity& Storage	Stored in 2~30℃ dry place away from light, valid for 18 months. Shall be used as soon as possible within 30 minutes after opening the bag.	The kit shall be stored at -20±5℃ and the period of validity is tentatively 6 months
Registration	In Pending	GXZZ20203400065
Batch No.	2006003-A	20200125C etc.
Manufacturer	Hangzhou Laihe Biotech Co.,Ltd.	Shanghai BioGerm Medical Technology Co., Ltd.

3.4 Method of Quality Control

- **On-site quality control of clinical trials**

In the course of this study, clinical supervisors appointed by the sponsor will make regular on-site inspection visits to the clinical trial research unit to ensure that all contents of the study protocol are strictly observed and the study data are filled in correctly.Participating researchers must undergo unified training, unified recording methods and judgment standards. The whole clinical trial process should be carried out under strict operation. All observations and findings in clinical trials should be verified to ensure the reliability of the data and to ensure that the

conclusions in clinical trials are derived from the original data. There are corresponding data management measures in clinical trial and data processing stage.

· Quality control of clinical specimens

1. Read the instructions carefully before the test, and the test operation shall be carried out strictly according to the instructions.

2. The collection of specimens shall be carried out in strict accordance with relevant clinical operation standards.

· **Quality control of reagents in clinical trials**

1. Read the reagent instructions carefully before the test, and the test operation shall be carried out strictly according to the instructions.

2. Negative and positive controls were set for each batch of tests. If no positive results were found during the positive quality control, the test would be invalid.

3. To read and interpret the result at a specified time.

4. All reagents used in the test are kept strictly in accordance with the manufacturer's requirements and used within the validity period.

3.5 Clinical evaluation method

· The reagents and clinical results are mainly represented in a four-grid table (as shown in Table 1). The table is self-explanatory, that is, it has table titles, table notes and number of cases

· SPSS software was used to conduct kappa consistency analysis on the test results of the assessment reagent in clinical results. Kappa value (k) was determined as $k>0.75$, showing good consistency. $0.40\leq k\leq 0.75$, good consistency; $K<0.40$, poor consistency.

3.6 Statistical analysis of clinical trial data

A single blind method was used in this clinical trial. Conduct conformity analysis, consistency analysis, correlation analysis and statistical difference analysis on the experimental data, and calculate clinical sensitivity, clinical specificity and total compliance rate; Calculate the kappa value.

(1) General analysis:

- The clinical sensitivity and specificity, total coincidence rate and 95% confidence interval (as compared with clinical diagnosis) of the test reagents were calculated as follows:

Table1.Test Results of_____Specimens

Lyher Kit	Clinical Results		Total
	Positive(+)	Negative(-)	
Positive(+)	A	B	A+B
Negative(-)	C	D	C+D
Total	A+C	B+D	A+B+C+D

Clinical sensitivity: A/(A+C) ×100%

Clinical specificity：D/(B+D)×100%

Total consistent rate：(A+D)/(A+B+C+D)×100%

The calculation formula of 95% confidence interval is: p±1.96×[p (1-p) /n]1/2 (where p is clinical sensitivity, clinical specificity or total coincidence rate, n is the number of samples, if p value is 100%, p=99.99% will be used for analysis).

- SPSS software was used to conduct Kappa consistency analysis (to report specific kappa values) on the data in table 1, to investigate the consistency between the assessment reagent and clinical diagnosis and nucleic acid test results, as well as the consistency between the assessment reagent and clinical diagnosis results.
- Cross interference test: Relevant tests were carried out on the specimens containing interfering substances and compared with the clinical diagnosis results, so as to verify the specificity of the assessment reagent from the clinical perspective.

3.7 Provisions for revision of clinical trial protocols

- In general, the clinical trial protocol should not be changed. Any modification of the scheme during the test shall be explained, and the time, reason, process and whether the change is recorded shall be explained in detail and its impact on the evaluation of the whole study result shall be demonstrated.
- The above modification instructions shall be submitted in written form by the registration applicant, and shall be put on record in the administrative body of the trial undertaking unit after being confirmed by each clinical implementation unit.

