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Evaluation of iLAMP Novel-COV19 detection kit From Ione BIO

Evaluation Report
Final

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Introduction

RT-LAMP (LOOP MEDIATED isothermal amplification) PCR can be used as an alternative to the qRT-PCR to detect SARS-CoV 2 in RNA. This report described the evaluation of the iLAMP Novel-COV19 from Ione BIO.

Material & Methods

A total of 66 samples were tested for iLAMP Novel-Cov19 detection kit. 49 samples were tested with a RNA carrier and 17 samples were tested without an RNA carrier. From each sample 200 µl mixed with 275 µl MagNA Pure lysis buffer with EAV included and 450 µl was extracted on a MagNA Pure 96 Instrument (Roche) using the MagNA Pure 96 DNA and Viral NA Small Volume Kit (Roche) and eluted in a volume of 50 µl.

The E-gene/EAV and RdRp-gene qRT-PCR PCRs were performed to compare the results with the iLAMP Novel-COV19 detection kit by using Fast Virus Master Mix (FVMM). Reaction condition of both PCR described in Table 1 and 2.

Table 1: A Master mix preparation iLAMP Novel-COV19

Master mix	**µl**
RT LAMP premix	14
Primer set	6
Specimen nucleic acid	5
Total volume	25

B: Master mix preparation FVMM

E-gene/EAV qRT PCR	**µl**	**RdRp**-gene qRT-PCR	**µl**
4x Taqman Fast Virus MM	5	**4x Taqman Fast Virus MM**	5
E+EAV Mix	3	**RdRp Mix**	3
PCR grade water	7	**PCR grade water**	7
Specimen nucleic acid	5	**Specimen nucleic acid**	5
Total volume	20	**Total volume**	20

Table 2 A *Amplification temperature protocol Biorad iLAMP Novel-COV19*

PCR Program	**Temp Target (°C)**	**Time**	Cycle
cDNA synthesis	**50**	**5 min**	**1**
Isothermal amplification	**67**	**30 sec**	**30**

B. Amplification temperature protocol LC480 mark II qRT-PCR

PCR Program	**Segment number**	**Temp Target (°C)**	**Hold Time (sec.)**	**Slope (°C/sec.)**	**Acquisition mode**	
Reverse Transcription	1	50	900	**EXTERNAL**		
Denaturation/Inactivation	1	95	120	**EXTERNAL**		
Denaturation	1	95	60	4.4	None	LC 480
Amplification	1	95	10	4.4	None	
(cycles:50)	2	60	30	2.2	Single	
Cooling	1	40	30	4.4	None	

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Results

The samples with RNA carrier were tested In iLAMP Novel-COV19 and E-gene and RdRP qRT-PCR to compare the results. The results are shown in table 3. Results of EAV (internal control) are not shown. Negative SARS-CoV2 samples are negative in both assays, but the sensitivity of iLAMP Novel COV2 is low. Just 7 of 31 positive SARS-CoV2 samples are detected in the iLAMP Novel COV2. Follows the interpretation described in iLAMP Novel-CoV19 test kit instruction for use, 4 samples (Ct>26) should be negative. Just 3 of 31 positive SARS-CoV2 samples are detected in the iLAMP Novel-COV19 detection kit. The sensitivity is 52% and specificity 100%.

Table 3: Results samples with RNA carrier

Name	qRT-PCR		IoneBIO	Result	With RNA carrier
	E	RdRp	N		
Sample 1	33.69	31.56	N/A	SARS-CoV2	
Sample 2	33.87	32.97	N/A	SARS-CoV2	
Sample 3	17.99	18.79	23.31	SARS-CoV2	
Sample 4	32.34	30.45	N/A	SARS-CoV2	
Sample 5	31.99	31.73	N/A	SARS-CoV2	
Sample 6	28.15	29.13	N/A	SARS-CoV2	
Sample 7	27.88	28.65	N/A	SARS-CoV2	
Sample 8	33.16	33.05	N/A	SARS-CoV2	
Sample 9	27.27	27.9	N/A	SARS-CoV2	
Sample 10	29.02	29.86	N/A	SARS-CoV2	
Sample 11	22.81	23.45	27.74	SARS-CoV2	
Sample 12	24.56	25.62	N/A	SARS-CoV2	
Sample 13	27.14	29.3	N/A	SARS-CoV2	
Sample 14	24.7	25.8	N/A	SARS-CoV2	
Sample 15	21.5	21.6	25.73	SARS-CoV2	
Sample 16	34.1	33	N/A	SARS-CoV2	
Sample 17	19.3	19.4	28.47	SARS-CoV2	
Sample 18	15.8	16	17.83	SARS-CoV2	
Sample 19	N/A	N/A	N/A	Non SARS-CoV2	
Sample 20	N/A	N/A	N/A	Non SARS-CoV2	
Sample 21	N/A	N/A	N/A	Non SARS-CoV2	
Sample 22	N/A	N/A	N/A	Non SARS-CoV2	
Sample 23	N/A	N/A	N/A	Non SARS-CoV2	
Sample 24	N/A	N/A	N/A	Non SARS-CoV2	
Sample 25	N/A	N/A	N/A	Non SARS-CoV2	
Sample 26	N/A	N/A	N/A	Non SARS-CoV2	

