COVID-19 Coronavirus Real Time PCR Kit

Stability Evaluation Report

1. Purpose

This protocol is designed to evaluate the stability of COVID-19 Coronavirus Real Time PCR Kit, including real-time stability studies (shelf life), transportation simulation studies and opening stability studies.

2. Application scope

COVID-19 Coronavirus Real Time PCR Kit

3. Reference laws and regulations

(1) Guidelines for the Technical Review of Registration of Multiple Nucleic Acid Detection Reagents for Respiratory Virus (No.80,2019), issued by the Technical Review Center for Medical Devices of the State Drug Administration of China
(2) Key Points for the Technical Review of the Registration of Novel Coronavirus Nucleic Acid Detection Reagents issued by the Technical Review Center for Medical Devices of the State Drug Administration of China
(3) WHO TGS2 Draft for Comment Establishing stability of an in vitro diagnostic for WHO Prequalification [2015]
(4) ISO ISO 23640:2011 In Vitro Diagnostic Medical Devices – Evaluation of Stability of In Vitro Diagnostic Reagents[2011]
(5) ASTM D4169 − 14 Standard Practice for Performance Testing of Shipping Containers and Systems [2014]
(6) CEN EN 13640:2002 Stability testing of in vitro diagnostic reagents [2002]

4. Test materials

4.1 Examination Reagents

Product Name	Novel Coronal Virus (SARS-CoV-2) nucleic acid detection kit (fluorescent PCR method)
Batch Lot	Lot20200104 (Expire date: 2021.01.20), Lot20200105 (Expire date: 2021.01.20), Lot20200106 (Expire date: 2021.01.20)
Specification	50TEST

4.2 Reference Panel

References used in this study include positive references (P1-P10), negative references (N1-N12), limit of detection references (L1), and precision references (J1-J2). The components of the references are shown in table 1-1.

Table 1-1. Components of reference panel

Sample Name	Code	Component	Characteristics	Concentration (copies/mL)	Batch No.	Specifications (mL/Tube)
Positive Reference	P1	2019-nCoV（402121）	Virus-like Particles	10^8~10^7	20200101	0.5
Positive Reference	P2	2019-nCoV（402122）	Virus-like Particles	10^8~10^7	20200101	0.5
Positive Reference	P3	2019-nCoV（402123）	Virus-like Particles	10^7~10^6	20200101	0.5
Positive Reference	P4	2019-nCoV（402124）	Virus-like Particles	10^7~10^6	20200101	0.5
Positive Reference	P5	2019-nCoV（402125）	Virus-like Particles	10^7~10^6	20200101	0.5
Positive Reference	P6	2019-nCoV（S01）	Viral Nucleic Acid	10^8~10^6	20200101	0.5
Positive Reference	P7	2019-nCoV（S02）	Viral Nucleic Acid	10^8~10^6	20200101	0.5
Positive Reference	P8	2019-nCoV（S03）	Viral Nucleic Acid	10^8~10^6	20200101	0.5
Positive Reference	P9	2019-nCoV（S04）	Viral Nucleic Acid	10^8~10^6	20200101	0.5
Positive Reference	P10	2019-nCoV（S05）	Viral Nucleic Acid	10^8~10^6	20200101	0.5
Negative Reference	N1	Influenza A (H1N1) virus (2009)	Cultured Virus	/	20200101	0.5
Negative Reference	N2	Influenza A (H3N2) virus	Cultured Virus	/	20200101	0.5
Negative Reference	N3	Influenza B virus	Cultured Virus	/	20200101	0.5
Negative Reference	N4	Parainfluenza Virus	Cultured Virus	/	20200101	0.5
Negative Reference	N5	Adenovirus	Cultured Virus	/	20200101	0.5
Negative Reference	N6	Respiratory syncytial virus	Cultured Virus	/	20200101	0.5
Negative Reference	N7	Coronavirus type 229E	Sample	/	20200101	0.5

Negative Reference	N8	Coronavirus type OC43	Sample	/	20200101	0.5
Negative Reference	N9	Coronavirus type HKU1	Sample	/	20200101	0.5
Negative Reference	N10	Coronavirus type NL63	Sample	/	20200101	0.5
Negative Reference	N11	SARS	Virus-like Particles	/	20200101	0.5
Negative Reference	N12	MERS	Virus-like Particles	/	20200101	0.5
LOD Reference	L1	2019-nCoV（402125）、RNP-1	Virus-like Particles	10^4	20200101	0.5
Precision Reference	J1	2019-nCoV（402125）、RNP-1	Virus-like Particles	10^8~10^6	20200101	0.5
	J2	2019-nCoV（402125）、RNP-1	Virus-like Particles	10^4	20200101	0.5

Source:

Cultured virus and samples are from Taizhou Centers for Disease Prevention and Control, Jiangsu Province

Virus-like particles are from Tsinghua University

4.3 Instruments

Fluorescence quantitative PCR instrument: ABI7500

Full-automatic nucleic acid extraction and liquid separation apparatus: SSNP-3000A

