Performance Evaluation Data

BT/FDA-PCR-09 rev.A/0

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Reviewed by Bowei Wang Date：Mar 13th,2020

Approved by Yongjun Han Date：Mar 13th,2020

Product Name：SARS-CoV-2 Nucleic Acid Detection Kit (Real Time PCR)

Tests have been validated but the FDA's independent review of this validation is pending.

1. Performance indicator

1.1 Appearance

1.1.1 The outer packing of the product is complete without damage, the components of the kit are complete and intact, and the inner tubes of each group are tightly sealed without leakage.

1.1.2 Package label complete, clear, no wear.

1.2 Accuracy

Test three reference samples with weak positive, medium positive and strong positive. The ORF1ab gene and N gene shall be positive, and the positive coincidence rate shall be 100%.

1.3 Analytical Specificity

Test two negative references. Both the ORF1ab gene and N gene shall be negative, and the negative coincidence rate shall be 100%.

1.4 Limit of Detection

Test the reference sample for limit detection with the minimum detection limit of 300 copies/ml. The positive results shall be no less than 19 times in the 20 repeated tests.

1.5 Within-run Precision

Test one weak positive reference sample by using kits with same lot number for 10 times. The results shall be positive for ORF1ab and N genes, the positive coincidence rate shall 100%, and the coefficient of variation (CV) shall be less than 10%.

1.6 Between-run Precision

Test one weak positive reference sample by three kits with different lot number for 10 times. The results shall be all positive for ORF1ab gene and N gene in 30 experiments. The positive coincidence rate shall be 100%, and the coefficient of variation (CV) shall be less than 10%.

1.7 Stability

After placing the kits in -20±5℃ for 6 months, do the above tests (2.1-2.5). The test results shall meet the technical requirements.

2. Test method

2.1 Appearance

2.1.1 Visual inspection under natural light, appearance should meet the requirements of 2.1.1.

2.1.2 Visual inspection under natural light, the packaging label shall conform to the requirements of 2.1.2.

2.2 Accuracy

Test three copies of reference sample with ORF1ab gene and N gene weak positive, medium positive and strong positive according to the product IFU. Each sample was tested for 3 times. The number of positive test results was denoted as M, and the total number of tests was 9. The coincidence rate R was calculated according to the following formula, and the results of each gene should meet the requirements of 2.2.

R=（M/9）× 100%

Result：

Repeat time	Gene	Weak positive	Moderate positive	Strong positive
1	ORF1ab	+	+	+
	N	+	+	+
2	ORF1ab	+	+	+
	N	+	+	+
3	ORF1ab	+	+	+
	N	+	+	+

ORF1ab coincidence rate R=（M/9）× 100%

=9/9 × 100%

=100%

N gene coincidence rate R=（M/9）× 100%

=9/9 × 100%

=100%

Conclusion: The accuracy was 100% in the tests of three quality control samples with ORF1ab gene and N gene weak positive, medium positive and strong positive, respectively.

2.3 Analytical Specificity

Test two negative reference samples with low or high concentration, according to the product specification. Each sample was tested three times. The number of negative test results was denoted as M. The total number of tests was 6. The coincidence rate R was calculated according to the following formula, and the results of each gene should meet the requirements of 2.3.

R=（M/6）× 100%

Result：

Repeated time	Gene	Positive	Negative
1	ORF1ab	-	-
	N	-	-
2	ORF1ab	-	-
	N	-	-
3	ORF1ab	-	-
	N	-	-

ORF1ab coincidence rate R=（M/6）× 100%

=6/6 × 100%

=100%

N gene coincidence rate R=（M/6）× 100%

=6/6 × 100%

=100%

Conclusion: The accuracy was 100% in the tests of two quality control products with ORF1ab gene and N gene negative.

2.4 Minimum Detectable Limit

According to the product instruction, each sample with the minimum detection limit of ORF1ab or N genes (300 copies / ml) was detected for 20 times.

Repeated time	ORF1ab	N
1	+	+
2	+	+
3	+	+
4	+	+
5	+	+
6	+	+
7	+	+
8	+	+
9	+	+

10	+	+
11	+	+
12	+	+
13	+	+
14	+	+
15	+	+
16	+	+
17	+	+
18	+	+
19	+	+
20	+	+

Conclusion: The results from 20 experiments showed that all the tests were positive.

