COVID-19 Antigen Test kit (Colloidal Gold)

Stability Study Data

Product Name:	COVID-19 Antigen Test Kit (Colloidal Gold)
Applicant	SINGUWAY BIOTECH INC.
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CONTENT

1. Purpose of stability study

Stability is an essential attribute of in vitro diagnostic reagents, and it is an important index to ensure the safety and effectiveness of products in use. This study provides novel coronavirus COVID-19 Antigen detection kit(Colloidal gold method) storage condition and validity period by conducting stability studies under different conditions, according to CLSI Standard EP25–Evaluation of Stability of In Vitro Diagnostic Reagents.

2. Specific methods of stability study

2.1 Stability study plan

2.1.1 Stability test at actual storage temperature

A novel coronavirus COVID-19 Antigen detection kit(Colloidal gold method) was developed for the three batches (20200603, 20200611 and 20200620) of the continuous trial production (hereinafter referred to as the COVID-19 Antigen detection kit). Each batch of 10 boxes was stored below 2-8 degrees and RT (room temperature), and each was conducted at 0, 3, 6, 9, 12, 15, 18, 24 and 26 months respectively. Using COVID-19 Antigen factory reference as the test sample, the change of the quality of the kit with the increase of storage time was investigated. The test process was strictly in accordance with the provisions of the user manual, and the test results were summarized in the attached table.

2.1.2 Simulated transport stability test

The three batches (20200603, 20200611, and 20200620) of COVID-19 Antigen test kits were put into 15 boxes of each batch, and then put into medium foam boxes with ice bags, and then packed in transport cartons. Place the transport carton with the kit in the trunk of the transport vehicle and walk with the vehicle every day. Take it out at the end of 5 day by air and 20day by ship. Use COVID-19 Antigen factory reference as the assessment sample, and use COVID-19 Antigen detection kit for detection.

2.1.3 Opening stability test

Take three batches (20200603, 20200611 and 20200620) of COVID-19 Antigen detection kits for continuous trial production, each batch of 12 kits are opened and stored at RT for 15days, i.e. One assessment test on the 0, 15, 30 and 45 days respectively. Using COVID-19 Antigen enterprise reference as the test sample, the change of the quality of the kit with the increase of storage time was investigated. The test process was strictly in accordance with the provisions of the user manual, and the test results were summarized in the attached table.

2.1.4 Freeze thaw simulation stability test

Take three batches (20200603, 20200611 and 20200620) of COVID-19 Antigen detection kits for continuous trial production, and put 12 kits of each batch at 37℃ for storage, and test the stability of 4, 8, 12 and 24 hours. Using COVID-19 Antigen factory reference product as the test sample, the accelerated stability of the kit quality was investigated, and the test process was operated in strict accordance with the provisions of the user manual, and the test results were summarized in the attached table.

2.2 Quality control of experiment

2.2.1 Negative control: the results were negative

2.2.2 Positive control: the results were positive

2.2.3 The above two items need to be met in one experiment at the same time, otherwise, this experiment is invalid, and the experiment should be carried out again.

2.3 Reagent qualification standard

2.3.1 Appearance

The packing box shall be clean and tidy, each component shall be complete, the inner packing tube cover shall be tight, the negative control shall have sedimentation after standing, the contents of other components shall be clear and free of sedimentation after melting, the instruction manual shall be clear and complete, the product name, batch number and validity period shall be clear.

2.3.2 Capacity

The contents of each component of the kit shall meet the requirements of the following table:

No.	Component name	Marked capacity	Rated capacity	Capacity verification
1	Lysis buffer	6.5ml×2 bottles	6.5mL	No less than 6ml
2	Test cards	25	25	No less than 25
3	Swabs	25	25	No less than 25
4	tubes	25	25	No less than 25
5	Tube handdle	1	1	No less than 1
6	Filter drip head	25	25	Filter drip head

2.3.3 Technical indicators

2.3.3.1 Minimum detection limit

Novel coronavirus Antigen reference material was detected in the reference material S. The test results should meet the following requirements: S1 is positive and S2, S3 only fr.

2.3.3.2 Compliance rate of negative reference

The detection of novel coronavirus Antigen reference materials in factory 8 negative reference materials N1~N8, the test results should be all negative; the coincidence rate is 100%.