4. Clinical trial results and analysis

A total of 585 patients were collected in this clinical trial, including 382 excluded cases and 203 confirmed cases.

Test Results of Lyher Kit	Clinical diagnosis(PCR results)		
	Positive(+)	Negative(-)	Total
Positive(+)	193	1	194
Negative(-)	10	381	391
Total	203	382	585

Clinical sensitivity：193/(193+10)×100%＝95.07%

95% CI：$p\pm1.96\times[p(1-p)/n]^{1/2}$=[91.13%，99.99%]

Clinical specificity：381/(381+1)×100%＝99.74%

95% CI：$p\pm1.96\times[p(1-p)/n]^{1/2}$=[98.55%，99.99%]

Total coincidence rate：(193+381)/(193+10+1+381)×100%=98.12%

95% CI：$p\pm1.96\times[p(1-p)/n]^{1/2}$=[97.02%，99.22%]

Consistency analysis by the value of Kappa：

Kappa=0.977(K ＞ 0.75),It can be considered that the examination reagents and clinical diagnosis results are consistent.

According to the calculation of test results, there was no statistically significant difference between the reagents to be tested and the clinical diagnosis results. Consistency analysis Kappa=0.881, indicating that Lyher Kit were highly consistent with the clinical test results.

5. Discussion and Conclusions

A total of 585 patients were collected in this clinical trial, including 203 confirmed cases and 382 excluded cases. The clinical sensitivity of Lyher Kit was 95.07%, the clinical specificity of Lyher Kit was 99.74%, and the total coincidence rate was 98.12%, Kappa=0.977. There was no statistically significant difference between the test results. And the test results of Lyher Kit and the clinical diagnosis results were highly consistent.

In this clinical evaluation, the equivalence between the test reagent and clinical diagnosis should be verified. The evaluation results showed that Novel Coronavirus (COVID-19) Antigen Test Kit(Colloidal Gold) produced by Hangzhou Laihe Biotech Co., Ltd had high consistency with the clinical diagnosis results, which means that the product had good clinical application performance.

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Stability testing of Novel Coronavirus (COVID-19) Antigen Test Kit (Colloidal Gold)

1. Objective

To study the stability of Novel Coronavirus (COVID-19) Antigen Test Kit (Colloidal Gold) produced by Hangzhou Laihe Biotech Co.,Ltd.

2. Equipment

2.1 Incubators

2.2 Thermometers

2.3 Refrigerators

3. Materials

3.1 Novel Coronavirus (COVID-19) Antigen Test Kit (Colloidal Gold) finished products("Lyher Kit"for short)

3.2 Enterprise reference products, negative and positive specimens

4. Testing procedures

See instructions for detailed operating procedures.

5. Study plan

5.1 Stability study of finished products

5.1.1 Accelerated stability testing

Three batches of Lyher Kits (140 pieces of each batch were placed in a incubator set at 60 ℃. Taken 20 pieces of each batch to test everyday until all the Lyher Kits were used out. At the same time, to observe the stability of the matched specimen extraction buffer of Lyher Kits that has been opened, all the specimen diluents were opened and placed at an ordinary condition for 30-45 minutes and resealed before moving them into the incubator.

Table 5.1.1-1: Results of Accelerated Stability Testing (Day 1, 2020.06.26)

Specimen	Test results of Lyher Kits		
	2006003	2006004	2006005
N01	Negative	Negative	Negative
N02	Negative	Negative	Negative
N03	Negative	Negative	Negative
N04	Negative	Negative	Negative

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N05	Negative	Negative	Negative
P01	Positive	Positive	Positive
P02	Positive	Positive	Positive
P03	Positive	Positive	Positive
P04	Positive	Positive	Positive
P05	Positive	Positive	Positive
L1	Negative	Negative	Negative
L2	Positive	Positive	Positive
L3	Positive	Positive	Positive

Table 5.1.1-2: Results of Accelerated Stability Testing (Day 2, 2020.06.27)