2.5 Within-run Precision

Each sample with ORF1ab gene or N gene weak positive was detect for 10 repeated times by using the kits with same lot number, according to the product instruction. The number of positive test results was denoted as M, and the total number of tests was 10. The coincidence rate R was calculated according to the following formula. Calculate the mean value of Ct and standard deviation SD of the 10 results. The coefficient of variation (CV) was calculated according to the following formula.

Result：

Repeated time	ORF1ab	N
1	+	+

2	+	+
3	+	+
4	+	+
5	+	+
6	+	+
7	+	+
8	+	+
9	+	+
10	+	+

ORF1ab coincidence rate R=（M/10）× 100%

=10/10 × 100%

=100%

N gene coincidence rate R=（M/10）× 100%

=10/10 × 100%

=100%

Repeated time	ORF1ab	N
1	35.68	35.76
2	37.29	34.64

3	37.15	35.04
4	36.44	36.22
5	36.22	36.41
6	35.41	35.28
7	36.67	34.16
8	37.09	34.57
9	37.34	36.19
10	37.06	34.56
Mean Value	36.635	35.283
Variance	0.683378	0.813976
CV %	1.87	2.31

Conclusion： The coincidence rate R of ORF1ab was calculated as 100%. The mean Ct value of ORF1ab was 36.635, the standard deviation was 0.683, and the coefficient of variation (CV) was calculated as 1.87%.

The coincidence rate R of N gene was 100%. The mean Ct value of N gene was 35.283, the standard deviation was 0.814, and the coefficient of variation (CV) was 2.31%.

2.6 Between-run precision

Each sample with ORF1ab gene and N gene weak positive was detected by three kits with different lot number for 10 times, according to the product instructions. The number of positive tests results was denoted as M, and the total number of tests was 30. The coincidence rate R was calculated according to the following formula. The mean value of Ct and standard deviation SD of the 30 results were calculated at the same time. The coefficient of variation (CV) was calculated according to the following formula. The results should meet the requirements of 2.6.

$R=（M/30）\times 100\%$

CV= SD/M × 100%

Result：

Repeated time	ORF1ab			N		
	UP20200301	UP20200301	UP20200301	UP20200301	UP20200301	UP20200301
1	+	+	+	+	+	+
2	+	+	+	+	+	+
3	+	+	+	+	+	+
4	+	+	+	+	+	+
5	+	+	+	+	+	+
6	+	+	+	+	+	+
7	+	+	+	+	+	+
8	+	+	+	+	+	+
9	+	+	+	+	+	+
10	+	+	+	+	+	+

ORF1ab coincidence rate R=（M/30）× 100%

=30/30 × 100%

=100%

N gene coincidence rate R=（M/30）× 100%

=30/30 × 100%

=100%

Weak positive CT value：

Repeated time	ORF1ab（CT value）			N（CT value）		
	UP20200301	UP20200302	UP20200303	UP20200301	UP20200302	UP20200303
1	37.32	35.18	35.27	36.18	36.87	35.37
2	35.87	36.43	36.73	36.42	35.24	36.75
3	36.07	35.94	35.92	35.28	37.83	37.81
4	35.23	36.87	35.12	35.21	35.62	35.53
5	36.42	37.35	36.49	35.87	36.04	35.81
6	35.24	36.81	37.21	35.12	35.02	37.05
7	37.81	35.49	36.82	37.84	37.34	36.84
8	36.95	36.58	35.91	36.58	35.81	35.08
9	36.21	35.91	37.29	35.67	36.42	37.44
10	37.27	36.84	35.27	37.26	37.52	37.46
Mean Value	36.33			36.34		
Variance	0.78			0.938		
CV %	2.14			2.58		

According to the calculation, the coincidence rate R of ORF1ab was 100%. The mean Ct value of ORF1ab was 36.33, the standard deviation was 0.78, and the coefficient of variation (CV) was 2.14%.

The coincidence rate R of N gene was 100%. The mean Ct value of N gene was 36.34, the standard deviation was 0.938, and the coefficient of variation (CV) was 2.58%.

