

COVID-19 Coronavirus Real Time PCR Kit

Robustness Research Report

1. Purpose

This protocol is designed to evaluate the detection effectiveness of COVID-19 Coronavirus Real Time PCR Kit under abnormal working conditions.

2. Application scope

COVID-19 Coronavirus Real Time PCR Kit

3. Reference laws and regulations

(1) FDA 1757 Guidance for Industry and Food and Drug Administration Staff : Applying Human Factors and Usability Engineering to Medical Devices [2016]

(2) ANSI/AAMI HE75:2009/(R)2013 2013 Human factors engineering – Design of medical devices[2013]

4. Test materials

4.1 Examination Reagents

Product Name	Specification	Batch Lot	Expire date
Novel Coronal Virus (SARS-CoV-2) nucleic acid detection kit (fluorescent PCR method)	50TEST	20200104	2021.01.20

4.2 Reference Panel

Two Novel Coronal Virus (SARS-CoV-2) simulated positive samples (virus-like particles diluted with negative nasopharyngeal swab sample) with a concentration of 3 times of limit of detection were included in the panel, marked as sample 1 and sample 2, respectively. A negative sample was included and marked as sample 3.

Nasopharyngeal swab sample and negative sample are from Taizhou Centers for Disease Prevention and Control, Jiangsu Province. Virus-like particles sample is 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

The experiment was conducted using 3 reagent lots of COVID-19 Coronavirus Real Time PCR Kit with 2 separate runs by 2 operators for each sample.

For sample integrity and preparing, the following conditions are included:

- (1) Sample type error e.g. whole blood①, serum②, the dilute matrix of virus-like particles is whole blood or serum.
- (2) Sample storage and transport error e.g. samples were left at room temperature for 24 hours③, samples were freeze-thawed for 7 times④, samples were transported in ice box for 6 days⑤.
- (3) Sample preparing error e.g. sample volume was 100μL⑥, sample volume was 400μL⑦.

For reagent integrity, the following conditions are included:

- (1) Reagent storage and transport error e.g. reagents were left at room temperature for 24 hours①, reagents were transported in ice box for 6 days②.
- (2) Reagent use error e.g. mixed use of reagents of different batches③, wrong usage amount of reaction solution④, wrong usage amount of enzyme mixture solution⑤, wrong usage amount of triple reaction solution⑥.

For instrument integrity, the following conditions are included:

- (1) Instrument placement error e.g. nonhorizontal placement①, placed too close to other instruments②.
- (2) Effect of dust and mold③.
- (3) Effect of power/voltage fluctuation④.

For environmental factor, the following conditions are included:

- (1) Light①
- (2) Humidity②
- (3) Temperature③
- (4) Air pressure (high altitude simulation) ④
- (5) Dust particles⑤

6. Acceptance criteria

No significant difference between the test results under abnormal working conditions and normal working conditions.

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. Related Records Form

For sample integrity and preparing, test results of 7 abnormal conditions are shown in table 1-1.

