Performance Characterization of Bio Basic's SARS-CoV-2 RT-PCR Detection Kit (Cat# CoV-2-RTPCR)

Study-1 Limit of Detection Analysis
Study-2 Clinical Performance Evaluation

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LoD Determination Summary

Limit of Detection (LoD)/analytical sensitivity is determined by spiking SARS-CoV-2 reference genome RNA into an artificial matrix with human total RNA. The initial study used a dilution series including 3 replicates for each concentration ranging from 5 copies per reaction to 1000 copies per reaction. The confirmatory study used 20 replicates of the final concentration. The final LoD is reported to be 10 copies/reaction.

1.1 Materials

The following items were used to determine the limit of detection (LoD) of the detection kit:

Product	Source	Cat#	Lot no.
SARS-CoV-2 RT-PCR Detection Kit	Bio Basic	COV-2-RTPCR	
SARS-CoV-2 reference genome RNA (8.07 X 10^3 copies/µl)	ATCC	ATCC-VR1986D	70034826
Human total RNA (100ng/µL)	abm	--	--
BioRad-CFX96	BioRad		

1.2 Experimental Procedure

Reactions were setup according to the datasheet from the kit provided, briefly:

1.2.1. Template was prepared by mixing the reference SARS-CoV-2 reference genome (ATCC-VR1986D) and human total RNA to mimic the real patient samples. The human RNA amount in each sample is 2 ng/µl. The SARS-CoV-2 RNA was used with different copies/µl to meet the need of LoD determination.

1.2.2. Completely thaw all reagents on ice and mix gently.

1.2.3. Prepare a reaction mix according to the table below to the PCR tube or plate:

Component	Sample	Positive control	NTC
EZ-RT-PCR Master Mix	1 µl	1 µl	1 µl
COVID-19 primer+buffer Mix	19 µl	19 µl	19 µl
COVID-19 Positive control	0 µl	5 µl	0 µl
Sample	5 µl	0 µl	0 µl
RT-PCR grade water	0 µl	0 µl	5 µl

1.2.4. Close reaction tube or plate, vortex for 5-10 seconds and spin down the tube or plate briefly.

1.2.5. Perform test according to the following Real-time PCR reaction condition:

Step	Temp.	Time	Cycle #
cDNA Synthesis	55°C	15 min	1 cycle
RT inactivation Pre-denaturation	95°C	30 sec	1cycle
Amplification	95°C	15 sec	45 cycles
	60°C	1 min	

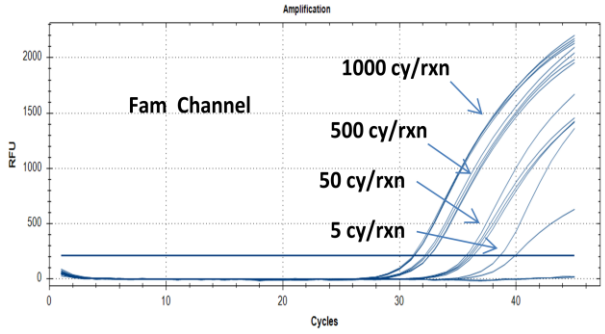
1.2.6. Interpretation of Results:

2019-nCoV ORF1ab (FAM)	+	+	-	-	-
2019-nCoV N (Vic or HEX)	+	-	+	-	-
IPC (Cy5)	+	+	+	+	-
Result	Positive	Positive	Positive	Negative	Invalid

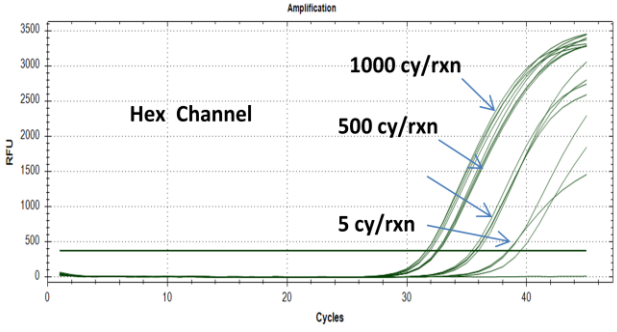
Note: (+): Ct value< 37, (-): Ct value>37

