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

Flex Studies

Operational limits of the device were tested in the following series of experiments. All of these experiments were performed by our engineers in Laihe laboratory.

Materials and Devices:

1) Lyher Novel Coronavirus(COVID-19) Antigen Test Kit (Colloidal Gold) (Lot No. 2010034) (hereinafter referred to as "Lyher Kits")
2) Positive specimens P1 (3×LoD) and P2 (1.5×LoD):

Preparation: Dilute the inactivated SARS-CoV-2 by PBS buffer to the concentration of $4.05\times10^2TCID_{50}$/mL(3×LoD) and $2.025\times10^2TCID_{50}$/mL(1.5×LoD) respectively. Then add 100μl of the above dilutions to negative specimens to build the positive specimen P1 and P2.

Inactivated SARS-CoV-2 in this studies were purchased from Hangzhou Clongene Biotech Co., Ltd. The virus strain was isolated from clinical positive samples and identified by whole genome sequencing

3) Negative Nasal specimens (N) were collected from healthy individuals, purchased from Hangzhou KingMed Diagnostics Laboratory Co.,Ltd.
4) Biosafety cabinet
5) Refrigerator
6) Heater
7) Humidifier
8) Timer
9) Oscillator, mallet, blank mold
10) High-speed centrifuge
11) Yellow plastic film

1. Sample not added to cassette immediately after removing foil bag.

1.1 The objective of the study

To confirm how long the Lyher kit can be exposed to the real use environment within which range the performance of Lyher kit will not be affected.

1.2 Test procedure

1） Take the 9 specimens using for this test: 3 negative specimens N, 3 positive specimen P1, 3 positive specimen P2

2） Treat the specimens according to the package insert.

3） Take 45 pcs of Lyher Kits, 9 pcs for each group, remove the foil bags and take out the kits to exposing into real environment;

4） Start the timer to count;

5） At the time points of 10, 20, 30, 40 and 50 minutes to test the specimens N, P1 and P2 by one group of Lyher Kits respectively.

6） Read the results according to the instructions and record.

1.3 **Results and analysis**

There are 45 test data in 5 groups(From Table 1-1 to Table 1-5), it shows that there is false negative results for Specimen P2(1.5×LoD) in the group which were tested at the time point of 50 minutes and the results of the other four groups are all correct. It indicates that the sample addition operation should be completed within 40 minutes after Lyher Kits are opened, otherwise it may lead to wrong results.

Table 1-1:Results of Delay of Specimen Adding(10 minutes)

Specimen\Time	1	2	3
N	Negative	Negative	Negative
P1	Positive	Positive	Positive
P2	Positive	Positive	Positive

Table 1-2:Results of Delay of Specimen Adding(20 minutes)

Specimen\Time	1	2	3
N	Negative	Negative	Negative
P1	Positive	Positive	Positive
P2	Positive	Positive	Positive

Table 1-3:Results of Delay of Specimen Adding(30 minutes)

Specimen\Time	1	2	3
N	Negative	Negative	Negative
P1	Positive	Positive	Positive
P2	Positive	Positive	Positive

Table 1-4:Results of Delay of Specimen Adding(40 minutes)

Specimen\Time	1	2	3
N	Negative	Negative	Negative
P1	Positive	Positive	Positive
P2	Positive	Positive	Positive

Table 1-5:Results of Delay of Specimen Adding(50 minutes)

Specimen\Time	1	2	3
N	Negative	Negative	Negative
P1	Positive	Positive	Positive
P2	Positive	Negative	Negative

2. Variable sample volumes added to cassette.

2.1 **The objective of the study**

To confirm variable sample volumes added to Lyher kits

2.2 **Test procedure**

1） Take the 9 specimens using for this test: 3 of negative specimens N, 3 of positive specimen P1, 3 of positive specimen P2;

2） Treat the specimens according to the package insert;
3） Take 45 pcs of Lyher Kits, 9 pcs for each group;
4） Take each group of Lyher Kits to test the specimens N, P1 and P2 according the package insert, read the results at 15 minutes.Variable sample volumes tested in this study were 1 drop, 2 drops, 3 drops, 4drops and 5drops.

2.3 **Results and analysis**

There are 45 test data in 5 groups(From Table 2-1 to Table 2-5), it can be seen that the incorrect results were obtained for P2 (1.5×LoD) when 1 drop of specimen was added. The results of this study demonstrated that false negative results are obtained if the volume less than 2 drops is used for sample addition to the kits. The results of this study demonstrated that the volume of specimen adding to Lyher Kit should not be less than 2 drops, otherwise it may result in false negative results.

