

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

### Study of Stability

#### 1. Objective

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

#### 2. Equipment

2.1 Incubators

2.2 Thermometers

2.3 Refrigerators

#### 3. Testing procedures

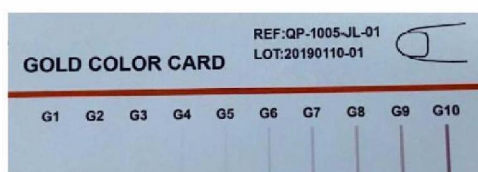
See instructions for detailed operating procedures.

#### 4. Study plan

##### 4.1 Stability study of finished products

##### Specimens:

- Negative Nasal swab specimens: collected from healthy individuals, purchased from Hangzhou KingMed Diagnostics Laboratory Co.,Ltd.
- Positive Nasal swab specimens:
  - L01-L03: prepared by adding inactivated SARS-CoV-2 virus to negative Nasal specimens with titers of  $0.675 \times 10^2$  TCID<sub>50</sub>/mL,  $1.35 \times 10^2$  TCID<sub>50</sub>/mL and  $2.7 \times 10^2$  TCID<sub>50</sub>/mL respectively, namely  $0.5 \times \text{LoD}$ ,  $1 \times \text{LoD}$ , and  $2 \times \text{LoD}$ .
  - P01-P05: prepared by adding inactivated SARS-CoV-2 virus (purchased from Changzhou Ouji Biotech Co.,Ltd) into negative Nasal swab specimens. Concentrations were determined by matching the color intensity of the positive line shown on the test strip against the color lines shown on the Gold Color Card (see in below). The concentration of P01 and P02 were in the range of G5-G6, the concentrations of P03 and P04 were in the range of G6-G8, and the concentrations of P05 were in the range of G9-G10.



Note: Due to pixel of camera, the color in this photo is a little different from the real color card.

#### 4.1.1 Accelerated stability testing

Three batches of Lyher kits (140 pieces of each batch were placed in an incubator set at 60 °C. 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 4.1.1-1: 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
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 4.1.1-2: 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 4.1.1-3: 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
L3	Positive	Positive	Positive

Table 4.1.1-4: 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 4.1.1-5: 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 4.1.1-6: 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
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 4.1.1-7: 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 °C 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°C. To ensure the validity of the products, the validity period of the product is set at 18 months tentatively.

#### 4.1.2 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 °C. If the temperature were lower than 2°C or higher than 30 °C, 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 4.1.2-1: 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

Table 4.1.2-2: Results of Stability Test under storage condition (Month 3, 2020.09.25)

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

Table 4.1.2-3: Results of Stability Test under storage condition (Month 6, 2020.12.25)

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.

#### 4.2 Stability testing of specimens

##### Specimens:

- Negative specimens:
  - Negative Nasal (Nasal) specimens: 3 specimens (collected from healthy individuals, purchased from Hangzhou KingMed Diagnostics Laboratory Co.,Ltd.)
- Positive specimens:
  - Positive Nasal (Nasal) specimens: 3 specimens (prepared by adding inactivated SARS-COV-2 virus to negative Nasal specimens with titers of  $2.7 \times 10^3$  TCID<sub>50</sub>/mL, namely 20×LoD)

##### 4.2.1 Stability testing of the specimens stored at 2-8°C condition.

Stored 6 (3 positive and 3 negative) of Nasal specimens which are near the Cut-off at 2-8 °C. Tested these 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 4.2.1-1 Results of Stability Testing of Specimens Stored At 2-8°C Condition. (Tested at 2020.06.26)

Specimen	Test results of Lyher kits		
	2006003	2006004	2006005
Negative Nasal swab1	Negative	Negative	Negative
Negative Nasal swab2	Negative	Negative	Negative
Negative Nasal swab3	Negative	Negative	Negative
Positive Nasal swab1	Positive	Positive	Positive
Positive Nasal swab2	Positive	Positive	Positive
Positive Nasal swab3	Positive	Positive	Positive
Cut-off Nasal swab1	Positive	Positive	Positive
Cut-off Nasal swab2	Positive	Positive	Positive
Cut-off Nasal swab3	Positive	Positive	Positive

Table 4.2.1-2 Results of Stability Testing of Specimens Stored At 2-8°C Condition. (Tested at 2020.06.29)

Specimen	Test results of Lyher kits		
	2006003	2006004	2006005
Negative Nasal swab1	Negative	Negative	Negative
Negative Nasal swab2	Negative	Negative	Negative
Negative Nasal swab3	Negative	Negative	Negative
Positive Nasal swab1	Positive	Positive	Positive
Positive Nasal swab2	Positive	Positive	Positive
Positive Nasal swab3	Positive	Positive	Positive
Cut-off Nasal swab1	Positive	Positive	Positive
Cut-off Nasal swab2	Positive	Positive	Positive
Cut-off Nasal swab3	Positive	Positive	Positive

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

#### 4.3 Transport stability testing

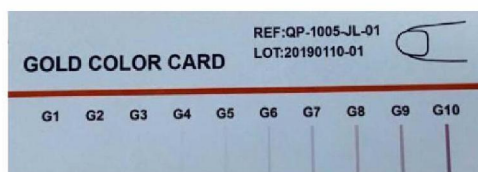
##### Specimens:

- Negative Nasal swab specimens: collected from healthy individuals, purchased from Hangzhou KingMed Diagnostics Laboratory Co.,Ltd.
- Positive Nasal swab specimens:
  - L01-L03: prepared by adding inactivated SARS-CoV-2 virus to negative Nasal specimens with titers of  $0.675 \times 10^2$  TCID<sub>50</sub>/mL,  $1.35 \times 10^2$  TCID<sub>50</sub>/mL and  $2.7 \times 10^2$  TCID<sub>50</sub>/mL respectively, namely  $0.5 \times \text{LoD}$ ,  $1 \times \text{LoD}$ , and  $2 \times \text{LoD}$ .
  - P01-P05: prepared by adding inactivated SARS-CoV-2 virus (purchased from Changzhou Ouji Biotech Co.,Ltd) into negative Nasal swab specimens. Concentrations were determined by matching the color intensity of the positive line shown on the test strip against




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the color lines shown on the Gold Color Card (see in below). The concentration of P01 and P02 were in the range of G5-G6, the concentrations of P03 and P04 were in the range of G6-G8, and the concentrations of P05 were in the range of G9-G10.



Note: Due to pixel of camera, the color in this photo is a little different from the real color card. 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 8, 2020 and returned to Hangzhou at December 23,2020.

Table 4.3-1 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 4.3-2 Test results of the Kits returned from Haikou (Will be tested at December 24,2021, the date has not yet arrived)

Table 4.3-3: Test results of the expired Kits returned from Chifeng (Tested at 2020.12.24)

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 4.3-4: 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.