1.3 Analysis of Data

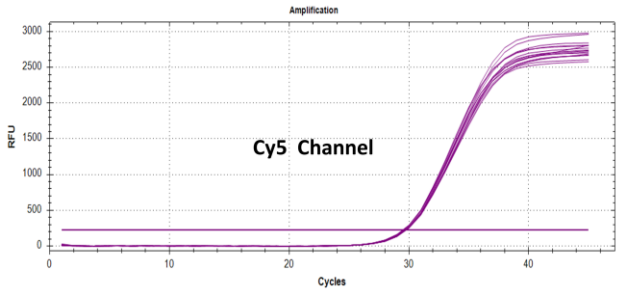
1.3.1 First round LoD test using human RNA at 10 ng/reaction, mixed with 1000, 500, 50 and 5 copies/reaction of SARS-CoV-2 reference genome RNA.



ORF1ab (FAM) Results:
All samples were positive except for 2 samples at 5 copies/rxn.



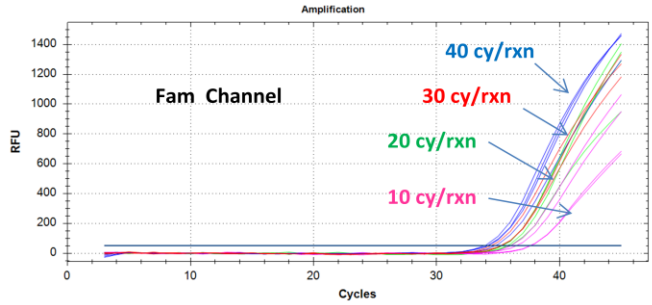
N (HEX) Results:
All samples were positive except for 1 sample at 5 copies/rxn.



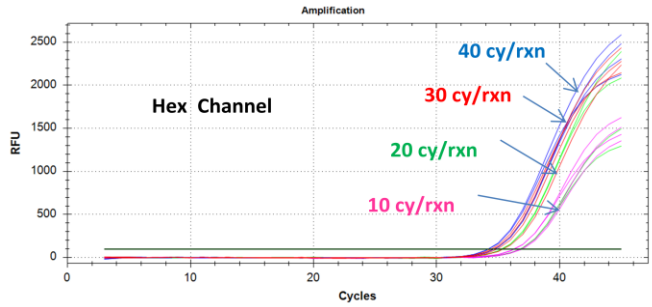
IPC (Cy5) Results:
All samples are the same as with 10 ng/rxn human RNA

Conclusion of first round of LoD test: Limit of detection is between 5-50 copies per reaction.

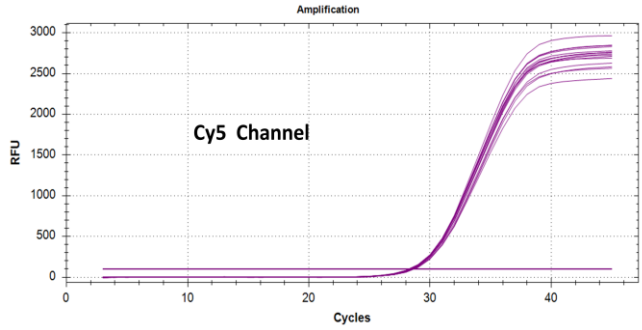
1.3.2 Second round LoD test using human RNA at 10ng/reaction, mixed with 40, 30, 20 and 10 copies/reaction of SARS-CoV-2 reference genome RNA.



ORF1ab (FAM) Results:
All samples were positive



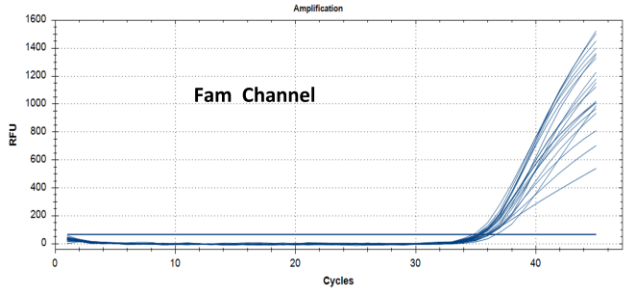
N (HEX) Results:
All samples were positive



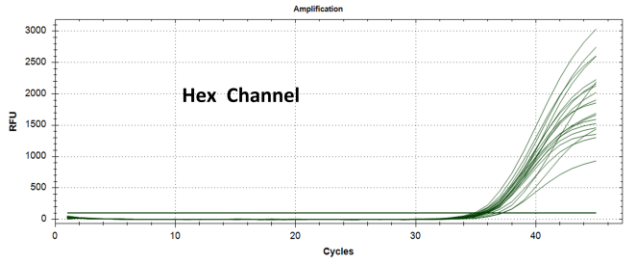
IPC (Cy5) Results:
All samples are the same as with 10ng/rxn human RNA

Conclusion of second round of LoD test: Limit of detection should be 10 copies/reaction.