2.3.3.3 Compliance rate of positive reference

Detection of novel coronavirus Antigen reference material in the enterprise 10 positive reference materials L, the results should be the coincidence rate is 100%.

2.3.3.4 Precision

Detection of novel coronavirus Antigen reference materials in the enterprise reference materials R1, R2, R3 repeated detection 10 times, the results of it will be positive.

3. Results of stability studies

3.1 Stability test results at storage temperature

According to the stability test scheme under storage temperature, 10 kits of COVID-19 Antigen detection kits of three batches (20200603 batch, 20200611 batch, 20200620 batch) of continuous trial production were stored in the temperature below 2-8℃ and room temperature (RT, 15-30℃) for each batch, and were tested once in the 0, 3, 6, 9, 12, 15, 18, 24 and 26 months respectively. See table 3-1 and 3-2 for the test results.

Table 3.1-1test results of test enterprise reference materials after the reagent box stored at 2-8℃ for 0-26months

Inspection items Lot/ Storage month		Appearance	reference N1~N5	Positive reference P1~P5	Limit the test to reference S1,S2,S3	Repeat reference 10 times
		Q/NG	Compliance rate(-/-)	Compliance rate(+/+)	Positive reference	Compliance rate(+/+)
20200603 Test result	0 month	Q	5/5	5/5	S1, S2	10/10
	3 months	Q	5/5	5/5	S1	10/10
	6 months	Q	5/5	5/5	S1	10/10
20200611 Test result	0 month	Q	5/5	5/5	S1	10/10
	3 months	Q	5/5	5/5	S1	10/10
20200620 Test result	0 month	Q	5/5	5/5	S1	10/10
	3 months	Q	5/5	5/5	S1	10/10

Table 3.1-2 test results of test enterprise reference materials after the reagent box stored at RT for 0-26 months

Inspection items Lot/ Storage month		Appearance	Negative reference N1~N5	Positive reference P1~P5	Limit the test to reference S1,S2,S3	Repeat reference 10 times
		Q/NG	Compliance rate(-/-)	Compliance rate(+/+)	Positive reference	Compliance rate(+/+)
20200603 Test result	0 month	Q	5/5	5/5	S1、S2	10/10
	3 months	Q	5/5	5/5	S1	10/10
	6 months	Q	5/5	5/5	S1	10/10
20200611 Test result	0 month	Q	5/5	5/5	S1	10/10
	3 months	Q	5/5	5/5	S1	10/10
20200620 Test result	0 month	Q	5/5	5/5	S1	10/10
	3 months	Q	5/5	5/5	S1	10/10

It can be concluded from table 3-1 that the COVID-19 Antigen detection kit developed and manufactured by our company can reach its performance index after being stored for 6 month under 2-30℃. Since the R & D is less than 6 months, only one batch has been tested for 9months stability, but it is still qualified.

3.2 Research results of simulated transport stability test

According to the simulation transportation stability test scheme, three batches (20200603, 20200611 and 20200620) of continuous trial production are selected as COVID-19 was put in 15 boxes of each batch in the medium foam box with ice bags. The two boxes were removed after first, third, fifth days of transportation. The remaining reagent was transported 5days by air and for 20 days by ship. The results were shown in table 3-2.

Table 3.2-1 simulation transport stability test results of reference products

Inspection items Lot/ Storage month		Appearance	Negative reference N1~N5	Positive reference P1~P5	Limit the test to reference S1, S2, S3	Repeat reference 10 times
		Q/NG	Compliance rate(-/-)	Compliance rate(+/+)	Positive reference	Compliance rate(+/+)
20200603 Test result	0 day	Q	5/5	5/5	S1、S2	10/10
	5 days	Q	5/5	5/5	S1、S2	10/10
	20days	Q	5/5	5/5	S1	10/10
20200611 Test result	0 day	Q	5/5	5/5	S1	10/10
	5 days	Q	5/5	5/5	S1	10/10
	20days	Q	5/5	5/5	S1	10/10
20200620	0 day	Q	5/5	5/5	S1	10/10

Test result	5 days	Q	5/5	5/5	S1	10/10
	20days	Q	5/5	5/5	S1	10/10

From table 3.2-1, it can be concluded that the experimental results of the COVID-19 Antigen detection kit designed by our company for the first, third and fifth day of transportation under the simulated transportation conditions and the test enterprise reference products after transportation are all within its performance indexes.