Name	qRT-PCR		IoneBIO	Result	With RNA carrier
	E	RdRp	N		
Sample 27	25.93	26.62	N/A	SARS-CoV2	
Sample 28	33.87	30.6	N/A	SARS-CoV2	
Sample 29	28.41	28.51	N/A	SARS-CoV2	
Sample 30	N/A	N/A	N/A	Non SARS-CoV2	
Sample 31	31.99	31.15	27.33	SARS-CoV2	
Sample 32	N/A	N/A	N/A	Non SARS-CoV2	
Sample 33	30.78	31.26	N/A	SARS-CoV2	
Sample 34	N/A	N/A	N/A	Non SARS-CoV2	
Sample 35	22.82	23.65	N/A	SARS-CoV2	
Sample 36	N/A	38.49	N/A	SARS-CoV2	
Sample 37	34.52	34.81	N/A	SARS-CoV2	
Sample 38	36.08	N/A	N/A	SARS-CoV2	
Sample 39	26.95	27.8	N/A	SARS-CoV2	
Sample 40	N/A	N/A	N/A	Non SARS-CoV2	
Sample 41	N/A	N/A	N/A	Non SARS-CoV2	
Sample 42	28.53	29.14	28.84	SARS-CoV2	
Sample 43	N/A	36.92	N/A	SARS-CoV2	
Sample 44	N/A	N/A	N/A	Non SARS-CoV2	
Sample 45	34.61	35.7	N/A	SARS-CoV2	
Sample 46	N/A	N/A	N/A	Non SARS-CoV2	
Sample 47	N/A	N/A	N/A	Non SARS-CoV2	
Sample 48	N/A	N/A	N/A	Non SARS-CoV2	
Sample 49	N/A	N/A	N/A	Non SARS-CoV2	
Positive control qRT-PCR	26.54	27.29			
Positive control			18.02		

Extracted RNA samples contain an RNA carrier, this may be a disturbing factor in the detection of SARS-CoV2 in the iLAMP Novel-CoV19 detection kit. To test this hypothesis, the positive control of the detection kit was tested with and without the RNA carrier.
Result are shown in figure 1. From this result it can be concluded that the RNA carrier has an inhibitory effect.

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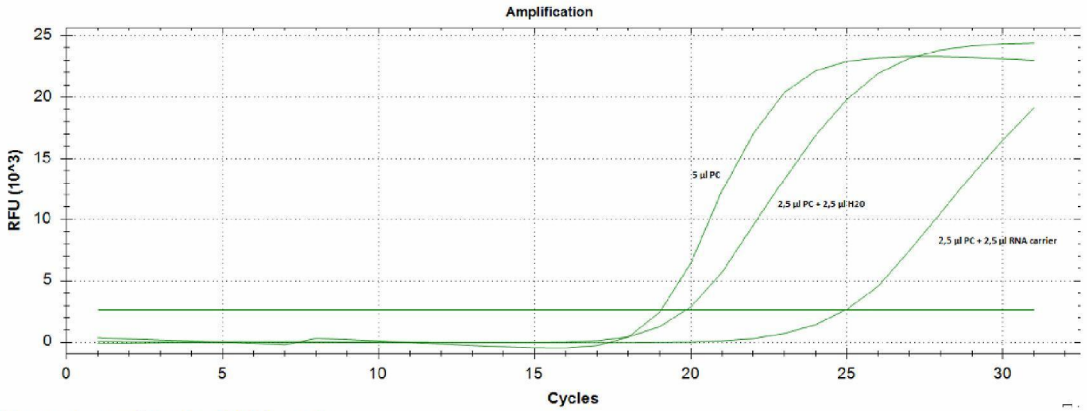

Figure 1: result test of RNA carrier

A total of 17 samples without RNA carrier where tested in the iLAMP Novel-COV19 detection kit and the E-gene qRT-PCR. Results are shown in table 4. There are 8 of 16 samples positive for SARS-CoV2. From Ct> 26 in the qRT-PCR the results are negative in the iLAMP Novel-COV19 detection kit. The sensitivity from iLAMP Novel-COV19 detection kit is 67%.

Table 4: Results samples without RNA carrier

Name	qRT-PCR	IoneBIO	Result	
	E	N		
Samples 50	25.27	28.31	SARS-CoV2	Without RNA carrier
Samples 51	30.73	N/A	SARS-CoV2	
Samples 52	30.5	N/A	SARS-CoV2	
Samples 53	27.45	24.82	SARS-CoV2	
Samples 54	25.94	N/A	SARS-CoV2	
Samples 55	29.3	N/A	SARS-CoV2	
Samples 56	28.76	N/A	SARS-CoV2	
Samples 57	17.22	14.20	SARS-CoV2	
Samples 58	20.26	17.36	SARS-CoV2	
Samples 59	18.49	15.38	SARS-CoV2	
Sample 60	N/A	N/A	Non SARS-CoV2	
Sample 61	23.81	17.66	SARS-CoV2	
Sample 62	26.33	N/A	SARS-CoV2	
Sample 63	33.73	N/A	SARS-CoV2	
Sample 64	31.76	16.24	SARS-CoV2	
Sample 65	23.26	23.43	SARS-CoV2	
Sample 66	21.49	18.49	SARS-CoV2	

Conclusion

The sensitivity is 52% when the RNA carrier that keep the RNA stable is used. When the RNA carrier is not used, more samples become positive , but the sensitivity is still only 67%. The RNA carrier has an inhibitory effect on the iLAMP Novel COV19 detection assay.
The specificity is only tested in the first experiment with RNA carrier. In this experiment the specificity is 100%. But because of the use of RNA carrier and know that it gives inhibition there is no guarantee that other viruses will not be positive in the iLAMP Novel COV19 detection kit.
Since the sensitivity is 67%, the use of the iLAMP Novel COV19 detection kit is not sensitive enough to detect SARS-CoV2.