Table 2-1:Results of the Study of Different Specimen Volume Added(1 drop)

Specimen\Time	1	2	3
N	Negative	Negative	Negative
P1	Positive	Positive	Positive
P2	Negative	Positive	Positive

Table 2-2: Results of the Study of Different Specimen Volume Added(2 drops)

Specimen\Time	1	2	3
N	Negative	Negative	Negative
P1	Positive	Positive	Positive
P2	Positive	Positive	Positive

Table 2-3: Results of the Study of Different Specimen Volume Added(3 drops)

Specimen\Time	1	2	3
N	Negative	Negative	Negative
P1	Positive	Positive	Positive
P2	Positive	Positive	Positive

Table 2-4: Results of the Study of Different Specimen Volume Added(4 drops)

Specimen\Time	1	2	3
N	Negative	Negative	Negative
P1	Positive	Positive	Positive
P2	Positive	Positive	Positive

Table 2-5: Results of the Study of Different Specimen Volume Added(5 drops)

Specimen\Time	1	2	3
N	Negative	Negative	Negative
P1	Positive	Positive	Positive
P2	Positive	Positive	Positive

3. Operator delays reading the cassette after test is complete.

3.1 **The objective of the study**

To confirm what is the best time point to read the result.

3.2 **Test procedure**

1） Take the 9 specimens using for this test: 3 of negative specimens N, 3 of positive specimen P1, 3 of positive specimen P2;

2） Treat the specimens according to the package insert;

3） Take 45 pcs of Lyher Kits, 9 pcs for each group;

4） To test the specimens according to the package insert but results are read off the kit at varying times following test completion. Time points tested are 10 minutes, 15 minutes, 20 minutes and 25 minutes.

3.3 **Results and analysis**

There are 36 test results in 4 groups(from Table 3-1 to Table 3-4). It can be seen that correct results for the tests were obtained for the time points of 15 minutes and 20 minutes after the test was completed. But the false negative was obtained for the positive specimen P2 at the time point of 10 minutes and the false positive result was obtained for the negative specimen N at the time point of 25 minutes. This study demonstrates that the test can provide a correct and valid result interpretation at the recommended read time (15 minutes).

Table 3-1:Results of Reading at Different Time after Sample Addition(10 minutes)

Specimen\Time	1	2	3
N	Negative	Negative	Negative
P1	Positive	Positive	Positive
P2	Positive	Negative	Negative

Table 3-2:Results of Reading at Different Time after Sample Addition(15 minutes)

Specimen\Time	1	2	3
N	Negative	Negative	Negative
P1	Positive	Positive	Positive
P2	Positive	Positive	Positive

Table 3-3:Results of Reading at Different Time after Sample Addition(20 minutes)

Specimen\Time	1	2	3
N	Negative	Negative	Negative
P1	Positive	Positive	Positive
P2	Positive	Positive	Positive

Table 3-4:Results of Reading at Different Time after Sample Addition(25 minutes)

Specimen\Time	1	2	3
N	Negative	Suspected Positive	Negative
P1	Positive	Positive	Positive
P2	Positive	Positive	Positive

4. Delay between sample collection and extracting in the provided buffer.

4.1 **The objective of the study**

To confirm how long it could be that the delay between sample collection and extracting in the provided buffer.

4.2 **Test procedure**

1） Take the specimens N, P1 and P2, 5 for each type, in total 15 aliquot. Arrange the 15 aliquot into 5 groups,each group contains 1of specimen N, 1 of specimen P1 and 1 of specimen P2.

2） Store the above swabs specimen at room temperature and wait for different time to elute the specimens.The following wait times are tested between preparation of specimens and extracting: 1 hour (SOP), 2 hours, 3 hour, 4 hours and 5 hours.

3） Using 45pcs of Lyher kits to test the above specimens and read the results according to the package insert and each specimen shall be test for 3 times.

For this study, samples were tested according to the package insert with one variable: the time between sample collection (sample spike onto swabs) and extracting; the control condition was immediate extracting and testing (SOP). The following wait times were tested between spike of sample onto swab and extracting: 0 minutes (SOP), 30 minutes, 1 hour, and 15 hours. Correct results for all 27 tests were obtained for the SOP condition, the 30 minute timepoint, and the one hour time point for both Influenza A and Influenza B samples. This study demonstrates that accurate test results can be obtained if the operator delays extracting the sample from the swab for up to one hour.