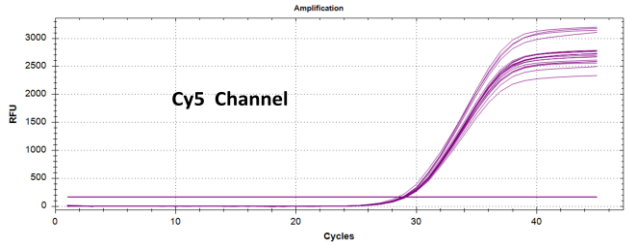
1.3.3 Third round (Confirmation) LoD test using 20 replicates of human RNA at 10 ng/reaction, mixed with 10 copies/reaction of SARS-CoV-2 reference genome RNA.



ORF1ab (FAM) Results:
All samples were positive



N (HEX) Results:
All samples were positive



IPC (Cy5) Results:
All samples are the same as with 10ng/rxn human RNA

[Ct Value Table to Follow on next page]

1.3.4 Ct value result of 20 replicates of LoD test

Samples	ORF1ab (FAM)	N (Hex)	IPC (Cy5)	Result
Sample 1	36.06	35.64	29.1	Pass
Sample 2	35.15	35.22	29.01	Pass
Sample 3	35.26	35.57	29.06	Pass
Sample 4	35.19	35.76	29.19	Pass
Sample 5	35.21	36.21	29.19	Pass
Sample 6	36.11	35.47	29.12	Pass
Sample 7	36.11	35.99	28.81	Pass
Sample 8	35.89	35.08	29.03	Pass
Sample 9	35.64	35.87	29.05	Pass
Sample 10	36.1	37.19	29.17	Pass
Sample 11	36.63	34.75	28.85	Pass
Sample 12	35.79	35.66	28.86	Pass
Sample 13	35.31	35.39	28.48	Pass
Sample 14	35.46	35.78	29.03	Pass
Sample 15	35.03	35.01	28.95	Pass
Sample 16	34.7	35.39	29.09	Pass
Sample 17	35.84	35.29	29.03	Pass
Sample 18	35.34	35.65	29.07	Pass
Sample 19	35.03	37.15	29.04	Pass
Sample 20	35.28	36.69	29.1	Pass
P.C.	18.7	18.43	25.23	Pass
NTC	Clean	Clean	Clean	Pass

Note: P.C. is positive control plasmids provided in the kit; NTC (no-template-control) is negative control.

Conclusion from confirmatory test: 10copies/rxn presented all positive signal from FAM and HEX channels, with 2 samples showing Ct>37 in Hex Channel. According to the result interpretation, these 2 are considered positive due to the positivity at FAM channel.

1.4 Conclusion

The LoD of SARS-CoV-2 RT-PCR Detection Kit was confirmed at 10 copies/reaction.

Clinical Performance Evaluation Summary

Clinical Performance Evaluation is performed with 30 reactive and 30 non-reactive specimens. Contrived clinical samples were prepared by mixing the reference SARS-CoV-2 reference genome (ATCC-VR1986D) and human total RNA. For reactive samples, 30 samples with 1X LoD, 3X samples with 10X LoD, 3X samples with 100X LoD were evaluated with the kit.

2.1 Materials

The following items were used to evaluate the clinical performance of the detection kit:

Product	**Source**	**Cat#**	**Lot no.**
SARS-CoV-2 RT-PCR Detection Kit	Bio Basic	COV-2-RTPCR	
SARS-CoV-2 reference genome RNA (8.07×10^3 copies/µl)	ATCC	ATCC-VR1986D	70034826
Human total RNA (100ng/µL)	abm	--	--
BioRad-CFX96	BioRad		

2.2 Experimental Procedure

Reactions were setup and result interpretation was performed according to the datasheet from the kit, similar to the experimental procedure 1.2 in the LoD Determination Test above.

