Analytical Sensitivity Study Test Report
(Limited of Detection, LoD)

- **Product Name** : nCoV-QS
- **Lot No. :** M224220AT1O
- **Manufacturing date :** 2020.01.31

	Name	Date	Signature
Created by:	Eunkyung Yu	2020 .02. 24	
Reviewed by:	Jihye Yoon	2020 .02. 24	
Approved by:	Hyonsun Kim	2020 .02. 24	

Revision History

Revision No.	Rev. Date	Revision Description
0.0	2020. 02. 05	First issue for design verification
1.0	2020. 02. 13	Change of target name
2.0	2020. 02. 24	Change according to additional test

Contents

1 Purpose

When using the Veri-Q PCR 316 instrument, it was performed to measure the detection limit of nCoV-QS.

2 References

CLSI, EP17-A2: 2012: Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline

3 Time schedule

3.1 Test period : 2020.01.31~2020.01.31, 2020.02.24

4 Device information

- Veri-Q PCR 316(제인 17-4426호) (S/N :17G027, 18A005, 18A008, 18F018, 18K001, 19C004, 19C027, 19C030, 19D009, 19D015)

5 Test information

5.1 Sample

5.1.1 Sample information

- nCoV-ORF: in vitro transcript RNA
- nCoV-N gene: in vitro transcript RNA

5.1.2 Sample concentration

	Test for detection range	Test for LoD
nCoV-ORF(copies/3 ㎕)	$5x10^0$, $5x10^1$, $5x10^2$, $5x10^3$, $5x10^4$, $5x10^5$	50, 25, 12.5, 6. 25, 3.125, 1.5625
nCoV-N(copies/3 ㎕)	$5x10^0$, $5x10^1$, $5x10^2$, $5x10^3$, $5x10^4$, $5x10^5$	50, 25, 12.5, 6. 25, 3.125

5.2 Test procedure

- The test is deigned to 3 replicates for checking of detection range and linearity.
- The test is deigned to 20 replicates for checking of Limit of detection.
- Following the instruction for use of the product (MCTF-2242_IFU_EN_rev.0.0)
-

5.3 Acceptance criteria

- Confirm the Ct values and amplified curves of the each concentrations.

5.4 Data analysis

- Confirm the Ct values and amplified curves of the each concentrations.
- N gene is determine the LoD concentration for 95% detection rate by probit analysis.
- ORF gene is determine the LoD concentration for 95% detection rate.(No probit analysis)
- Set the threshold line as below table before checking the Ct value.

PPM	Target	Fluorophore	Threshold	Cut-off Ct value for positive
nCoV-QS-PPM1	COVID19	FAM	1000	< 40
	IPC	Cy5	1500	< 40
nCoV-QS-PPM2	COVID19	Cy5	1500	< 40
	IPC	HEX	500	< 40

6 Results

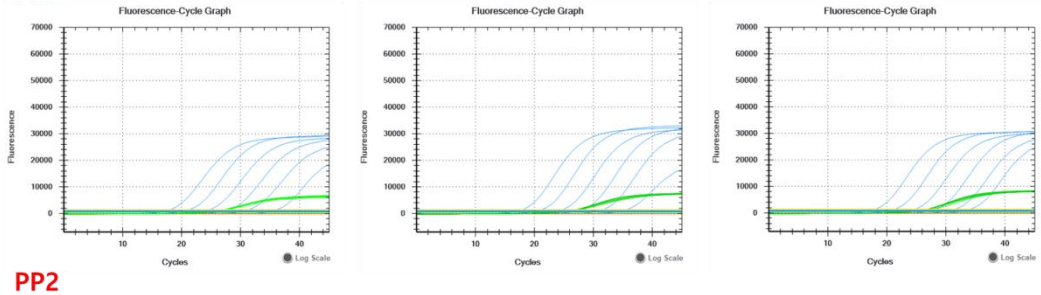
6.1 Detection range and linearity

The detection range was confirmed by repeating three times. When the Ct value according to each concentration was confirmed by the linearity, the R^2 value of 0.99 was shown.