4.3 **Results and analysis**

There are 45 test results in 5 groups(from Table 4-1 to Table 4-5). It can be seen that incorrect results for the specimen P2 (1.5×LoD) are obtained at the 5 hours timepoint under the SOP condition. This study demonstrates that accurate test results can be obtained if the operator delays extracting the sample from the swab for up to 4 hours.

Table 4-1:Results of Delay between sample collection and extracting(1 hour)

Specimen\Time	1	2	3
N	Negative	Negative	Negative
P1	Positive	Positive	Positive
P2	Positive	Positive	Positive

Table 4-2:Results of Delay between sample collection and extracting(2 hours)

Specimen\Time	1	2	3
N	Negative	Negative	Negative
P1	Positive	Positive	Positive
P2	Positive	Positive	Positive

Table 4-3:Results of Delay between sample collection and extracting(3 hours)

Specimen\Time	1	2	3
N	Negative	Negative	Negative
P1	Positive	Positive	Positive
P2	Positive	Positive	Positive

Table 4-4:Results of Delay between sample collection and extracting(4 hours)

Specimen\Time	1	2	3
N	Negative	Negative	Negative
P1	Positive	Positive	Positive
P2	Positive	Positive	Positive

Table 4-5:Results of Delay between sample collection and extracting(5 hours)

Specimen\Time	1	2	3
N	Negative	Negative	Negative
P1	Positive	Positive	Positive
P2	Negative	Positive	Positive

5. **Operator uses method(s) other than the SOP for extracting the swab**

5.1 **The objective of the study**

To confirm the tolerance of Lyher kits to the deviation of the SOP for extracting the swab

5.2 **Test procedure**

1） Take the specimens N, P1 and P2, 5 for each type, in total 15 aliquot. Arrange the 15 aliquot into 5 groups,each group contains 1of specimen N, 1 of specimen P1 and 1 of specimen P2.

2） Take 45 pcs of Lyher kits and apart them into 5 groups and 9 pcs each group;

3） The above specimens are treated in five different methods:

① Squeeze the swab 5 times and let it stand for 1 minute;

② Squeeze the swab 7 times and let it stand for 1.5 minutes;

③ Squeeze the swab 10 times and let it stand for 2 minutes;

④ Squeeze the swab 14 times and let it stand for 3 minutes;

⑤ Squeeze the swab 18 times and let it stand for 5 minutes;

4） Test the above specimens by Lyher kits and read the results according to the package inserts in the above four different usage scenarios. Each specimen shall be tested for 3 times.

5.3 **Results and analysis**

There are 45 test results in 5 groups (from Table 6-1 to Table 6-5). It can be seen that there are false negative results being obtained for the specimens P1(3×LoD)and P2(1.5×LoD)being squeezed 5 times and let stand for 1 minute. And there are false negative results still being obtained for the specimen P2 (1.5×LoD)being squeezing for 7 times and let stand for 1.5 minutes.The results of this study indicate that the appropriate treatment of the specimen used for Lyher Kits is to squeeze the swab at least for 10 times and let it stand for 2 minutes.

5.4 **Deviation treatment**

Due to the poor fault tolerance of the sample processing program and its key to the test results, incorrect sample processing is likely to occur during the use of the product. In order to avoid the wrong results, video training materials for sample

processing are provided and eye-catching positions such as the instruction manual will prompt "strictly follow the requirements of the instruction for sample processing".

Table 6-1:Results of the specimens treated by different methods(Squeeze 5 times, Stand for 1 minutes)

Specimen\Time	1	2	3
N	Negative	Negative	Negative
P1	Positive	Negative	Positive
P2	Negative	Negative	Negative

Table 6-2:Results of the specimens treated by different methods(Squeeze 7 times, Stand for 1.5 minutes)

Specimen\Time	1	2	3
N	Negative	Negative	Negative
P1	Positive	Positive	Positive
P2	Positive	Positive	Negative

Table 6-3:Results of the specimens treated by different methods(Squeeze 10 times, Stand for 2 minutes)

Specimen\Time	1	2	3
N	Negative	Negative	Negative
P1	Positive	Positive	Positive
P2	Positive	Positive	Positive

Table 6-4:Results of the specimens treated by different methods(Squeeze 14 times, Stand for 3 minutes)

Specimen\Time	1	2	3
N	Negative	Negative	Negative
P1	Positive	Positive	Positive
P2	Positive	Positive	Positive

Table 6-5:Results of the specimens treated by different methods(Squeeze 18 times, Stand for 5 minutes)

Specimen\Time	1	2	3
N	Negative	Negative	Negative
P1	Positive	Positive	Positive
P2	Positive	Positive	Positive

6. Test is performed at extremes of temperature and humidity.

6.1 **The objective of the study**

To confirm the influence of extreme environmental temperature and humidity on the test results.