2.7 Cross-reaction in Silico Analysis

Cross-reaction between SARS-CoV-2 and microbial organisms by Detection Kit

Virus/Bacteria /Parasite	Strain	Source / Sample type	Concentration	Result (+ or -)
High priority organisms likely in the circulating area				
Influenza B	B/Victoria	Inactivated culture	1.0×10^{6} $TCID_{50}$/mL	-
Influenza B	B/Yamagata	Inactivated culture	7.5×10^{7} $TCID_{50}$/mL	-
Influenza A	H1N1	Inactivated culture	1.0×10^{7} $TCID_{50}$/mL	-
Influenza A	H3N2	Inactivated culture	1.0×10^{8} $TCID_{50}$/mL	-
Neisseria meningitidis	N/A	isolate	10^{6} PFU/mL	-
Haemophilus influenzae	N/A	isolate	10^{6} PFU/mL	-
Staphylococcus aureus	N/A	isolate	10^{6} PFU/mL	-
Streptococcus pneumoniae	N/A	isolate	10^{6} PFU/mL	-
Rubella virus	N/A	isolate	10^{6} PFU/mL	-
Mumps virus	N/A	isolate	10^{6} PFU/mL	-
Adenovirus 3	N/A	isolate	10^{6} PFU/mL	-
Adenovirus 7	N/A	isolate	10^{6} PFU/mL	-
Respiratory syncytial virus, type B	N/A	isolate	10^{6} PFU/mL	-
Parainfluenza 2	N/A	isolate	10^{6} PFU/mL	-
Other high priority pathogens from the sample genetic family				
Influenza B	B/Victoria	Culture	1.0×10^{6} $TCID_{50}$/mL	-
Influenza B	B/Yamagata	Culture	7.5×10^{7} $TCID_{50}$/mL	-
Influenza A	H1N1	Culture	1.0×10^{7} $TCID_{50}$/mL	-
Influenza A	H3N2	Culture	1.0×10^{8} $TCID_{50}$/mL	-

Cross-reactions between Detection Kit and Pathogens including human MERS-CoV and SARS-CoV

Test results of MERS-coronavirus and SARS-coronavirus used in the study:

Virus/ organisms	Strain	Concentration (copies/mL)	2019-nCoV Test Result
MERS-coronavirus	EMC	1.0E05	Negative
SARS-coronavirus	Urbani	1.0E05	Negative

In addition to MERS-coronavirus and SARS-coronavirus, the 7 organisms listed.

Virus/ organisms	Strain	Source/ Sample type	Concentration (CFU/mL)
Streptococcus pyrogenes	BNCC 186346	Culture	1.00E+06
Bordetella pertussis	BNCC 337541	Culture	1.00E+06
Streptococcus pneumoniae	BNCC 337114	Culture	1.00E+06
Candida albicans	BNCC 186382	Culture	1.00E+06
Pseudomonas aeruginosa	BNCC 125486	Culture	1.00E+06
Staphylococcus epidermis	BNCC 102555	Culture	1.00E+06
Haemophilus influenzae	BNCC 337544	Culture	1.00E+06

Final concentration and test results of each organism used in the study:

Virus/ organisms	Final Concentration (CFU/mL)	2019-nCoV Test Result
Streptococcus pyrogenes	1.00E+06	Negative
Bordetella pertussis	1.00E+06	Negative
Streptococcus pneumoniae	1.00E+06	Negative
Candida albicans	1.00E+06	Negative
Pseudomonas aeruginosa	1.00E+06	Negative
Staphylococcus epidermis	1.00E+06	Negative
Haemophilus influenzae	1.00E+06	Negative

SARS-CoV-2 RT-qPCR test of all extracted nucleic acids indicated negative signals for all 7 organisms, suggesting no cross-reaction to seven microbes tested by the detection kit.

Cross-reactivity was evaluated based on in silico analysis of organisms, among the tested organisms, none of the tested organisms showed the homology for primers and probe of N gene. Candida glabrate, Cryptococcus neoformans, and SARS coronavirus showed > 80% homology with forward primer of ORF 1ab gene, 55% homology with reverse primer and 37% to 60% homology with probe. Therefore, the risk of non-specific amplification is low.

2.8 Clinical Evaluation

Clinical Evaluation Information

Sample collection site	Sample testing site	Positive	Negative	Percentage (%)
A third-party clinical laboratory	Shanghai Biotecan Pharmaceuticals Co. , Ltd	47	95	Positive = 33.1% Negative = 66.9%

2.9 Clinical performance study compared with ddPCR（droplet digital PCR）

142 respiratory specimens were selected to be tested with ddPCR to confirm the reported results of testing with the Detection Kit. The PPA and NPA analysis was used to evaluate the performance.The results were summarized in the Table:

Biotecan	ddPCR		Total
	Positive	Negative	
Positive	45	2	47
Negative	5	90	95
Total	50	92	142

PPA (%) = 100% × 45 /（45+5）= 90%

NPA (%) = 100% × 90 /（90+2）= 97.8%

3. Conclusion

Through the performance verification, it was found that the product performance meets all the requirements.

Tests have been validated but the FDA's independent review of this validation is pending.