Result interpretation:

2019-nCoV ORF1ab (FAM)	+	+	-	-	-
2019-nCoV N (Vic or HEX)	+	-	+	-	-
IPC (Cy5)	+	+	+	+	-
Result	Positive	Positive	Positive	Negative	Invalid

Note: (+): Ct value< 37, (-): Ct value>37

Contrived clinical samples were prepared by mixing the reference SARS-CoV-2 reference genome (ATCC-VR1986D) and human total RNA. In order to evaluate the performance of this detection kit with the contrived clinical samples, 30 samples with 1X LoD, 3X samples with 10X LoD, 3X samples with 100X LoD, and 40 negative samples, all carrying 2ng/µl human RNA, were tested with this kit. The result was listed in the below table.

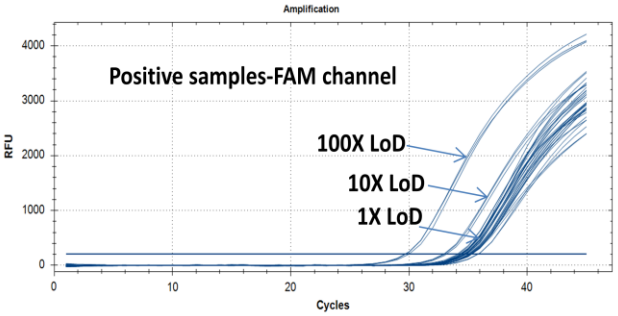
[Ct Value Table to Follow on next page]

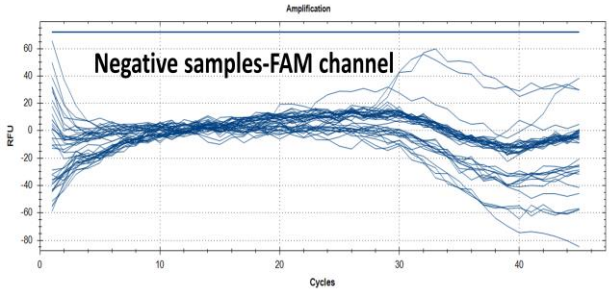
Samples	ORF1ab (FAM)	N (Hex)	IPC (Cy5)
1X LOD	35.23	35.93	29.28
1X LOD	34.75	35.77	29.33
1X LOD	34.14	36.51	29.35
1X LOD	34.56	36.87	29.31
1X LOD	34.81	36.98	29.35
1X LOD	35.03	36.01	29.44
1X LOD	34.41	36.16	29.47
1X LOD	34.32	36.3	29.37
1X LOD	35.25	36.49	29.46
1X LOD	34.65	36.26	29.33
1X LOD	34.56	36.25	29.36
1X LOD	34.61	36.15	29.35
1X LOD	35.21	35.45	29.4
1X LOD	35.15	35.84	29.32
1X LOD	34.33	35.71	29.39
1X LOD	34.39	36.6	29.38
1X LOD	35.27	36.43	29.4
1X LOD	35.78	36.16	29.42
1X LOD	35.18	36.23	29.35
1X LOD	34.63	36.5	29.44
1X LOD	34.25	36.2	29.44
1X LOD	35.18	35.65	29.4
1X LOD	34.41	36.17	29.36
1X LOD	35.77	36.01	29.35
1X LOD	34.97	36.16	29.39
1X LOD	35.11	35.85	29.34
1X LOD	34.38	35.71	29.44
1X LOD	35.05	36.19	29.34
1X LOD	34.72	35.98	29.31
1X LOD	34.05	35.97	29.2
10X LOD	32.86	34.09	30.05
10X LOD	32.98	34.32	30.08
10X LOD	33.12	33.89	29.98
100X LOD	29.94	31.27	28.88
100X LOD	29.65	31.18	28.93
100X LOD	29.59	31.2	28.88
P.C.	18.11	18.37	25.36
P.C.	18.15	18.38	25.27
NTC	N/A	N/A	N/A
NTC	N/A	N/A	N/A