5. Assessment methodology

According to the Guidelines for the Technical Review of Registration of Multiple Nucleic Acid Detection Reagents for Respiratory Virus (No.80,2019) ,the Key Points for the Technical Review of Registration of Novel Coronavirus Nucleic Acid Detection Reagents issued by the Technical Review Center for Medical Devices of the State Drug Administration, WHO TGS2 Draft for Comment Establishing stability of an in vitro diagnostic for WHO Prequalification[2015]、ISO 23640:2011 In Vitro Diagnostic Medical Devices – Evaluation of Stability of In Vitro Diagnostic Reagents [2011], the following researches are carried out:

Real-time stability studies

Three batches of kits were stored in the refrigerators(-20±5℃) and were took out on the 0^{th} day, the 3^{th}, 6^{th}, 9^{th}, 12^{th} and 14^{th} month to evaluate the performance of Positive coincidence rate, Negative coincidence rate, Limit of detection and Precision.

Transportation simulation studies

Three batches of kits were sealed with ice packs in foam boxes and set aside at room

temperature. Then simulate transport conditions for 0day, 2days, 4days and 6days. Performance of Positive coincidence rate, Negative coincidence rate, Limit of detection and Precision were evaluated after the simulation. Test kits that simulated for 4days were stored in the refrigerators(-20±5℃) and were took out on the 0th day, the 3th, 6th, 9th, 12th and 14th month to evaluate the performance of Positive coincidence rate, Negative coincidence rate, Limit of detection and Precision.

Opening stability studies

Unwrap the packing of three batches of kits, unscrew the tube cover of each components and then tightly capped the cover. Store the kits in the refrigerators(-20±5℃) and took out on the 0^{th} day, the 3^{th}, 6^{th}, 9^{th}, 12^{th} and 14^{th} month to evaluate the performance of Positive coincidence rate, Negative coincidence rate, Limit of detection and Precision.

6. Acceptance criteria

Positive coincidence rate:10/10(+/+)

Negative coincidence rate: 12/12(-/-)

Limit of Detection: Test the LOD Reference(L1) for 20 times, the detection rate is greater than 95% at the virus concentration of 1×10^3copies/mL

Precision: Test the Precision References (J1 and J2) for 10 times, the detection results of ORF1ab gene and N gene of SARS-CoV-2 are positive and the %CV of Ct value is less than 5%

7. Quality Control and Safety

(1) The kit is an in vitro test device. Operators should be professionally trained and experienced.

(2) Strictly divided the experiment into areas. To avoid pollution, lab supplies and work clothes of each area are exclusive use, shall not be cross used. Clean the laboratory table immediately after the experiment.

(3) The kit should be thoroughly thaw out at room temperature, mixed and centrifuged instantaneously before use.

(4) Blank control and positive control should be set for each experiment. Do not mix use reagents of different batches. Use the kit before the expiration date.

(5) The freeze stored RNA samples should be thoroughly thaw out at room temperature, mixed and centrifuged instantaneously before use.

(6) The samples to be tested in this kit shall be considered as infectious substances and shall be handled in accordance with the ministry of health's "general guidelines for biosafety in microbial biomedical laboratories" and "regulations on clinical waste management".

8. Supplementary items

None.

9. Results

Real-time stability studies

Three batches of kits were stored in the refrigerators(-20±5℃) and the results of the 0th day are shown in table 1-2. The results of storage for 3 months, 6 months, 9 months, 12 months, 14 months are to be verified.

Table 1-2. Test results of kits stored at -20±5℃ for 0 day

Batch	Shelf time (Day)	Positive coincidence rate	Negative coincidence rate	Limit of Detection（copies/mL）	Precision（CV，%）					
					J1			J2		
					FAM	VIC	CY5	FAM	VIC	CY5
Batch 1	0	10/10 (100%)	12/12 (100%)	1000	0.91	1.11	1.58	0.75	1.06	0.94
Batch 2	0	10/10 (100%)	12/12 (100%)	1000	1.13	1.07	1.21	0.90	0.88	0.87
Batch 3	0	10/10 (100%)	12/12 (100%)	1000	1.07	1.29	0.86	0.80	0.88	0.94

As illustrated in table 1-2, after storage at -20±5℃ for 0 day, the performance of Positive coincidence rate, Negative coincidence rate, Limit of detection and Precision of the three batches of kits meets the acceptance criteria. The result of the 0^{th} day is set as control and the results of the other storage time are to be verified.

Transportation simulation studies

Three batches of kits were sealed with ice packs in foam boxes and simulated transportation for 0day, 2days, 4days and 6days, the test results are shown in table 1-3.