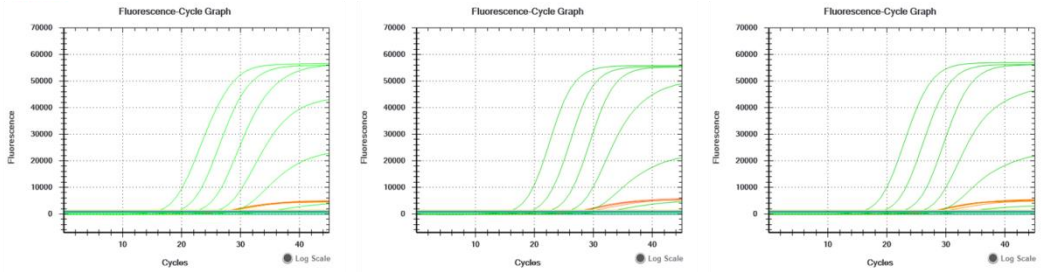
[Veri-Q PCR316]

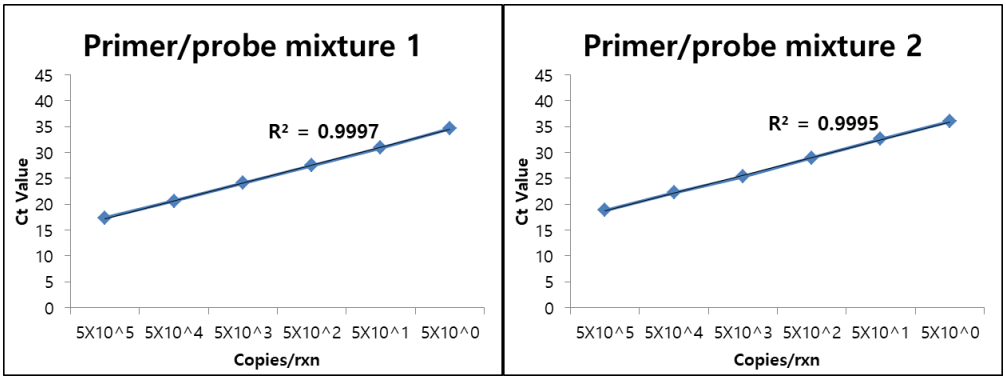
		nCoV-QS-PPM1							
		FAM(COVID19-ORF)				CY5 (IPC)			
	Cp/rxn	1TEST	2TEST	3TEST	Average	1TEST	2TEST	3TEST	Average
Ct	5X10^5	17.37	17.34	17.4	17.4	27.87	27.67	27.62	27.7
	5X10^4	20.67	20.57	20.69	20.6	27.42	27.42	27.02	27.3
	5X10^3	24.09	24.13	24.16	24.1	27.39	27.33	27.1	27.3
	5X10^2	27.39	27.46	27.64	27.5	27.21	27.14	26.95	27.1
	5X10^1	30.75	31.03	30.86	30.9	27.36	27.3	27	27.2
	5X10^0	35.4	34.97	33.37	34.6	27.22	27.16	27.01	27.1
	PC	26.89	25.52	26.41	26.3	27.19	27.24	N/D	27.2
	NC	N/D	N/D	N/D	–	27.17	27.14	26.84	27.1
		nCoV-QS-PPM2							
		HEX(IPC)				CY5(COVID19-N)			
	Cp/rxn	1TEST	2TEST	3TEST	Average	1TEST	2TEST	3TEST	Average
Ct	5X10^5	25.53	26.42	26.73	26.23	18.78	19.13	18.56	18.82
	5X10^4	25.57	25.68	25.54	25.60	22.32	22.28	22.06	22.22
	5X10^3	25.41	25.41	25.72	25.51	25.51	26.02	24.56	25.36
	5X10^2	25.5	25.69	25.58	25.59	29.02	29.2	28.62	28.95
	5X10^1	25.23	25.7	25.73	25.55	32.97	32.73	32.2	32.63
	5X10^0	25.34	25.62	25.57	25.51	35.95	N/D	36.02	35.99
	PC	25.14	25.78	25.37	25.43	24.78	24.93	24.07	24.59
	NC	25.56	25.6	25.37	25.51	N/D	N/D	N/D	–