3.3 Research results of opening stability test

According to the opening stability test scheme, take three batches (20200603, 20200611 and 20200620) of COVID-19 Antigen detection kits, each of which has 12 boxes, which are opened and stored at RT for 45 days. See table 3.3-1 for the test results.

Table 3.3-1 Opening stability test results of reference products

Inspection items Lot/ Storage month		Appearance	Negative reference N1~N5	Positive reference P1~P5	Limit the test to reference S1, S2, S3	Repeat reference 10 times
		Q/NG	Compliance rate(-/-)	Compliance rate(+/+)	Positive reference	Compliance rate(+/+)
20200603 Test result	0 day	Q	5/5	5/5	S1	10/10
	15 days	Q	5/5	5/5	S1	10/10
	30days	Q	5/5	5/5	S1	10/10
	45days	Q	5/5	5/5	S1	10/10
20200611 Test result	0 day	Q	5/5	5/5	S1	10/10
	5 days	Q	5/5	5/5	S1	10/10
	20days	Q	5/5	5/5	S1	10/10
	45days	Q	5/5	5/5	S1	10/10
20200620 Test result	0 day	Q	5/5	5/5	S1	10/10
	5 days	Q	5/5	5/5	S1	10/10
	20days	Q	5/5	5/5	S1	10/10
	45days	Q	5/5	5/5	S1	10/10

It can be concluded from table 3.3-1 that the COVID-19 Antigen detection kit developed and produced by our company is within its performance index when it is opened and stored at RT for 0 to 45 days.

3.4 Results of accelerated validity stability test

According to the simulated freeze-thaw stability experiment scheme, three batches (20200603, 20200611 and 20200620 batches) of COVID-19 Antigen detection kit in continuous trial production were taken and each batch of 12 boxes were stored below 37℃, 50℃and 65℃ they were divided into four groups and stored for 12, 24, 36, 48 and 60 days, respectively. The test

results are shown in table 3.4-1.

Table 3.4-1 Results of accelerated stability test of reference products-37℃

Inspection items Lot/ Storage month		Appearance	Negative reference N1~N5	Positive reference P1~P5	Limit the test to reference S1, S2, S3	Repeat reference 10 times
		Q/NG	Compliance rate(-/-)	Compliance rate(+/+)	Positive reference	Compliance rate(+/+)
20200603 Test result	12 day	Q	5/5	5/5	S1	10/10
	24 days	Q	5/5	5/5	S1	10/10
	36days	Q	5/5	5/5	S1	10/10
	48days	Q	5/5	5/5	S1	10/10
	60days	Q	5/5	5/5	S1	10/10
20200611 Test result	12 day	Q	5/5	5/5	S1	10/10
	24 days	Q	5/5	5/5	S1	10/10
	36days	Q	5/5	5/5	S1	10/10
	48days	Q	5/5	5/5	S1	10/10
	60days	Q	5/5	5/5	S1	10/10
20200620 Test result	12 day	Q	5/5	5/5	S1	10/10
	24 days	Q	5/5	5/5	S1	10/10
	36days	Q	5/5	5/5	S1	10/10
	48days	Q	5/5	5/5	S1	10/10
	60days	Q	5/5	5/5	S1	10/10

Table 3.4-2 Results of accelerated stability test of reference products-50℃

Inspection items Lot/ Storage month		Appearance	Negative reference N1~N5	Positive reference P1~P5	Limit the test to reference S1,S2,S3	Repeat reference 10 times
		Q/NG	Compliance rate(-/-)	Compliance rate(+/+)	Positive reference	Compliance rate(+/+)
20200603 Test result	12 day	Q	5/5	5/5	S1	10/10
	24 days	Q	5/5	5/5	S1	10/10
	36days	Q	5/5	5/5	S1	10/10
	48days	Q	5/5	5/5	S1	10/10
	60days	Q	5/5	5/5	S1	10/10
20200611 Test result	12 day	Q	5/5	5/5	S1	10/10
	24 days	Q	5/5	5/5	S1	10/10
	36days	Q	5/5	5/5	S1	10/10