Table 1-1. Test results of sample integrity and preparing

Conditio ns	Operat or	Batch	Sample1			Sample 2			Sample 3		
			FAM	VIC	CY5	FAM	VIC	CY5	FAM	VIC	CY5
Control group	1	1	32.99	32.62	26.28	32.39	32.25	26.06	Undet.	Undet.	26.65
		2	32.33	32.05	26.95	32.05	32.89	26.38	Undet.	Undet.	26.85
		3	32.23	32.78	26.54	32.14	32.37	26.76	Undet.	Undet.	26.15
	2	1	32.33	32.80	26.89	32.51	32.56	26.32	Undet.	Undet.	26.69
		2	32.31	32.65	26.96	32.69	32.76	26.47	Undet.	Undet.	26.28
		3	32.84	32.34	26.41	32.23	32.64	26.50	Undet.	Undet.	26.89
①	1	1	32.70	32.21	25.79	32.76	31.86	26.13	Undet.	Undet.	26.68
		2	32.32	32.47	26.70	32.54	32.80	26.22	Undet.	Undet.	26.52
		3	32.17	32.90	26.57	32.15	32.35	26.28	Undet.	Undet.	26.14
	2	1	31.92	33.10	26.84	32.20	32.39	26.15	Undet.	Undet.	26.66
		2	32.73	32.37	26.68	32.61	32.85	26.46	Undet.	Undet.	25.86
		3	32.59	32.78	26.02	32.23	32.14	26.83	Undet.	Undet.	26.68
②	1	1	33.91	33.67	26.89	33.32	33.56	26.53	Undet.	Undet.	26.58
		2	33.88	33.15	27.53	33.79	34.61	26.80	Undet.	Undet.	26.61
		3	33.32	34.34	26.39	33.32	33.69	26.97	Undet.	Undet.	26.69
	2	1	33.40	33.92	26.44	33.54	33.41	25.76	Undet.	Undet.	27.01
		2	33.79	33.79	27.20	34.05	34.54	26.60	Undet.	Undet.	26.12
		3	33.66	33.76	26.43	33.98	33.68	26.81	Undet.	Undet.	26.93
③	1	1	34.63	34.01	27.69	33.72	33.23	27.19	Undet.	Undet.	27.59
		2	33.72	33.10	27.84	33.54	33.96	28.17	Undet.	Undet.	28.51
		3	33.93	33.61	27.89	33.14	33.38	27.83	Undet.	Undet.	27.17
	2	1	33.46	34.04	28.15	34.19	33.40	27.26	Undet.	Undet.	27.60
		2	33.79	34.29	28.51	34.45	33.91	27.52	Undet.	Undet.	27.64
		3	34.30	33.90	27.22	33.42	33.51	28.09	Undet.	Undet.	28.26
④	1	1	33.02	32.88	26.51	32.39	32.63	26.56	Undet.	Undet.	27.15
		2	32.00	31.81	27.21	32.26	32.39	26.36	Undet.	Undet.	26.83
		3	32.22	33.14	26.09	32.04	32.84	27.21	Undet.	Undet.	26.60
	2	1	32.30	32.45	26.84	33.01	32.65	26.65	Undet.	Undet.	27.02
		2	31.89	32.26	27.12	33.15	33.11	26.16	Undet.	Undet.	25.97
		3	32.63	32.55	25.94	32.39	33.10	26.99	Undet.	Undet.	27.38
⑤	1	1	32.83	32.12	26.07	32.57	32.28	25.97	Undet.	Undet.	26.92
		2	32.48	31.55	26.67	31.80	33.22	26.79	Undet.	Undet.	27.12
		3	31.78	33.04	26.52	32.38	32.47	26.26	Undet.	Undet.	26.16
	2	1	32.30	32.71	26.91	32.16	32.99	26.60	Undet.	Undet.	26.96

		2	32.35	32.62	26.80	33.12	32.47	26.96	Undet.	Undet.	26.71
		3	33.09	32.84	26.25	32.17	32.28	26.37	Undet.	Undet.	26.47
⑥	1	1	34.20	33.66	26.79	33.48	33.26	26.59	Undet.	Undet.	27.24
		2	33.39	32.85	27.66	33.83	33.89	26.95	Undet.	Undet.	27.49
		3	33.54	34.06	26.93	32.99	33.27	27.54	Undet.	Undet.	26.82
	2	1	33.18	33.94	27.38	34.13	34.24	26.85	Undet.	Undet.	27.26
		2	33.94	33.72	27.26	33.61	33.81	27.09	Undet.	Undet.	27.07
		3	34.09	33.15	26.91	33.32	33.88	27.28	Undet.	Undet.	27.58
⑦	1	1	31.68	31.05	25.52	30.87	31.20	25.38	Undet.	Undet.	25.98
		2	30.67	30.44	26.42	30.78	31.66	25.72	Undet.	Undet.	26.32
		3	30.54	31.77	25.95	30.55	31.40	26.16	Undet.	Undet.	25.63
	2	1	30.90	31.61	26.27	31.09	31.41	25.75	Undet.	Undet.	26.05
		2	31.39	31.30	26.32	31.23	31.38	26.01	Undet.	Undet.	25.51
		3	31.17	30.96	25.82	31.07	31.84	25.94	Undet.	Undet.	26.40

For sample integrity and preparing, compared with the test results under normal conditions, the below conditions have no effect on the performance of COVID-19 Coronavirus Real Time PCR Kit: wrong sample type of whole blood and serum, samples left at room temperature for 24 hours, samples freeze-thawed 7 times, samples transported in ice box for 6 days, sample volume was 100μL, sample volume was 400μL.

For reagent integrity, test results of 6 abnormal conditions are shown in table 1-2.