6.2 **Test procedure**

1） Take the specimens N, P1 and P2, 4 for each type, in total 12 aliquot. Arrange the 12 aliquot into 4 groups,each group contains 1of specimen N, 1 of specimen P1 and 1 of specimen P2.

2） Take 36 pcs of Lyher kits and apart them into 4 groups and 9 pcs each group;

3） Set up four usage scenarios with different temperatures and humidity:

① Store the specimens and test kits in a refrigerator set at 2-8°C;

② Adjust the ambient temperature to 16°C and control the relative humidity below 30% with air conditioner and dehumidifier;

③ Use heater and humidifier to adjust temperature to 30°C and relative humidity to 80%;

④ Set the temperature to 40°C and the relative humidity to above 95%;

4） Test the specimens by Lyher kits and read the results according to the package inserts in the above four different usage scenarios. Each specimen shall be tested for 3 times.

6.3 **Results and analysis**

There are 36 test results in 4 groups (from Table 7-1 to Table 7-4). It can be seen that if specimens and test kits are taken out of the refrigerator and tested immediately, the C-line will not be displayed or displayed normally, resulting in the failure of the test while the other temperatures and humidity will not affect the performance of Lyher Kit.In order to avoid such errors, it should be stated in the instructions that the specimens and test kits should be balanced to room temperature before operation.

Table 7-1:The influence of different temperature and humidity on the test results (Taken out of the refrigerator and tested immediately)

Specimen\Time	1	2	3
N	Abnormal	Abnormal	Abnormal
P1	Abnormal	Abnormal	Abnormal
P2	Abnormal	Abnormal	Abnormal

Table 7-2:The influence of different temperature and humidity on the test results(16°C, RH% < 30%)

Specimen\Time	1	2	3
N	Negative	Negative	Negative
P1	Positive	Positive	Positive
P2	Positive	Positive	Positive

Table 7-3:The influence of different temperature and humidity on the test results(30°C, RH%=80%)

Specimen\Time	1	2	3
N	Negative	Negative	Negative
P1	Positive	Positive	Positive
P2	Positive	Positive	Positive

Table 7-4:The influence of different temperature and humidity on the test results(40°C, RH% > 95%)

Specimen\Time	1	2	3
N	Negative	Negative	Negative
P1	Positive	Positive	Positive
P2	Positive	Positive	Positive

7. Test is performed with a dropped or damaged cassette.

7.1 **The objective of the study**

To confirm the influence of general contamination (the device drop to the floor) and outside shell damage on test results.

7.2 **Test procedure**

1） Take 1 of the specimens N, P1 and P2;

2） Take 18 pcs of Lyher kits and apart them into 2 groups and 9 pcs each group;

3） Set up two usage scenarios:

① Open the foil bag and drop the cassettes to the floor and pick them up ;

② Open the foil bag and damage the shells of the cassettes with a hammer;

4） Test the specimens by Lyher kits and read the results according to the package inserts in the above two different usage scenarios. Each specimen shall be tested for 3 times.

7.3 **Results and analysis**

There are 18 test results in 2 groups(from Table 8-1 to Table 8-2). It can been seen that the performance of Lyher kits will not be affected no matter they fall to the ground or their shells are damaged.

Table 8-1:Results of the Lyher Kit which once dropped to the ground

Specimen\Time	1	2	3
N	Negative	Negative	Negative
P1	Positive	Positive	Positive
P2	Positive	Positive	Positive

Table 8-2:Results of the Lyher Kits which are damaged outside

Specimen\Time	1	2	3
N	Negative	Negative	Negative
P1	Positive	Positive	Positive
P2	Positive	Positive	Positive

8. Test is performed and results are read under sub-optimal lighting.

8.1 **The objective of the study**

To confirm the influence of light conditions on the interpretation of test results.