PP1


Fluorescence-Cycle Graph
Fluorescence
Cycles
Log Scale

PP2


Fluorescence-Cycle Graph
Fluorescence
Cycles
Log Scale


Primer/probe mixture 1
R² = 0.9997
Ct Value
Copies/rxn
5X10^5 5X10^4 5X10^3 5X10^2 5X10^1 5X10^0
Primer/probe mixture 2
R² = 0.9995
Ct Value
Copies/rxn
5X10^5 5X10^4 5X10^3 5X10^2 5X10^1 5X10^0

6.3 Limit of detection

Analytical sensitivity (limit of detection) of nCoV-QS defines each target gene as 95% detectable concentration (copies / ㎕). This test was replicated 20 times for each concentration using Veri-Q PCR 316, QD-P100. As a result of analyzing the analytical sensitivity, the limit of detection of ORF gene is 2.083 copies/㎕ and the limit of detection of N gene is 4.239 copies/㎕.

[Veri-Q PCR316]

-Raw data

	Cp/rxn	50	25	12.5	6.25	3.125	1.5625
PPM1 (COVID19-ORF)	1TEST	30.58	31.77	33.26	34.11	N/D	35.4
	2TEST	30.63	32.02	33.41	33.41	34.76	34
	3TEST	31.23	33.88	32.69	35.16	38.79	37.1
	4TEST	31.29	33.2	33.46	33.87	37.22	N/D
	5TEST	30.48	32.42	32.99	34.24	34.1	N/D
	6TEST	31.01	31.41	33.17	34.65	34.65	N/D
	7TEST	30.94	31.46	34.14	34.51	35.22	36.3
	8TEST	31.02	31.7	32.3	34.3	36.15	39.7
	9TEST	30.55	31.54	32.18	33.37	38.96	N/D
	10TEST	30.97	31.37	32.73	34.99	38.24	N/D
	11TEST	31.06	31.65	32.91	35.27	40.63	N/D
	12TEST	30.41	32.21	33.56	33.78	34.53	N/D
	13TEST	30.79	31.74	33.03	33.37	N/D	36.3
	14TEST	31	32.38	32.51	N/D	37.18	N/D
	15TEST	31.36	32.17	33.53	34.88	36.44	35.3
	16TEST	30.76	33.35	32.11	33.39	37.98	35
	17TEST	30.32	32.13	33.17	34.36	35.46	36.7
	18TEST	30.9	31.57	32.54	34.57	34.81	N/D
	19TEST	30.12	31.5	33.6	35.2	39.22	36.5
	20TEST	31.1	31.82	33.62	33.51	39.68	34.3
	AVR	**30.83**	**32.06**	**33.05**	**34.26**	**36.89**	**36.04**
	STDEV	**0.34**	**0.69**	**0.55**	**0.67**	**2.05**	**1.56**
	CV(%)	**1%**	**2%**	**2%**	**2%**	**6%**	**4%**
	Count	**20**	**20**	**20**	**19**	**18**	**11**
	Detection rate	**100%**	**100%**	**100%**	**95%**	**90%**	**55%**

	Cp/rxn	50	25	12.5	6.25	3.125
PPM2 (COVID19-N)	1TEST	29.44	30.81	34.51	33.34	N/D
	2TEST	30.4	34.55	34.88	36.03	N/D
	3TEST	29.92	30.67	N/D	N/D	N/D
	4TEST	30.39	31.32	N/D	N/D	N/D
	5TEST	30.03	31.8	33.5	N/D	N/D
	6TEST	30.21	31.92	35.78	N/D	N/D
	7TEST	30.04	31.55	33.48	N/D	N/D
	8TEST	30.55	32.02	33.69	N/D	N/D
	9TEST	29.86	31.61	N/D	N/D	N/D
	10TEST	29.97	32.8	N/D	N/D	N/D
	11TEST	30.02	31.02	N/D	N/D	N/D
	12TEST	30.06	32.58	34.26	N/D	N/D
	13TEST	30.19	32.64	N/D	N/D	N/D
	14TEST	29.85	32.9	N/D	N/D	N/D
	15TEST	29.57	32.6	34.4	N/D	N/D
	16TEST	30.68	31.17	N/D	33.71	N/D
	17TEST	30.2	31.12	N/D	N/D	N/D
	18TEST	29.75	31.96	N/D	N/D	N/D
	19TEST	29.88	30.96	N/D	N/D	N/D
	20TEST	30.85	30.79	N/D	N/D	N/D
	AVR	**30.09**	**31.84**	**34.31**	**34.36**	-
	STDEV	**0.35**	**0.96**	**0.78**	**1.46**	-
	CV(%)	**1%**	**3%**	**2%**	**4%**	-
	Count	**20**	**20**	**8**	**3**	**0**
	Detection rate	**100%**	**100%**	**40%**	**15%**	**0%**