Specimen	Test results of Lyher Kits		
	2006003	2006004	2006005
N01	Negative	Negative	Negative
N02	Negative	Negative	Negative
N03	Negative	Negative	Negative
N04	Negative	Negative	Negative
N05	Negative	Negative	Negative
P01	Positive	Positive	Positive
P02	Positive	Positive	Positive
P03	Positive	Positive	Positive
P04	Positive	Positive	Positive
P05	Positive	Positive	Positive
L1	Negative	Negative	Negative
L2	Positive	Positive	Positive
L3	Positive	Positive	Positive

Table 5.1.1-3:Results of Accelerated Stability Testing (Day 3, 2020.06.28)

Specimen	Test results of Lyher Kits		
	2006003	2006004	2006005
N01	Negative	Negative	Negative
N02	Negative	Negative	Negative
N03	Negative	Negative	Negative
N04	Negative	Negative	Negative
N05	Negative	Negative	Negative
P01	Positive	Positive	Positive
P02	Positive	Positive	Positive
P03	Positive	Positive	Positive
P04	Positive	Positive	Positive
P05	Positive	Positive	Positive
L1	Negative	Negative	Negative
L2	Positive	Positive	Positive

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L3	Positive	Positive	Positive

Table 5.1.1-4:Results of Accelerated Stability Testing (Day 4, 2020.06.29)

Specimen	Test results of Lyher Kits		
	2006003	2006004	2006005
N01	Negative	Negative	Negative
N02	Negative	Negative	Negative
N03	Negative	Negative	Negative
N04	Negative	Negative	Negative
N05	Negative	Negative	Negative
P01	Positive	Positive	Positive
P02	Positive	Positive	Positive
P03	Positive	Positive	Positive
P04	Positive	Positive	Positive
P05	Positive	Positive	Positive
L1	Negative	Negative	Negative
L2	Positive	Positive	Positive
L3	Positive	Positive	Positive

Table 5.1.1-5:Results of Accelerated Stability Testing (Day 5, 2020.06.30)

Specimen	Test results of Lyher Kits		
	2006003	2006004	2006005
N01	Negative	Negative	Negative
N02	Negative	Negative	Negative
N03	Negative	Negative	Negative
N04	Negative	Negative	Negative
N05	Negative	Negative	Negative
P01	Positive	Positive	Positive
P02	Positive	Positive	Positive
P03	Positive	Positive	Positive
P04	Positive	Positive	Positive
P05	Positive	Positive	Positive
L1	Negative	Negative	Negative
L2	Positive	Positive	Positive
L3	Positive	Positive	Positive

Table 5.1.1-6:Results of Accelerated Stability Testing (Day 6, 2020.07.01)

Specimen	Test results of Lyher Kits		
	2006003	2006004	2006005
N01	Negative	Negative	Negative
N02	Negative	Negative	Negative
N03	Negative	Negative	Negative

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N04	Negative	Negative	Negative
N05	Negative	Negative	Negative
P01	Positive	Positive	Positive
P02	Positive	Positive	Positive
P03	Positive	Positive	Positive
P04	Positive	Positive	Positive
P05	Positive	Positive	Positive
L1	Negative	Negative	Negative
L2	Positive	Positive	Positive
L3	Positive	Positive	Positive

Table 5.1.1-7:Results of Accelerated Stability Testing (Day 7, 2020.07.02)

Specimen	Test results of Lyher Kits		
	2006003	2006004	2006005
N01	Negative	Negative	Negative
N02	Negative	Negative	Negative
N03	Negative	Negative	Negative
N04	Negative	Negative	Negative
N05	Negative	Negative	Negative
P01	Positive	Positive	Positive
P02	Positive	Positive	Positive
P03	Positive	Positive	Positive
P04	Positive	Positive	Positive
P05	Positive	Positive	Positive
L1	Negative	Negative	Negative
L2	Positive	Positive	Positive
L3	Positive	Positive	Positive

From the above test results, it can be seen that under accelerated condition of 60 ℃ after 7 days, the product still meets the requirements. It is speculated that the stability of Lyher Kits is no less than 21 months under the storage condition of 2-30℃. To ensure the validity of the products, the validity period of the product is set at 18 months tentatively.