8.2 **Test procedure**

1） Take 1 of the specimens N, P1 and P2;

2） Take 18 pcs of Lyher kits and apart them into 2 groups and 9 pcs each group;

3） Set up two usage scenarios:

① Cover the bulb with a yellow plastic film to turn the light into yellow;

② Turn on all lights in the lab to create a highlight environment with illumination above 600;

4） Test the specimens by Lyher kits and read the results according to the package inserts in the above two different usage scenarios. Each specimen shall be tested for 3 times.

8.3 **Results and analysis**

There are 18 test results in 2 groups(from Table 9-1 to Table 9-2). It can be seen that incorrect results are obtained for the Specimen P2(1.5×LoD)at the weak light condition and there are no incorrect results being obtained at strong light condition. In order to avoid misjudgment of results due to insufficient light, the test results should be clearly read in the position of instruction manual reading results and matters needing attention and should be carried out under sufficient lighting conditions.

Table 9-1:Result interpretation under different illumination conditions(Weak light)

Specimen\Time	1	2	3
N	Negative	Negative	Negative
P1	Positive	Positive	Positive
P2	Positive	Negative	Negative

Table 9-2:Result interpretation under different illumination conditions(Strong Light)

Specimen\Time	1	2	3
N	Negative	Negative	Negative
P1	Positive	Positive	Positive
P2	Positive	Positive	Positive

9. Test is performed on non-level surface

9.1 **The objective of the study**

To confirm the influence of the tilt of the kits on the test results.

9.2 **Test procedure**

1） Take 1 of the specimens N, P1 and P2;

2） Take 36 pcs of Lyher kits and apart them into 4 groups and 9 pcs each group;

3） Set up four usage scenarios with different tilt of the cassettes:

① Raise the upper end of the cassette up with a blank mould ;

② Raise the sample hole's end of the cassette up;

③ Raise the right side of the cassettes up;

④ Raise the left side of the cassettes up;

4） Test the specimens by Lyher kits and read the results according to the package inserts in the above four different usage scenarios. Each specimen shall be tested for 3 times.

9.3 **Results and analysis**

There are 36 test results in 4 groups(from Table 10-1 to Table 10-4). It can be seen that the performance of Lyher kit will not be affected no matter which side of the cassette being raised up to make it non-level. However, if the tilt continues to increase, the sample may flow out of the sampling hole, resulting in insufficient sample volume and affecting the test results.

Table 10-1:Test results with different tilt(C-line's end being raised up)

Specimen\Time	1	2	3
N	Negative	Negative	Negative
P1	Positive	Positive	Positive
P2	Positive	Positive	Positive

Table 10-2:Test results with different tilt(Sample hole's being end raised up)

Specimen\Time	1	2	3
N	Negative	Negative	Negative
P1	Positive	Positive	Positive
P2	Positive	Positive	Positive

Table 10-3:Test results with different tilt(Left side being raised up)

Specimen\Time	1	2	3
N	Negative	Negative	Negative
P1	Positive	Positive	Positive
P2	Positive	Positive	Positive

Table 10-4:Test results with different tilt(Right side being raised up)

Specimen\Time	1	2	3
N	Negative	Negative	Negative
P1	Positive	Positive	Positive
P2	Positive	Positive	Positive

10. Test is performed on Disturbance.

10.1 **The objective of the study**

To confirm the influence of common laboratory noise and vibration on test results

10.2 **Test procedure**

1） Take 1 of the specimens N, P1 and P2;

2） Take 18 pcs of Lyher kits and apart them into 2 groups and 9 pcs each group;

3） Set up two usage scenarios:

① Opening an oscillator to make the whole process of the testing is in a state of slight vibration;

② Opening a high speed centrifuge to make the whole test process is in a high decibel environment;

4） Test the specimens by Lyher kits and read the results according to the package inserts in the above two different usage scenarios. Each specimen shall be tested for 3 times.

10.3 **Results and analysis**

There are 18 test results in 2 groups(from Table 11-1 to Table 11-2). It can be seen that neither vibration nor noise had any effect on the test results.

Table 11-1:Test results In Interference Environment(Vibration)

Specimen\Time	1	2	3
N	Negative	Negative	Negative
P1	Positive	Positive	Positive
P2	Positive	Positive	Positive

Table 11-1:Test results In Interference Environment(Noise)

Specimen \Time	1	2	3
N	Negative	Negative	Negative
P1	Positive	Positive	Positive
P2	Positive	Positive	Positive