Reaction system for Novel Coronavirus (COVID-19) Antigen Test Kit (Colloidal Gold)

Assay method used

The immune colloidal gold technique is used in the assay to detect antigens of COVID-19. The reagent binding pad is coated with anti-SARS-CoV-2 monoclonal antibodies which is labeled with colloidal gold marker, respectively. A nitrocellulose membrane in test area of a strip is coated with anti-SARS-CoV-2 antibodies. The quality control area within the nitrocellulose membrane is coated with goat anti-mouse IgG antibodies. When testing, the antibodies againstCOVID-19formimmuno-complexes with the antigen protein of the virus in the specimen to be tested. As a result of chromatography, immuno-complexes move along the membrane and will be captured by the anti-SARS-CoV-2 antibodies coated in the test area to form a visible line with red color (T-line). The free colloidal gold marker or immune complexes continue to move forward and specifically bind to the goat anti-mouse antibody coated in the quality control area to form a visible line (C line). If the specimen does not contain the antigen of COVID-19, no test line will show, only quality control line(C-line) will appear.

The validation protocol and report

The protocol were validated by 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 (1.0×LoD):
   Preparation: Dilute the inactivated SARS-CoV-2 by PBS buffer to the concentration of $4.05\times10^{2}TCID_{50}$/mL(3×LoD) and $1.35\times10^{2}TCID_{50}$/mL(1.0×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 specimens (N) were collected from healthy individuals, purchased from Hangzhou KingMed Diagnostics Laboratory Co.,Ltd.
4. Biosafety cabinet
5. Refrigerator
6. Timer

1. Optimal observation time of test result

1.1 **The objective of the study**

To confirm Optimal observation time of test results

1.2 **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 5 minutes, 10 minutes, 15 minutes, 20 minutes and 25 minutes respectively.

1.3 **Results and analysis**

There are 45 test data in 5 groups(From Table 1-1 to Table 1-5), it can be seen that all of the results were conformed to expectation when reading the results in 15 minutes. This assay demonstrated that reading the results in 15 minutes will get the best results.

Table 1-1: Results of the Study of Different time to read the results (5 minutes)

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

Table 1-2: Results of the Study of Different time to read the results (10 minutes)

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

Table 1-3: Results of the Study of Different time to read the results (15 minutes)

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

Table 1-4: Results of the Study of Different time to read the results (20 minutes)

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

Table 1-5: Results of the Study of Different time to read the results (25 minutes)

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

2. Optimal sample volumes

2.1 **The objective of the study**

To confirm Optimal 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, 4 drops and 5 drops.

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 all of the results were conformed to expectation when 3 drop of specimen was added. This assay demonstrated that 3 drops of sample will get the best results.

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

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

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

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

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

Specimen\Times	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	Negative	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	Negative	Negative	Negative

3. Optimal Specimen extraction buffer volumes for eluting the swab.

3.1 **The objective of the study**

To confirm the Optimal volumes specimen extraction buffer for eluting the swab.

3.2 **Test procedure**

1） Take the specimens N, P1 and P2, 6 for each type, in total 18 aliquot. Arrange the 18 aliquot into 6 groups, each group contains 1of specimen N, 1 of specimen P1 and 1 of specimen P2.

2） Treat the specimens according to the package insert but with different volume of extraction buffer, the following volumes of extraction buffer are used: 7 drops, 8 drops, 9drops, 10 drops, 11 drops and 12 drops;

3） Using 54 pcs 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.

3.3 **Results and analysis**

There are 54 test results in 6 groups (from Table 3-1 to Table 3-6). It can be seen that10 drops of extraction buffer is prefect for the sample extraction.

Table 3-1: Results of the Specimens Treated by Different Volume of Extraction Buffer (7 drops)

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

Table 3-2: Results of the Specimens Treated by Different Volume of Extraction Buffer (8 drops)

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

Table 3-3: Results of the Specimens Treated by Different Volume of Extraction Buffer (9 drops)

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

Table 3-4: Results of the Specimens Treated by Different Volume of Extraction Buffer (10 drops)

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

Table 3-5: Results of the Specimens Treated by Different Volume of Extraction Buffer (11 drops)

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

Table 3-6: Results of the Specimens Treated by Different Volume of Extraction Buffer(12 drops)

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

4. Optimal SOP for eluting the swab

4.1 The objective of the study

To confirm the Optimal SOP of eluting the swab

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） 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.

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 squeezed more than 10 times and let stand for more than 1 minute will get the best results.

Table 4-1: 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 4-2: 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 4-3: 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 4-4: 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 4-5: 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