5.1.2 Stability testing under storage condition(Real-time Stability)

Three batches of Lyher Kits(140 pieces of each batch) were taken to store in QC laboratory and recorded the temperatures of the storage condition everyday to ensure that the storage temperature of these products always be kept between 2 and 30 ℃. If the temperature were lower than 2℃ or higher than 30 ℃, the air

conditioning should be turned on to adjust the room temperature. Then tests will be performed at 1, 3, 6, 9, 12,15,18 and 21 months. At the same time, to observe the stability of the matched specimen specimen extraction buffer of Lyher Kits that has been opened, all the specimen diluents were opened and placed at an ordinary condition for 30-45 minutes and resealed before this testing item began.

Table 5.1.2-1: Results of Stability Test under storage condition (Month 1, 2020.07.25)

Specimen	Test results of Lyher Kits		
	2006003	2006004	2006005
N01	Negative	Negative	Negative
N02	Negative	Negative	Negative
N03	Negative	Negative	Negative
N04	Negative	Negative	Negative
N05	Negative	Negative	Negative
P01	Positive	Positive	Positive
P02	Positive	Positive	Positive
P03	Positive	Positive	Positive
P04	Positive	Positive	Positive
P05	Positive	Positive	Positive
L1	Negative	Negative	Negative
L2	Positive	Positive	Positive
L3	Positive	Positive	Positive

The real-time stability of Lyher kit was only tested for 1 month, and the real-time stability will be continuously tracked.

5.2 Stability testing of specimens

5.2.1 Stability testing of the specimens stored at 2-8℃ condition.

Stored 6 (3 positive and 3 negative) of Nasopharyngeal and Oropharyngeal specimens respectively, and 3 of nasopharyngeal and oropharyngeal specimens respectively which are near the cut-off value at 2-8 ℃. Tested this specimens by the selected three batches of Lyher Kits respectively at first day and repeated the operation every 3 days thereafter until the test result were unqualified.

Table 5.2.1-1 Results of Stability Testing of Specimens Stored At 2-8℃ Condition.(Tested at 2020.06.26)

Specimen	Test results of Lyher Kits		
	2006003	2006004	2006005

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	Negative oropharyngeal swab1	Negative	Negative	Negative
	Negative oropharyngeal swab2	Negative	Negative	Negative
	Negative oropharyngeal swab3	Negative	Negative	Negative
	Negative nasopharyngeal swab1	Negative	Negative	Negative
	Negative nasopharyngeal swab2	Negative	Negative	Negative
	Negative nasopharyngeal swab3	Negative	Negative	Negative
	Positive oropharyngeal swab1	Positive	Positive	Positive
	Positive oropharyngeal swab2	Positive	Positive	Positive
	Positive oropharyngeal swab3	Positive	Positive	Positive
	Positive nasopharyngeal swab1	Positive	Positive	Positive
	Positive nasopharyngeal swab2	Positive	Positive	Positive
	Positive nasopharyngeal swab3	Positive	Positive	Positive
	Near cut-off value oropharyngeal swab1	Positive	Positive	Positive
	Near cut-off value oropharyngeal swab2	Positive	Positive	Positive
	Near cut-off value oropharyngeal swab3	Positive	Positive	Positive
	Near cut-off value nasopharyngeal swab1	Positive	Positive	Positive
	Near cut-off value nasopharyngeal swab2	Positive	Positive	Positive
	Near cut-off value nasopharyngeal swab3	Positive	Positive	Positive

Table 5.2.1-2 Results of Stability Testing of Specimens Stored At 2-8℃ Condition.(Tested at 2020.06.29)