Table 1-3. Test results of kits after simulate transportation for different days

Batch	Transportation time（Day）	Positive coincidence rate	Negative coincidence rate	Limit of Detection（copies/mL）	Precision（CV，%）					
					J1			J2		
					FAM	VIC	CY5	FAM	VIC	CY5
Batch 1	0	10/10 (100%)	12/12 (100%)	1000	0.91	1.11	1.58	0.75	1.06	0.94
	2	10/10 (100%)	12/12 (100%)	1000	1.22	1.49	1.48	0.43	0.61	0.84
	4	10/10 (100%)	12/12 (100%)	1000	1.47	1.03	1.51	0.83	0.74	0.87
	6	10/10 (100%)	12/12 (100%)	1000	1.67	1.12	1.57	0.77	0.70	0.94
Batch 2	0	10/10 (100%)	12/12 (100%)	1000	1.13	1.07	1.21	0.90	0.88	0.87
	2	10/10 (100%)	12/12 (100%)	1000	1.24	0.98	1.78	0.43	0.59	0.89
	4	10/10 (100%)	12/12 (100%)	1000	1.44	1.60	1.49	0.93	1.02	0.87
	6	10/10 (100%)	12/12 (100%)	1000	1.37	1.74	1.48	0.98	0.91	1.03
Batch 3	0	10/10 (100%)	12/12 (100%)	1000	1.07	1.29	0.86	0.80	0.88	0.94
	2	10/10 (100%)	12/12 (100%)	1000	1.59	1.12	1.42	0.44	0.55	0.88
	4	10/10	12/12	1000	1.19	1.20	1.64	0.75	0.95	0.96

		(100%)	(100%)							
	6	10/10 (100%)	12/12 (100%)	1000	1.10	1.38	1.56	0.76	0.80	0.93

As illustrated in table 1-3, after sealed with ice packs in foam boxes and simulated transportation for 0day, 2days, 4days and 6days, the performance of Positive coincidence rate, Negative coincidence rate, Limit of detection and Precision of the three batches of kits meets the acceptance criteria.

Test kits that simulate transportation for 4days were stored in the refrigerators(-20±5℃) and were took out on the 0th day, the 3th, 6th, 9th, 12th and 14th month to evaluate the performance of Positive coincidence rate, Negative coincidence rate, Limit of detection and Precision. The test results are shown in table 1-4.

Table 1-4. Test results of kits stored at -20±5℃ after 4days of transportation simulation

Batch	Shelf time (Day)	Positive coincidence rate	Negative coincidence rate	Limit of Detection（copies/mL）	Precision（CV，%）					
					J1			J2		
					FAM	VIC	CY5	FAM	VIC	CY5
Batch1	0	10/10 (100%)	12/12 (100%)	1000	1.28	1.31	1.72	0.91	1.10	1.00
Batch2	0	10/10 (100%)	12/12 (100%)	1000	1.08	1.61	1.17	1.27	1.31	1.29
Batch3	0	10/10 (100%)	12/12 (100%)	1000	1.40	1.09	1.41	1.31	0.85	1.43

As illustrated in table 1-4, after 4 days' transportation simulation and the subsequent 0 day's storage at -20±5℃, the performance of Positive coincidence rate, Negative coincidence rate, Limit of detection and Precision of the three batches of kits meets the acceptance criteria. The result of the 0th day is set as control and the results of the other storage time are to be verified.

Opening stability studies

Unwrap the packing of three batches of kits, unscrew the tube cover of each components and then tightly capped the cover. Store the kits in the refrigerators(-20±5℃) and took out on the 0th day, the 3th, 6th, 9th, 12th and 14th month to evaluate the performance of Positive coincidence rate, Negative coincidence rate, Limit of detection and Precision. Only the test results of the 0^{th} day are available at present as shown in table 1-5.

Table 1-5. Test results of kits at the storage temperature of -20±5℃ after unwrapped

Batch	Shelf time (Month)	Positive coincidence rate	Negative coincidence rate	Limit of Detection（copies/mL）	Precision（CV，%）					
					J1			J2		
					FAM	VIC	CY5	FAM	VIC	CY5
Batch1	0	10/10	12/12	1000	1.35	1.06	1.35	0.89	0.87	0.72
	3									
	6									
	9									
	12									
	14									
Batch2	0	10/10	12/12	1000	1.21	1.20	1.10	0.83	0.79	0.81

	3									
	6									
	9									
	12									
	14									
Batch3	0	10/10	12/12	1000	1.57	1.52	1.54	0.41	0.79	1.05
	3									
	6									
	9									
	12									
	14									

As illustrated in table 1-5, after unwrapping the packing of three batches of kits, unscrewing the tube cover of each components, capping the cover and the subsequent 0 day's storage at -20±5℃, the performance of Positive coincidence rate, Negative coincidence rate, Limit of detection and Precision of the three batches of kits meets the acceptance criteria. The result of the 0^{th} day is set as control and the results of the other storage time are to be verified.

10. Conclusion

The shelf life evaluation of COVID-19 Coronavirus Real Time PCR Kit is inadequate at present, meanwhile, opening stability after 4 days' transportation simulation need to be studied. According to the stability evaluation results of other similar products on the fluorescence quantitative PCR technology platform of our company, the stability parameters of the kit are tentative as below:

(1) Stored at -20±5℃ avoid light, Valid for 12 months.

(2) Stored at -20±5℃ after unwrapped have no effect on the expiry date.

(3) Transported with ice bags for 4 days have no effect on the expiry date.

The corresponding data will be improved after sufficient verification.