Table 1-2. Test results of reagent integrity

Conditions	Operator	Batch	Sample 1			Sample 2			Sample 3		
			FAM	VIC	CY5	FAM	VIC	CY5	FAM	VIC	CY5
Control group	1	1	32.99	32.62	26.28	32.39	32.25	26.06	Undet.	Undet.	26.65
		2	32.33	32.05	26.95	32.05	32.89	26.38	Undet.	Undet.	26.85
		3	32.23	32.78	26.54	32.14	32.37	26.76	Undet.	Undet.	26.15
	2	1	32.33	32.80	26.89	32.51	32.56	26.32	Undet.	Undet.	26.69
		2	32.31	32.65	26.96	32.69	32.76	26.47	Undet.	Undet.	26.28
		3	32.84	32.34	26.41	32.23	32.64	26.50	Undet.	Undet.	26.89
①	1	1	34.35	33.72	27.19	33.74	33.49	26.89	Undet.	Undet.	27.83
		2	33.55	33.82	28.00	33.52	33.75	27.19	Undet.	Undet.	28.10
		3	33.67	34.17	28.23	33.61	33.59	27.60	Undet.	Undet.	27.55
	2	1	33.66	33.88	28.51	34.27	34.18	27.61	Undet.	Undet.	27.92
		2	33.14	34.35	27.96	34.17	33.66	28.13	Undet.	Undet.	27.48
		3	33.69	33.44	27.48	33.37	34.13	27.46	Undet.	Undet.	27.84
②	1	1	33.19	32.60	25.78	32.63	31.79	26.08	Undet.	Undet.	26.72
		2	32.33	32.36	27.44	31.58	33.23	26.25	Undet.	Undet.	26.86
		3	32.59	32.40	26.79	32.17	31.89	27.24	Undet.	Undet.	25.71
	2	1	31.89	32.68	26.75	32.11	32.93	25.83	Undet.	Undet.	27.05
		2	32.77	32.29	26.58	33.17	32.84	26.01	Undet.	Undet.	26.41
		3	32.86	32.15	26.16	32.03	32.59	26.73	Undet.	Undet.	26.54

③	1	1	34.40	33.60	27.42	33.33	33.26	27.64	Undet.	Undet.	28.15
		2	33.99	32.92	28.58	33.44	34.13	28.09	Undet.	Undet.	27.94
		3	33.26	34.50	27.84	33.12	33.66	28.48	Undet.	Undet.	27.94
	2	1	33.50	33.87	27.84	33.49	33.87	28.02	Undet.	Undet.	28.30
		2	33.56	33.73	28.60	34.27	33.75	28.11	Undet.	Undet.	27.66
		3	34.21	33.73	27.83	33.94	34.26	27.46	Undet.	Undet.	28.50
④	1	1	34.09	33.57	27.42	33.49	33.26	27.15	Undet.	Undet.	28.22
		2	33.55	33.38	28.73	33.18	34.49	27.75	Undet.	Undet.	27.68
		3	33.24	34.34	27.52	33.51	34.03	27.67	Undet.	Undet.	27.00
	2	1	33.22	34.54	28.45	33.53	33.94	27.51	Undet.	Undet.	28.48
		2	33.92	34.17	28.57	34.11	34.40	27.81	Undet.	Undet.	27.26
		3	34.10	33.74	28.10	33.90	33.66	27.86	Undet.	Undet.	28.59
⑤	1	1	34.72	33.96	27.61	33.68	34.04	27.00	Undet.	Undet.	27.58
		2	33.95	33.62	28.31	33.45	34.68	27.42	Undet.	Undet.	27.85
		3	33.96	33.98	27.82	33.89	33.41	28.40	Undet.	Undet.	27.47
	2	1	33.48	34.18	28.34	33.77	33.57	28.05	Undet.	Undet.	28.11
		2	33.24	34.22	28.46	34.20	33.70	27.92	Undet.	Undet.	27.32
		3	34.17	34.01	27.64	33.16	33.68	28.26	Undet.	Undet.	28.28
⑥	1	1	34.16	33.97	28.04	33.34	33.80	27.19	Undet.	Undet.	27.47
		2	33.23	33.24	28.31	33.00	34.11	28.03	Undet.	Undet.	27.74
		3	33.45	33.84	27.58	33.08	34.03	28.34	Undet.	Undet.	27.75
	2	1	33.43	33.71	28.08	33.71	34.12	27.35	Undet.	Undet.	27.56
		2	33.50	34.16	28.25	33.84	34.31	27.37	Undet.	Undet.	27.81
		3	34.18	33.96	27.63	33.20	33.76	28.05	Undet.	Undet.	28.11

For regent integrity, compared with the test results under normal conditions, regents transported in ice box for 6 days have no effect on the performance of COVID-19 Coronavirus Real Time PCR Kit. However, regents left at room temperature for 24 hours, mixed use of regents of different batches, wrong usage amount of reaction solution, wrong usage amount of enzyme mixture solution, wrong usage amount of triple reaction solution have significant effect on the performance.