Specimen	Test results of Lyher Kits		
	2006003	2006004	2006005
Negative oropharyngeal swab1	Negative	Negative	Negative
Negative oropharyngeal swab2	Negative	Negative	Negative
Negative oropharyngeal swab3	Negative	Negative	Negative
Negative nasopharyngeal swab1	Negative	Negative	Negative
Negative nasopharyngeal swab2	Negative	Negative	Negative
Negative nasopharyngeal swab3	Negative	Negative	Negative
Positive oropharyngeal swab1	Positive	Positive	Positive
Positive oropharyngeal swab2	Positive	Positive	Positive
Positive oropharyngeal swab3	Positive	Positive	Positive
Positive nasopharyngeal swab1	Positive	Positive	Positive
Positive nasopharyngeal swab2	Positive	Positive	Positive
Positive nasopharyngeal swab3	Positive	Positive	Positive
Near cut-off value oropharyngeal swab1	Positive	Negative	Positive
Near cut-off value oropharyngeal swab2	Positive	Positive	Positive
Near cut-off value oropharyngeal swab3	Positive	Positive	Positive
Near cut-off value nasopharyngeal swab1	Positive	Positive	Negative
Near cut-off value nasopharyngeal swab2	Positive	Positive	Negative
Near cut-off value nasopharyngeal swab3	Positive	Positive	Positive

It can be seen from the above detection results, at 2-8℃, the test results of the untreated nasopharyngeal and oropharyngeal specimens being stored at 2-8℃ for three days may be false-negative, so the specimens should not be stored for more than 24 hours.

5.2.2 Stability testing of the samples stored at -20℃ condition

Stored 6 (3 positive and 3 negative) of nasopharyngeal and oropharyngeal specimens respectively, and 3 of nasopharyngeal and oropharyngeal specimens respectively which are near the cut-off value in a refrigerator set at -20 ℃. Tested this specimens by the selected three batches of Lyher Kits respectively at first day and repeated the operation every 30 days thereafter until the test result were unqualified.

Table 5.2.2-1 Results of Stability Testing of Specimens Stored At -20℃ Condition. (Tested at 2020.06.26)

Specimen	Test results of Lyher Kits		
	2006003	2006004	2006005
Negative oropharyngeal swab1	Negative	Negative	Negative
Negative oropharyngeal swab2	Negative	Negative	Negative
Negative oropharyngeal swab3	Negative	Negative	Negative
Negative nasopharyngeal swab1	Negative	Negative	Negative
Negative nasopharyngeal swab2	Negative	Negative	Negative
Negative nasopharyngeal swab3	Negative	Negative	Negative
Positive oropharyngeal swab1	Positive	Positive	Positive
Positive oropharyngeal swab2	Positive	Positive	Positive
Positive oropharyngeal swab3	Positive	Positive	Positive
Positive nasopharyngeal swab1	Positive	Positive	Positive
Positive nasopharyngeal swab2	Positive	Positive	Positive
Positive nasopharyngeal swab3	Positive	Positive	Positive
Near cut-off value oropharyngeal swab1	Positive	Positive	Positive
Near cut-off value oropharyngeal swab2	Positive	Positive	Positive
Near cut-off value oropharyngeal swab3	Positive	Positive	Positive
Near cut-off value nasopharyngeal swab1	Positive	Positive	Positive
Near cut-off value nasopharyngeal swab2	Positive	Positive	Positive
Near cut-off value nasopharyngeal swab3	Positive	Positive	Positive

Table 5.2.2-2 Results of Stability Testing of Specimens Stored At -20℃ Condition.(Tested at 2020.07.27)

