COVID-19 Coronavirus Real Time PCR Kit

Analytical sensitivity test report

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 kit 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 test the analytical sensitivity of COVID-19 Coronavirus Real Time PCR Kit

Risk assessment

The risk caused by the analytical sensitivity of the kit is evaluated and summarized in risk assessment file where possible effects of analytical sensitivity on accuracy of the kit have been analyzed.

Acceptance criteria

Positive results rate at limit of detection (LOD) shall be higher than 95%. Coefficients of variation in repeatability test shall be lower than 5% (%CV<5%).

Study design

Reference standards:

(1) NMPA technical review center: 《Guidelines on registration examination of multiple nucleic acid detection of respiratory virus (2019 No.80)》
(2) NMPA technical review center: 《Key points for technical review of 2019 novel coronal virus detection reagent registration》
(3) EN 13612:2002/AC:2002 Performance evaluation of in vitro diagnostic medical devices
(4) CLSI EP05-A3:Evaluation of Precision ofQuantitative Measurement Procedures;Approved Guideline-Third Edition.
(5) Diagnostic Assessment. Principles for Performance studies, TGS–3. Geneva: World Health Organization; 2016
(6) 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.

(7) GHTF/SG1/N68:2012 Essential Principles of Safety and Performance of Medical Devices

(8) Guidance for Industry and FDA Staff; In Vitro Diagnostic (IVD) Device Studies -Frequently Asked Questions.

Test samples

No.	Sample type	Resource	Remark
Sample 1	Nasopharyngeal swab	Jiangsu Taizhou CDC*	Deactivated
Sample 2	Nasopharyngeal swab	Jiangsu Taizhou CDC	Deactivated
Sample 3	Nasopharyngeal swab	Jiangsu Taizhou CDC	Deactivated
Sample 4	Nasopharyngeal swab	Jiangsu Taizhou CDC	Deactivated
Sample 5	Nasopharyngeal swab	Jiangsu Taizhou CDC	Deactivated
Sample 6	Nasopharyngeal swab	Jiangsu Taizhou CDC	Deactivated
Sample 7	Sputum	Jiangsu Taizhou CDC	Deactivated
Sample 8	Sputum	Jiangsu Taizhou CDC	Deactivated
Sample 9	Bronchoalveolar lavage fluid	Jiangsu Taizhou CDC	Deactivated
Sample 10	Bronchoalveolar lavage fluid	Jiangsu Taizhou CDC	Deactivated

***CDC：** Center for Disease Control and Prevention

Methods

Limit of detection (LOD

(1) Determination of LOD

Three quantified clinical samples of nasopharyngeal swab type are pooled from sample list. Five concentrations are made by serial dilution of samples, 1×10^5 copies /mL, 1×10^4 copies /mL, 1×10^3 copies /mL, 1×10^2copies /mL and 1×10^1 copies /mL. Each dilution is tested by kits of those three lots for 9 times on QuantStudio™ 5 fluorescent PCR instrument. The lowest concentration over which more than 95% positive rate is demonstrated is determined as the LOD.

(2) Verification of LOD

Serial dilution of other three positive nasopharyngeal swab samples of different origins is made to reach 1×10^3 copies /mL concentration. Each dilution is tested for 20 times by each of those three kit lot on QuantStudio™ 5 fluorescent PCR instrument. The LOD is verified when at least 95% positive rate is demonstrated.

Detection coverage for samples with various time and regional characteristics

(1) Verification of LOD

Serial dilution of 10 positive samples with different time and regional characteristics is made to

reach 1×10^3 copies /mL concentration. Each dilution is divided into 10 tubes. To calculate positive rate of detection for each sample, each tube is tested for 10 times by each of those three kit lot on QuantStudio™ 5 fluorescent PCR instrument.

(2) Repeatability verification

Serial dilution of 10 positive samples with different time and regional characteristics is made to reach 1×10^4 copies /mL concentration. Each dilution is divided into 10 tubes. To calculate the coefficient of variation (CV) of Ct values for each sample, each tube is tested for 10 times by each of those three kit lot on QuantStudio™ 5 fluorescent PCR instrument. The sensitivity evaluation is based on the results from those 10 samples by using kits of three lots.

Results

Data for determination of LOD is shown in table 1.