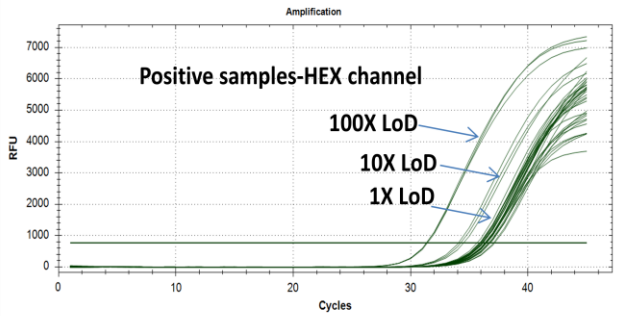
Samples	ORF1ab (FAM)	N (Hex)	IPC (Cy5)
Negative	N/A	N/A	29.29
Negative	N/A	N/A	29.17
Negative	N/A	N/A	29.21
Negative	N/A	N/A	29.14
Negative	N/A	N/A	29.16
Negative	N/A	N/A	29.18
Negative	N/A	N/A	29.29
Negative	N/A	N/A	29.27
Negative	N/A	N/A	29.25
Negative	N/A	N/A	29.16
Negative	N/A	N/A	29.22
Negative	N/A	N/A	29.31
Negative	N/A	N/A	29.28
Negative	N/A	N/A	29.37
Negative	N/A	N/A	29.37
Negative	N/A	N/A	29.31
Negative	N/A	N/A	29.3
Negative	N/A	N/A	29.36
Negative	N/A	N/A	29.41
Negative	N/A	N/A	29.42
Negative	N/A	N/A	29.32
Negative	N/A	N/A	29.35
Negative	N/A	N/A	29.27
Negative	N/A	N/A	29.21
Negative	N/A	N/A	29.26
Negative	N/A	N/A	29.28
Negative	N/A	N/A	29.2
Negative	N/A	N/A	29.26
Negative	N/A	N/A	29.18
Negative	N/A	N/A	29.25
Negative	N/A	N/A	29.33
Negative	N/A	N/A	29.28
Negative	N/A	N/A	29.24
Negative	N/A	N/A	29.3
Negative	N/A	N/A	29.3
Negative	N/A	N/A	29.29
Negative	N/A	N/A	29.21
Negative	N/A	N/A	29.17
Negative	N/A	N/A	29.12
Negative	N/A	N/A	29.18

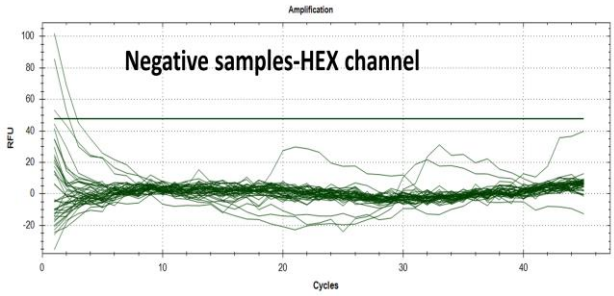
Note: P.C. is positive control plasmids provided in the kit; NTC (no-template-control) is negative control.

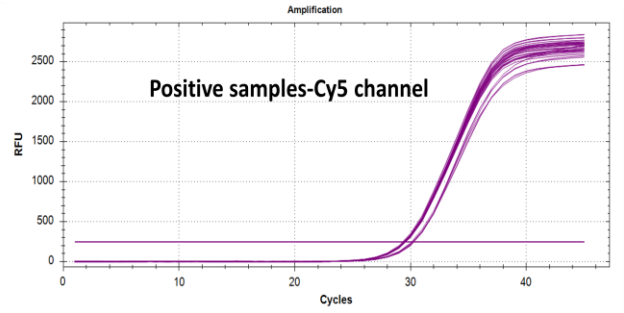
2.3. Representative Amplification Plots

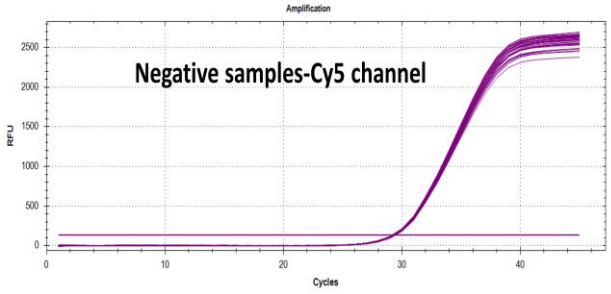












2.4 Conclusion:

Clinical performance of SARS-CoV-2 RT-PCR Detection Kit was confirmed at 100% at LoD of 10 copies/reaction.