-Probit analysis

Veri-Q PCR316_PPM2							
Confidence limits							
percentage		95% confidence limits for the copy			95% confidence limits for the log[a]		
		Estimates	Lower	Upper	Estimates	Lower	Upper
PROBIT	.010	1.979	0.960	2.782	0.296	-0.018	0.444
	.020	2.248	1.178	3.065	0.352	0.071	0.486
	.030	2.438	1.340	3.262	0.387	0.127	0.513
	.040	2.591	1.475	3.419	0.414	0.169	0.534
	.050	2.723	1.595	3.554	0.435	0.203	0.551
	.060	2.840	1.704	3.674	0.453	0.232	0.565
	.070	2.947	1.806	3.783	0.469	0.257	0.578
	.080	3.047	1.901	3.885	0.484	0.279	0.589
	.090	3.140	1.992	3.980	0.497	0.299	0.600
	.100	3.228	2.080	4.070	0.509	0.318	0.610
	.150	3.621	2.479	4.477	0.559	0.394	0.651
	.200	3.967	2.841	4.843	0.598	0.453	0.685
	.250	4.290	3.183	5.197	0.632	0.503	0.716
	.300	4.603	3.516	5.553	0.663	0.546	0.745
	.350	4.913	3.842	5.925	0.691	0.585	0.773
	.400	5.226	4.166	6.320	0.718	0.620	0.801
	.450	5.548	4.490	6.752	0.744	0.652	0.829
	.500	5.885	4.817	7.231	0.770	0.683	0.859
	.550	6.242	5.148	7.772	0.795	0.712	0.891
	.600	6.626	5.490	8.392	0.821	0.740	0.924
	.650	7.049	5.846	9.116	0.848	0.767	0.960
	.700	7.524	6.227	9.978	0.876	0.794	0.999
	.750	8.072	6.645	11.036	0.907	0.822	1.043
	.800	8.729	7.121	12.383	0.941	0.853	1.093
	.850	9.563	7.696	14.208	0.981	0.886	1.153
	.900	10.727	8.455	16.949	1.030	0.927	1.229
	.910	11.029	8.646	17.695	1.043	0.937	1.248
	.920	11.366	8.856	18.545	1.056	0.947	1.268
	.930	11.749	9.092	19.531	1.070	0.959	1.291
	.940	12.192	9.360	20.698	1.086	0.971	1.316
	.950	12.718	9.674	22.120	1.104	0.986	1.345
	.960	13.364	10.054	23.922	1.126	1.002	1.379
	.970	14.204	10.537	26.349	1.152	1.023	1.421
	.980	15.403	11.211	29.977	1.188	1.050	1.477
	.990	17.501	12.348	36.769	1.243	1.092	1.565
a. Logarithm=10							

7 Conclusion

- The analytical sensitivity (Limit of Detection: LoD) of the N gene is defined as the concentration (copies/ul of the eluate) that can be detected with a positive rate ≥ 95 % using the probit analysis.
- The analytical sensitivity (Limit of Detection: LoD) of the ORF gene is defined as the concentration (copies/ul of the eluate) that can be detected with a positive rate ≥ 95 % (No probit analysis)
- Testing was carried out on 20 replicates at each concentration.
- The analytical sensitivity (LoD) of nCoV-QS is as follow.

	ORF gene	N gene
Veri-Q PCR316	2.083 copies/㎕ (6.25 copies/rxn)	4.239 copies/㎕ (12.718 copies/ rxn)