Specimen	Test results of Lyher Kits		
	2006003	2006004	2006005

LYHER®

Negative oropharyngeal swab1	Negative	Negative	Negative
Negative oropharyngeal swab2	Negative	Negative	Negative
Negative oropharyngeal swab3	Negative	Negative	Negative
Negative nasopharyngeal swab1	Negative	Negative	Negative
Negative nasopharyngeal swab2	Negative	Negative	Negative
Negative nasopharyngeal swab3	Negative	Negative	Negative
Positive oropharyngeal swab1	Positive	Positive	Positive
Positive oropharyngeal swab2	Positive	Positive	Positive
Positive oropharyngeal swab3	Positive	Positive	Positive
Positive nasopharyngeal swab1	Positive	Positive	Positive
Positive nasopharyngeal swab2	Positive	Positive	Positive
Positive nasopharyngeal swab3	Positive	Positive	Positive
Near cut-off value oropharyngeal swab1	Positive	Positive	Positive
Near cut-off value oropharyngeal swab2	Positive	Positive	Positive
Near cut-off value oropharyngeal swab3	Positive	Positive	Positive
Near cut-off value nasopharyngeal swab1	Positive	Positive	Positive
Near cut-off value nasopharyngeal swab2	Positive	Positive	Positive
Near cut-off value nasopharyngeal swab3	Positive	Positive	Positive

Table 5.2.2-3 Results of Stability Testing of Specimens Stored At -20℃ Condition.(Tested at 2020.08.26)

Specimen	Test results of Lyher Kits		
	2006003	2006004	2006005
Negative oropharyngeal swab1	Negative	Negative	Negative
Negative oropharyngeal swab2	Negative	Negative	Negative
Negative oropharyngeal swab3	Negative	Negative	Negative
Negative nasopharyngeal swab1	Negative	Negative	Negative
Negative nasopharyngeal swab2	Negative	Negative	Negative
Negative nasopharyngeal swab3	Negative	Negative	Negative
Positive oropharyngeal swab1	Positive	Positive	Positive
Positive oropharyngeal swab2	Positive	Positive	Positive
Positive oropharyngeal swab3	Positive	Positive	Positive
Positive nasopharyngeal swab1	Positive	Positive	Positive
Positive nasopharyngeal swab2	Positive	Positive	Positive
Positive nasopharyngeal swab3	Positive	Positive	Positive
Near cut-off value oropharyngeal swab1	Positive	Positive	Positive
Near cut-off value oropharyngeal swab2	Positive	Positive	Positive
Near cut-off value oropharyngeal swab3	Positive	Positive	Positive
Near cut-off value nasopharyngeal swab1	Positive	Positive	Positive
Near cut-off value nasopharyngeal swab2	Positive	Positive	Positive
Near cut-off value nasopharyngeal swab3	Positive	Positive	Positive

This study was only tested for 1 month, and the real-time stability will be continuously tracked.

5.2.3. Stability testing of specimens stored at -20℃ that repeated freezing and thawing

Stored 6 (3 positive and 3 negative) of nasopharyngeal and oropharyngeal specimens respectively, and 3 of nasopharyngeal and oropharyngeal specimens respectively which are near the cut-off value in a refrigerator set at -20 ℃.Tested these specimens by three batches of Lyher Kits respectively.The specimens shall be balanced to room temperature for 1 hour before testing, then restore the specimens in refrigerator at -20℃ at least for 4 hours.And repeated the above operation until the test result were unqualified

Table 5.2.3.-1 Results of stability testing of specimens stored at -20℃ that repeated freezing and thawing (Tested at 2020.06.26, 9:02)

Specimen	Test results of Lyher Kits		
	2006003	2006004	2006005
Negative oropharyngeal swab1	Negative	Negative	Negative
Negative oropharyngeal swab2	Negative	Negative	Negative
Negative oropharyngeal swab3	Negative	Negative	Negative
Negative nasopharyngeal swab1	Negative	Negative	Negative
Negative nasopharyngeal swab2	Negative	Negative	Negative
Negative nasopharyngeal swab3	Negative	Negative	Negative
Positive oropharyngeal swab1	Positive	Positive	Positive
Positive oropharyngeal swab2	Positive	Positive	Positive
Positive oropharyngeal swab3	Positive	Positive	Positive
Positive nasopharyngeal swab1	Positive	Positive	Positive
Positive nasopharyngeal swab2	Positive	Positive	Positive
Positive nasopharyngeal swab3	Positive	Positive	Positive
Near cut-off value oropharyngeal swab1	Positive	Positive	Positive
Near cut-off value oropharyngeal swab2	Positive	Positive	Positive
Near cut-off value oropharyngeal swab3	Positive	Positive	Positive
Near cut-off value nasopharyngeal swab1	Positive	Positive	Positive
Near cut-off value nasopharyngeal swab2	Positive	Positive	Positive
Near cut-off value nasopharyngeal swab3	Positive	Positive	Positive