For instrument integrity, test results of 4 abnormal conditions are shown in table 1-3.

Table 1-3. Test results of instrument integrity

Conditions	Operator	Batch	Sample 1			Sample 2			Sample 3		
			FAM	VIC	CY5	FAM	VIC	CY5	FAM	VIC	CY5
Control group	1	1	32.99	32.62	26.28	32.39	32.25	26.06	Undet.	Undet.	26.65
		2	32.33	32.05	26.95	32.05	32.89	26.38	Undet.	Undet.	26.85
		3	32.23	32.78	26.54	32.14	32.37	26.76	Undet.	Undet.	26.15
	2	1	32.33	32.80	26.89	32.51	32.56	26.32	Undet.	Undet.	26.69
		2	32.31	32.65	26.96	32.69	32.76	26.47	Undet.	Undet.	26.28
		3	32.84	32.34	26.41	32.23	32.64	26.50	Undet.	Undet.	26.89
①	1	1	34.45	34.00	27.12	33.40	33.07	27.25	Undet.	Undet.	28.14
		2	34.13	33.71	28.24	33.33	34.35	27.93	Undet.	Undet.	28.14

	2	3	33.11	33.89	27.73	33.81	34.09	28.55	Undet.	Undet.	27.54
		1	33.98	34.20	28.55	34.27	33.73	28.00	Undet.	Undet.	28.11
		2	33.72	34.03	28.55	33.60	34.14	27.96	Undet.	Undet.	28.06
		3	33.85	33.93	28.03	33.27	33.84	27.46	Undet.	Undet.	27.89
②	1	1	34.35	34.24	27.37	33.22	33.95	27.39	Undet.	Undet.	28.42
		2	33.86	33.22	28.27	33.72	34.35	27.24	Undet.	Undet.	27.74
		3	33.85	33.67	27.83	33.93	33.90	28.55	Undet.	Undet.	27.48
	2	1	33.70	34.57	27.82	33.31	33.78	28.00	Undet.	Undet.	28.32
		2	33.85	33.58	28.60	33.91	34.53	27.35	Undet.	Undet.	27.79
		3	34.44	33.35	27.47	33.71	34.22	28.01	Undet.	Undet.	28.22
③	1	1	32.57	32.43	25.96	32.07	31.97	26.32	Undet.	Undet.	26.28
		2	32.78	32.13	26.79	32.47	33.03	26.71	Undet.	Undet.	26.68
		3	31.88	32.29	26.28	31.74	32.20	27.02	Undet.	Undet.	26.37
	2	1	31.91	32.64	26.70	32.65	32.51	26.30	Undet.	Undet.	26.97
		2	32.42	32.18	27.21	33.01	32.62	26.27	Undet.	Undet.	25.96
		3	32.34	32.78	26.06	32.72	32.78	26.14	Undet.	Undet.	26.69
④	1	1	34.66	34.36	27.23	33.47	33.63	27.03	Undet.	Undet.	27.92
		2	33.83	33.82	28.24	33.75	34.61	27.98	Undet.	Undet.	27.66
		3	33.19	33.86	27.84	33.00	33.97	27.94	Undet.	Undet.	27.62
	2	1	33.30	33.65	28.27	33.76	33.81	27.27	Undet.	Undet.	27.97
		2	33.59	33.55	28.47	33.65	34.32	28.26	Undet.	Undet.	27.14
		3	33.68	33.49	28.00	33.06	34.07	27.74	Undet.	Undet.	27.87

For instrument integrity, compared with the test results under normal conditions, instruments nonhorizontal placement, instruments placed too close to other instruments, power/voltage fluctuation, dust and mold have no effect on the performance of COVID-19 Coronavirus Real Time PCR Kit.

For environmental factor, test results of 5 abnormal conditions are shown in table 1-4.

