COVID-19 Coronavirus Real Time PCR Kit

Applicable Models Research Report

Research report: Applicable models research of COVID-19 Coronavirus Real Time PCR Kit
Protocol: Experiment protocol for applicable models research of COVID-19 Coronavirus Real Time PCR Kit

Final report: Final evaluation report on applicable models research of COVID-19 Coronavirus Real Time PCR Kit

Research period: 2020.01-2020.02

Product lot examined: Lot1：20200104 (expire date: 2021.01.20)，Lot2：20200105 (expire date: 2021.01.20), Lot3：20200106 (expire date: 2021.01.20). Enzyme system, primers and probes used in those three kits lots were provided by Jiangsu Shuoying biotechnology co. Ltd from their three independent batches.

Instruction version: v1.0

Research institution/organization: R&D department, Jiangsu Bioperfectus Technologies Co., Ltd.

Test location: on-site real-time test

Purpose： This study is to study the applicable models of COVID-19 Coronavirus Real Time PCR Kit

Risk assessment

The risk caused by varied models is evaluated and summarized in risk assessment file where possible effects of measurement precision on accuracy of the kit has been analyzed.

Acceptance criteria

The Limit of Detection rate on the 5 instruments are greater than 95%; The variable coefficient of Precision results on the 5 instruments are less than 95%. The Positive coincidence rate and Negative coincidence rate on the 5 instruments are 100%.

Study design

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) EN 13612:2002/AC:2002 Performance evaluation of in vitro diagnostic medical devices.

(4) CLSI EP05-A3: Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline-Third Edition.

(5) Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline—Second Edition This. EP17-A2 Vol. 32 No. 8 Replaces EP17-A Vol. 24 No. 34.

(6) CLSI EP15-A2: User verification of performance for precision and trueness; Approved Guideline
-Second Edition.

(7) ISO3534-1 2006: Statistics -- Vocabulary and symbols -- Part 1: General statistical terms and terms used in probability.

(8) Diagnostic Assessment. Principles for Performance studies, TGS–3. Geneva: World Health Organization; 2016

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	N1	Influenza A	Cultured Virus	/	20200101	0.5

Reference		(H1N1) virus (2009)				
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

Assessment methodology

The performance of three batches of kits, including Limit of Detection, Accuracy and Precision were evaluated on 5 instruments. The Limit of Detection experiment was carried out by testing a LOD Reference(L1) for 20 times. The Precision experiment was a 5×5×5 evaluation

method—calling for measurements on 5 days, with 5 different instruments at a single site, with 5 replicates per run for a high concentration sample, a low concentration sample, a negative sample and two Precision Reference(J1 and J2). The Accuracy experiment was carried out by testing positive references(P1-P10) and negative references(N1-N12).

Results

The analysis of LOD test results shows in Table 1.

Table 1. LOD test results

Batch	Coincidence Rate				
	ABI7500	QuantStudio™ 5	Roche LightCycler®480	Bio-Rad CFX96™	Hongshitech SLAN-96P/S
Batch 1	100%（20/20）	100%（20/20）	100%（20/20）	100%（20/20）	100%（20/20）
Batch2	100%（20/20）	100%（20/20）	100%（20/20）	100%（20/20）	100%（20/20）
Batch 3	100%（20/20）	100%（20/20）	100%（20/20）	100%（20/20）	100%（20/20）

As illustrated in table 1, LOD Reference(L1) were tested and the result shows the detection rate of three batches of kits on ABI7500, QuantStudio™ 5, Roche LightCycler®480, Bio-RadCFX96™ and Hongshitech SLAN-96P/S are all greater than 95%

The analysis of Precision test results shows in Table 2-4.

Table 1. Precision test results

Sample Name	Channel	average	Within-Run Precision		In-Lab Precision		Between Instruments Precision	
			SD	%CV	SD	%CV	SD	%CV
Sample1	FAM	24.92	0.39	1.58%	0.55	2.19%	0.71	2.86%
	VIC	25.34	0.61	2.39%	0.87	3.43%	1.03	4.07%
Sample2	FAM	31.03	0.53	1.69%	0.63	2.02%	0.82	2.64%
	VIC	31.12	0.58	1.87%	0.65	2.08%	0.74	2.39%
Precision Reference J1	FAM	21.31	0.34	1.61%	0.42	1.97%	0.55	2.56%
	VIC	21.31	0.40	1.86%	0.42	1.96%	0.54	2.52%
Precision Reference J2	FAM	33.89	0.62	1.84%	0.75	2.20%	0.86	2.53%
	VIC	33.80	0.65	1.92%	0.72	2.13%	0.80	2.37%