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Table 5.2.3.-2 Results of stability testing of specimens stored at -20°C that repeated freezing and thawing (Tested at 2020.06.26, 14:06)

Specimen	Test results of Lyher Kits		
	2006003	2006004	2006005
Negative oropharyngeal swab1	Negative	Negative	Negative
Negative oropharyngeal swab2	Negative	Negative	Negative
Negative oropharyngeal swab3	Negative	Negative	Negative
Negative nasopharyngeal swab1	Negative	Negative	Negative
Negative nasopharyngeal swab2	Negative	Negative	Negative
Negative nasopharyngeal swab3	Negative	Negative	Negative
Positive oropharyngeal swab1	Positive	Positive	Positive
Positive oropharyngeal swab2	Positive	Positive	Positive
Positive oropharyngeal swab3	Positive	Positive	Positive
Positive nasopharyngeal swab1	Positive	Positive	Positive
Positive nasopharyngeal swab2	Positive	Positive	Positive
Positive nasopharyngeal swab3	Positive	Positive	Positive
Near cut-off value oropharyngeal swab1	Positive	Positive	Positive
Near cut-off value oropharyngeal swab2	Positive	Positive	Positive
Near cut-off value oropharyngeal swab3	Positive	Positive	Positive
Near cut-off value nasopharyngeal swab1	Positive	Positive	Positive
Near cut-off value nasopharyngeal swab2	Positive	Positive	Positive
Near cut-off value nasopharyngeal swab3	Positive	Positive	Positive

Table 5.2.3.-3 Results of stability testing of specimens stored at -20°C that repeated freezing and thawing (Tested at 2020.06.26, 20:01)

Specimen	Test results of Lyher Kits		
	2006003	2006004	2006005
Negative oropharyngeal swab1	Negative	Negative	Negative
Negative oropharyngeal swab2	Negative	Negative	Negative
Negative oropharyngeal swab3	Negative	Negative	Negative
Negative nasopharyngeal swab1	Negative	Negative	Negative
Negative nasopharyngeal swab2	Negative	Negative	Negative
Negative nasopharyngeal swab3	Negative	Negative	Negative
Positive oropharyngeal swab1	Positive	Positive	Positive
Positive oropharyngeal swab2	Positive	Positive	Positive
Positive oropharyngeal swab3	Positive	Positive	Positive
Positive nasopharyngeal swab1	Positive	Positive	Positive
Positive nasopharyngeal swab2	Positive	Positive	Positive
Positive nasopharyngeal swab3	Positive	Positive	Positive
Near cut-off value oropharyngeal swab1	Positive	Positive	Positive
Near cut-off value oropharyngeal swab2	Positive	Positive	Positive

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Near cut-off value oropharyngeal swab3	Positive	Positive	Positive
Near cut-off value nasopharyngeal swab1	Positive	Positive	Positive
Near cut-off value nasopharyngeal swab2	Positive	Positive	Positive
Near cut-off value nasopharyngeal swab3	Positive	Positive	Positive

Table 5.2.3.-4 Results of stability testing of specimens stored at -20°C that repeated freezing and thawing (Tested at 2020.06.27, 8:35)