Table 1 Determination of LOD

sample	Concentration（copies /mL）	Number of positive results (n)			Total test (N)	Positive rate (%)
		Lot 1	Lot 2	Lot 3		
sample 1	10^5	9	9	9	27	100.0
	10^4	9	9	9	27	100.0
	10^3	8	9	9	27	96.3
	10^2	3	2	2	27	25.9
	10^1	0	0	1	27	3.7
sample 2	10^5	9	9	9	27	100.0
	10^4	9	9	9	27	100.0
	10^3	9	8	9	27	96.3
	10^2	3	3	2	27	29.6
	10^1	0	0	1	27	3.7
sample 3	10^5	9	9	9	27	100.0
	10^4	9	9	9	27	100.0
	10^3	8	9	9	27	96.3
	10^2	2	2	2	27	22.2
	10^1	0	0	0	27	0

As seen in table 1, all positive rates for detection of all the three nasopharyngeal swab samples at concentration of 10^3 copies /mL are higher than 95%, resulting a rough determination of LOD to be 10^3 copies /mL which has been further verified by using different types of samples as shown in table 2.

Table 2 Verification of LOD

Concentration (copies /mL)	sample	Number of positive results (n)			Total test (N)	Positive rate (%)
		Lot 1	Lot 2	Lot 3		
10^3	Clinical sample 4	19	20	20	60	98.3
	Clinical sample 5	19	19	20	60	96.7
	Clinical sample 6	20	19	20	60	96.7
	Clinical sample 7	10	10	10	30	100.0
	Clinical sample 8	10	10	10	30	100.0
	Clinical sample 9	10	10	10	30	100.0
	Clinical sample 10	10	10	10	30	100.0

As seen in table 2, all positive rates for detection of all the 10 samples of different types including nasopharyngeal swab, sputum and bronchoalveolar larvage fluid at the concentration of 10^3 copies /mL are higher than 95%. As a result, LOD is determined as the concentration of 10^3

copies /mL.

Table 3 and table 4 show the detection results of samples of various time and regional characteristics.

Table 3. Verification of LOD for samples of various time and regional characteristics

Sample (at the concentration of 10^3copies /mL)	Number of positive results (n)			Total test (N)	Positive rate (%)
	Lot 1	Lot 2	Lot 3		
sample 1	10	10	10	30	100.0
sample 2	10	10	10	30	100.0
sample 3	9	10	10	30	96.7
sample 4	10	10	10	30	100.0
sample 5	10	10	10	30	100.0
sample 6	10	9	10	30	96.7
sample 7	10	10	10	30	100.0
sample 8	10	10	10	30	100.0
sample 9	10	10	10	30	100.0
sample 10	10	10	10	30	100.0

As seen in table 3, all positive rates for detection of all 10 samples of various time and regional characteristics are higher than 95% at the concentration of 10^3 copies /mL, showing broad detection coverage of this kit to be applied to samples of various regional characteristics.

Table 4. Verification of repeatability for samples of various time and regional characteristics

Sample (at the concentration of 10^3copies /mL)	Number of positive results (n)			Total test (N)	%CV (%)
	Lot 1	Lot 2	Lot 3		
sample 1	10	10	10	30	1.86%
sample 2	10	10	10	30	1.63%
sample 3	10	10	10	30	1.56%
sample 4	10	10	10	30	1.64%
sample 5	10	10	10	30	1.58%
sample 6	10	10	10	30	1.57%
sample 7	10	10	10	30	1.58%
sample 8	10	10	10	30	1.65%
sample 9	10	10	10	30	1.85%
sample 10	10	10	10	30	1.68%

As seen in table 4, all %CV for detection of all 10 samples of various time and regional characteristics are lower than 5% at the concentration of 10^4 copies /mL, showing good repeatability of detection performance of this kit to be applied to samples of various regional characteristics.

Conclusion

The results of analytical sensitivity study shows that the LOD of the Novel Coronal Virus (SARS-CoV-2) nucleic acid detection kit (fluorescent PCR method) is determined as 1×10^3 copies /mL and that good detection coverage of this kit for sample of various time and regional characteristics is also verified, leading to a reasonable estimation that performance indicators of the sensitivity can meet the demands of users.