Table 1-4. Test results of environmental factor

Conditio ns	Operat or	Batch	Sample1			Sample 2			Sample 3		
			FAM	VIC	CY5	FAM	VIC	CY5	FAM	VIC	CY5
Control group	1	1	32.99	32.62	26.28	32.39	32.25	26.06	Undet.	Undet.	26.65
		2	32.33	32.05	26.95	32.05	32.89	26.38	Undet.	Undet.	26.85
		3	32.23	32.78	26.54	32.14	32.37	26.76	Undet.	Undet.	26.15
	2	1	32.33	32.80	26.89	32.51	32.56	26.32	Undet.	Undet.	26.69
		2	32.31	32.65	26.96	32.69	32.76	26.47	Undet.	Undet.	26.28
		3	32.84	32.34	26.41	32.23	32.64	26.50	Undet.	Undet.	26.89
①	1	1	32.52	32.75	26.45	32.53	31.87	26.05	Undet.	Undet.	26.79
		2	32.59	32.23	27.00	32.04	32.59	26.38	Undet.	Undet.	26.82
		3	31.76	32.78	26.82	32.25	32.36	26.54	Undet.	Undet.	25.76
	2	1	31.94	32.95	27.25	32.34	32.90	25.94	Undet.	Undet.	26.56
		2	32.73	32.81	27.11	32.91	33.05	26.34	Undet.	Undet.	26.19

		3	33.05	31.93	26.29	32.65	32.69	26.35	Undet.	Undet.	26.76
②	1	1	34.21	33.66	27.89	33.26	33.25	27.07	Undet.	Undet.	28.14
		2	33.23	33.75	27.98	33.22	34.44	27.25	Undet.	Undet.	28.20
		3	33.23	34.54	27.42	33.47	34.00	27.97	Undet.	Undet.	27.74
	2	1	33.60	33.88	28.65	34.06	33.78	27.80	Undet.	Undet.	28.25
		2	33.38	33.66	28.65	33.83	34.34	27.33	Undet.	Undet.	27.39
		3	34.03	33.49	27.55	33.13	34.28	27.58	Undet.	Undet.	28.09
③	1	1	32.52	32.82	26.54	32.21	32.57	26.53	Undet.	Undet.	26.78
		2	31.86	31.68	26.59	32.22	32.48	26.12	Undet.	Undet.	26.82
		3	32.28	32.44	26.39	32.44	32.44	26.82	Undet.	Undet.	26.12
	2	1	32.73	32.72	26.81	32.34	32.23	26.50	Undet.	Undet.	26.94
		2	32.08	32.31	26.67	32.79	32.69	26.49	Undet.	Undet.	26.41
		3	33.16	32.17	26.74	32.40	32.63	26.23	Undet.	Undet.	27.02
④	1	1	33.49	32.95	26.73	32.43	32.60	26.48	Undet.	Undet.	26.26
		2	31.95	31.78	27.26	32.12	32.75	25.97	Undet.	Undet.	27.24
		3	31.78	33.09	26.15	31.71	32.24	27.16	Undet.	Undet.	25.86
	2	1	32.37	32.76	26.72	32.47	32.47	26.23	Undet.	Undet.	26.50
		2	32.29	32.78	27.18	32.75	32.33	26.63	Undet.	Undet.	25.97
		3	33.30	32.64	26.34	32.32	32.15	26.54	Undet.	Undet.	27.07
⑤	1	1	34.05	33.69	27.35	33.39	33.09	27.09	Undet.	Undet.	28.05
		2	33.74	33.64	28.65	33.17	34.17	27.34	Undet.	Undet.	28.19
		3	33.13	33.67	27.74	33.04	33.89	28.54	Undet.	Undet.	27.31
	2	1	33.84	34.57	28.27	33.32	33.44	27.72	Undet.	Undet.	28.15
		2	33.80	34.00	28.69	34.31	34.17	28.13	Undet.	Undet.	27.37
		3	34.02	33.48	28.16	33.54	34.26	27.93	Undet.	Undet.	28.46

For environmental factor, compared with the test results under normal conditions, light, humidity, temperature, air pressure (high altitude simulation) and dust particles have no effect on the performance of COVID-19 Coronavirus Real Time PCR Kit.

10. Conclusion

Operate strictly according to the instructions for use of COVID-19 Coronavirus Real Time PCR Kit and use under normal working conditions is recommended to ensure the reliability of test results.