Specimen	Test results of Lyher Kits		
	2006003	2006004	2006005
Negative oropharyngeal swab1	Negative	Negative	Negative
Negative oropharyngeal swab2	Negative	Negative	Negative
Negative oropharyngeal swab3	Negative	Negative	Negative
Negative nasopharyngeal swab1	Negative	Negative	Negative
Negative nasopharyngeal swab2	Negative	Negative	Negative
Negative nasopharyngeal swab3	Negative	Negative	Negative
Positive oropharyngeal swab1	Positive	Positive	Positive
Positive oropharyngeal swab2	Positive	Positive	Positive
Positive oropharyngeal swab3	Positive	Positive	Positive
Positive nasopharyngeal swab1	Positive	Positive	Positive
Positive nasopharyngeal swab2	Positive	Positive	Positive
Positive nasopharyngeal swab3	Positive	Positive	Positive
Near cut-off value oropharyngeal swab1	Positive	Positive	Positive
Near cut-off value oropharyngeal swab2	Positive	Positive	Positive
Near cut-off value oropharyngeal swab3	Positive	Positive	Positive
Near cut-off value nasopharyngeal swab1	Positive	Positive	Positive
Near cut-off value nasopharyngeal swab2	Positive	Positive	Positive
Near cut-off value nasopharyngeal swab3	Positive	Positive	Positive

Table 5.2.3.-4 Results of stability testing of specimens stored at -20°C that repeated freezing and thawing (Tested at 2020.06.27, 14:13)

Specimen	Test results of Lyher Kits		
	2006003	2006004	2006005
Negative oropharyngeal swab1	Negative	Negative	Negative
Negative oropharyngeal swab2	Negative	Negative	Negative
Negative oropharyngeal swab3	Negative	Negative	Negative
Negative nasopharyngeal swab1	Negative	Negative	Negative
Negative nasopharyngeal swab2	Negative	Negative	Negative
Negative nasopharyngeal swab3	Negative	Negative	Negative
Positive oropharyngeal swab1	Positive	Positive	Positive
Positive oropharyngeal swab2	Positive	Positive	Positive
Positive oropharyngeal swab3	Positive	Positive	Positive
Positive nasopharyngeal swab1	Positive	Positive	Positive
Positive nasopharyngeal swab2	Positive	Positive	Positive

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Positive nasopharyngeal swab3	Positive	Positive	Positive
Near cut-off value oropharyngeal swab1	Positive	Positive	Positive
Near cut-off value oropharyngeal swab2	Positive	Positive	Positive
Near cut-off value oropharyngeal swab3	Positive	Negative	Positive
Near cut-off value nasopharyngeal swab1	Positive	Positive	Positive
Near cut-off value nasopharyngeal swab2	Positive	Negative	Negative
Near cut-off value nasopharyngeal swab3	Positive	Positive	Positive

From the above test results, the treated nasopharyngeal and oropharyngeal specimens can be frozen and thawed up to 4 times at -20℃. To ensure sample validity, repeated freezing-thawing should not exceed 3 times.

5.3 Transport stability testing

Sent three batches of Lyher Kits by express to Haikou City and Chifeng City and sent them back. Tested specimens by these kits and to test specimens with the expired Kits of these three batches.All the test results shall be accordance with the requirements.

Haikou City: Sent to Haikou City started at April 27,2020 and returned to Hangzhou at July 13,2020.

Chifeng City: Will be sent to Chifeng City at December 26, 2020.

Table 5.3-1 Test results of the Kits returned from Haikou (Tested at 2020.07.14)

Specimen	Test results of Lyher Kits		
	2006003	2006004	2006005
N01	Negative	Negative	Negative
N02	Negative	Negative	Negative
N03	Negative	Negative	Negative
N04	Negative	Negative	Negative
N05	Negative	Negative	Negative
P01	Positive	Positive	Positive
P02	Positive	Positive	Positive
P03	Positive	Positive	Positive
P04	Positive	Positive	Positive
P05	Positive	Positive	Positive
L1	Negative	Negative	Negative
L2	Positive	Positive	Positive
L3	Positive	Positive	Positive

Table 5.3-2 Test results of the Kits returned from Haikou (Will be tested at December 24,2021,The date has not yet arrived)

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Table 5.3-3:Test results of the expired Kits returned from Chifeng (The date has not yet arrived)

Table 5.3-4:Test results of the expired Kits returned from Chifeng (Will be tested at December 24,2021,The date has not yet arrived)

The above test results show that the transportation has no adverse effect on the product quality, but accurate judgment still needs to wait for the test results after the products reach the effective period.