	48days	Q	5/5	5/5	S1	10/10
	60days	Q	5/5	5/5	S1	10/10
20200620 Test result	12 day	Q	5/5	5/5	S1	10/10
	24 days	Q	5/5	5/5	S1	10/10
	36days	Q	5/5	5/5	S1	10/10
	48days	Q	5/5	5/5	S1	10/10
	60days	Q	5/5	5/5	S1	10/10

Table 3.4-3 Results of accelerated stability test of reference products-60℃

Inspection items Lot/ Storage month		Appearance	Negative reference N1~N5	Positive reference P1~P5	Limit the test to reference S1,S2,S3	Repeat reference 10 times
		Q/NG	Compliance rate(-/-)	Compliance rate(+/+)	Positive reference	Compliance rate(+/+)
20200603 Test result	12 day	Q	5/5	5/5	S1	10/10
	24 days	Q	5/5	5/5	S1	10/10
	36days	Q	5/5	5/5	N	10/10
	48days	Q	5/5	5/5	N	10/10
	60days	Q	5/5	5/5	N	10/10
20200611 Test result	12 day	Q	5/5	5/5	S1	10/10
	24 days	Q	5/5	5/5	S1	10/10
	36days	Q	5/5	4/5	N	10/10
	48days	Q	5/5	3/5	N	9/10
	60days	Q	5/5	3/5	N	9/10
20200620 Test result	12 day	Q	5/5	5/5	S1	9/10
	24 days	Q	5/5	5/5	S1	10/10
	36days	Q	5/5	5/5	N	10/10
	48days	Q	5/5	5/5	N	10/10
	60days	Q	5/5	4/5	N	7/10

It can be concluded from table 3.4-1, 3.4-2, 3.4-3that the experimental results of the COVID-19 Antigen detection kit developed and produced by our company are all within its performance indexes after being stored at 37℃ and 50℃for 60 days. Therefore, it can be considered that the product can be stored at 60℃ for 24 days

The differential forms of Arrhenius do the indefinite integral:

$$lnk = \frac{-E_a}{RT} + C$$

You can see by the type, with LNK for 1\ T drawing should be a straight line, the slope - Ea\ R.

For the majority of chemical reaction, have such relation. If the temperature T1 and T2, respectively, the reaction rate constant k1 and k2, respectively, then:

$$\ln k_1 \quad -\frac{E_a}{RT_1}+C, \ln k_2 \quad -\frac{E_a}{RT_2}+C$$

$$\ln\frac{k_2}{k_1} \quad -\frac{E_a}{R}\left(\frac{1}{T_2}-\frac{1}{T_1}\right) \quad -\frac{E_a}{R}\frac{T_1-T_2}{T_1T_2}$$

$$lgx=\frac{lnx}{ln10}, ln10\approx 2.303$$

$$lg\frac{k_1}{k_2}=\frac{E_a}{2.303R}\frac{T_1-T_2}{T_1T_2}$$

When it is 60℃(333.15K) it can be stored at least 25days; when it is 50℃ (323.15K) it can be stored at least 60 days. To the above formula:

$$\frac{Ea}{2.303R}=\lg\frac{k1}{k2}\frac{T1T2}{T1-T2}=\lg\frac{1/60}{1/25}*\frac{323.15*333.15}{323.15-333.15}=4284$$

$$\lg\frac{k1}{k3}=\frac{Ea}{2.303R}\frac{T1-T3}{T1*T3}=4284*\frac{323.15-298.15}{323.15*298.15}=1.11$$

$$\frac{k1}{k3}=10^{1.11}=12.9$$

D3=60*12.9=774

When it is RT(25℃ (298.15K), the validity of the theory is at least 774 days. So under the condition of 25℃, it can be stored at least 24months (720days).

4. Stability test summary and conclusion

Based on the above research results, the following conclusions can be drawn:

1) The kit is stored under the actual storage condition for 6 months with stable performance; According to the principle that the time limit for stable storage is less than two months; the validity period of this kit at the actual storage temperature is determined to be 24 months.

2) After 5 days /20 days' transportation, the kit's performance is stable. Therefore, the foam box and ice bag are used to seal the transportation. The transportation time does not affect the performance of the kit.

3) The performance of the kit is stable after it is opened and stored at RT for 45 days. Therefore, the shelf life of the kit is 30 days when it is stored in RT.