Table3：Confidence interval Results

Sample Name	Analysis Parameters	Within-Run Precision		In-Lab Precision		Between Instruments Precision	
		FAM	VIC	FAM	VIC	FAM	VIC
Sample1 Sample2	Standard Deviation (SD)	0.39	0.61	0.55	0.87	0.71	1.03
	DF	80.00	80.00	22.14	21.34	4.25	5.07
	$x^2(1-\alpha/2)\ ,DF$	57.153	57.153	10.982	10.283	0.484	0.831
	$x^2(\alpha/2)\ ,DF$	101.879	101.879	33.924	32.671	9.488	11.071
Precision Reference J1	Standard Deviation (SD)	0.53	0.58	0.63	0.65	0.82	0.74
	DF	80.00	80.00	27.31	32.79	4.17	5.43
Precision Reference J2	$x^2(1-\alpha/2)\ ,DF$	57.153	57.153	14.573	18.291	0.484	0.831
	$x^2(\alpha/2)\ ,DF$	101.879	101.879	40.113	46.194	9.488	11.071

Sample1 Sample2	Standard Deviation (SD)	0.34	0.40	0.42	0.42	0.55	0.54
	DF	80.00	80.00	26.03	39.52	4.23	4.19
	$x^2(1-\alpha/2)\ ,DF$	57.153	57.153	13.844	24.433	0.484	0.484
	$x^2(\alpha/2)\ ,DF$	101.879	101.879	38.885	55.758	9.488	9.488
Precision Reference J1	Standard Deviation (SD)	0.62	0.65	0.75	0.72	0.86	0.80
	DF	80.00	80.00	27.34	32.78	5.43	6.07
	$x^2(1-\alpha/2)\ ,DF$	57.153	57.153	14.573	19.047	0.831	1.237
	$x^2(\alpha/2)\ ,DF$	101.879	101.879	40.113	47.400	11.071	12.592

Table4：95% Confidence Interval

Sample Name	Channel	Within-Run Precision		In-Lab Precision		Between Instruments Precision	
		SD	%CV	SD	%CV	SD	%CV
Sample1	FAM	0.35-0.47	1.40%-1.87%	0.44-0.77	1.77%-3.11%	0.48-2.11	1.91%-8.47%
	VIC	0.54-0.72	2.12%-2.83%	0.70-1.25	2.78%-4.95%	0.70-2.55	2.75%-10.05%
Sample2	FAM	0.47-0.62	1.50%-2.00%	0.52-0.86	1.67%-2.77%	0.54-2.41	1.75%-7.76%
	VIC	0.52-0.69	1.66%-2.22%	0.55-0.87	1.75%-2.78%	0.52-1.90	1.67%-6.10%
Precision Reference J1	FAM	0.30-0.41	1.43%-1.91%	0.34-0.58	1.61%-2.70%	0.36-1.61	1.71%-7.56%
	VIC	0.35-0.47	1.65%-2.20%	0.35-0.53	1.65%-2.50%	0.36-1.58	1.67%-7.42%
Precision Reference J2	FAM	0.55-0.74	1.63%-2.18%	0.62-1.02	1.82%-3.01%	0.60-2.19	1.77%-6.46%
	VIC	0.57-0.77	1.70%-2.27%	0.60-0.94	1.77%-2.79%	0.56-1.78	1.65%-5.26%

As illustrated in table 2-4, it shows that the test results of negative sample on the 5 instruments are all negative, the variation coefficient of test results of high concentration sample, low concentration sample Precision References (J1 and J2) are less than 95%. All the results meet the requirement of consistency, indicating the test kit have consistent precision performance on the 5 instruments.

The analysis of Accuracy test results shows in Table 5.

Table 5. Accuracy test results

Batch	Coincidence Rate				
	ABI7500	QuantStudio™ 5	Roche LightCycler®480	Bio-Rad CFX96™	Hongshitech SLAN-96P/S
Batch 1	100%（10/10）	100%（10/10）	100%（10/10）	100%（10/10）	100%（10/10）
Batch2	100%（12/12）	100%（12/12）	100%（12/12）	100%（12/12）	100%（12/12）
Batch 3	100%（20/20）	100%（20/20）	100%（20/20）	100%（20/20）	100%（20/20）

As illustrated in table 5, positive references(P1-P10) and negative references(N1-N12) were tested and the result shows that accuracy coincidence rate of three batches of kits on ABI7500, QuantStudio™ 5, Roche LightCycler®480, Bio-RadCFX96™ and Hongshitech SLAN-96P/S are all 100%.

Conclusion

The experimental results of LOD, Precision and Accuracy test shows that the detection rate of

three batches of kits on the 5 instruments are greater than 95%, the coefficient of variation is less than 5% and the coincidence rate of positive/negative references are 100%, indicating the COVID-19 Coronavirus Real Time PCR Kitis applicable on ABI7500, QuantStudio™ 5, Roche LightCycler®480, Bio-RadCFX96™ and Hongshitech SLAN-96P/S .