

Validation of ILRI in-house reconstituted kit for RT-qPCR diagnosis of SARS-CoV-2 virus



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ILRI PROJECT REPORT

Validation of ILRI in-house reconstituted kit for RT-qPCR diagnosis of SARS-CoV-2 virus

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Cover photo— Scientists preparing samples in biosafety level III facilities at ILRI laboratory (photo credit: ILRI/Paul Karaimu).

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Contents

Summary.....	iv
Purpose.....	1
Methodology.....	1
Deviations	3
Results.....	4
Discussion	13
Conclusion	13

Summary

Samples	Samples used for the validation were extracted using TANbead Maelstrom 9600 according to the viral nucleic acid extraction protocol and the Qiagen, RNeasy Kit.	
RT-qPCR	qPCR set-up for the diagnosis of SARS-COV-2 was done in Applied Biosystems Quant Studio 5 Instrument.	
Consumables	Applied Biosystem 96-well plates and optical sealers were used for the set-up.	
Conditions	ILRI In-House	Reverse transcription - 50°C for 10 min, Initial - 95°C for 2 min, cycling stage – 45 cycles of 95°C for 3 sec, 60°C for 30 sec.
	TaqPath Kit	Activation - 25°C for 2 min, Reverse transcription - 53°C for 10 min, Initial - 95°C for 2 min, cycling stage – 40 cycles of 95°C for 3 sec, 60°C for 30 sec.
	Da An Gene Kit	Reverse transcription - 50°C for 15 min, Initial - 95°C for 15 min, cycling stage – 45 cycles of 94°C for 15 sec, 55°C for 45 sec.

Purpose

The purpose of the experiment was to validate International Livestock Research Institute (ILRI) In-House reconstituted mastermix, primers and probes (IDT) that target the virus genes as an alternative to commercially constituted kits for COVID-19 RT-qPCR testing. This validation exercise is to generate data that can be used to decide whether an ILRI In-House reconstituted kit can be used place of the Da An Gene COVID-19 commercial kit –the main RT-PCR-reagent for the routine diagnosis of COVID-19 at ILRI. In the workflow, two commercial kits, Da An gene Kit and TaqPath™ COVID-19 RT-PCR Kit, were used as Gold standard comparators. The experiment was conducted under a precondition that only RT-qPCR-reagents - primer-probe mix and PCR thermocycling (temperature profile) conditions - change while the instruments, reaction volume, and other parameters remain unchanged. All PCR assays conducted were real-time PCR systems based on hydrolyzing probe chemistry (Taqman™). Each assay was targeting at least two viral genes N and ORF1ab with the exemption of TaqPath which in addition detects the viral spike protein. Furthermore, both the ILRI In-House reconstitution assay and the commercial Da An Gene kit target Human Rnase P gene as a control to the entire testing workflow – extraction, qPCR and the integrity of the oral-nasal pharyngeal sampling process.

Methodology

All tests were conducted in parallel using the three different protocols for the same pool of RNA. In all the set-ups, 5 µl of RNA was added to the master mix -primer-probe cocktail to achieve a final volume of 25 µl. Below are plans used for the extraction and qPCR's respectively.

TANBead Extraction Plate Layout												
3rd March 2021		Plate Number	1 - Auto Plates				Kit Lot No					
COVID-19												
		Samples	94									
1	2	3	4	5	6	7	8	9	10	11	12	
COVC22965	COVC22966	COVC22968	COVC22969	COVC22971	COVC22974	COVC22975	COVC22979	COVC23001	COVC23002	COVC23171	COVC23182	
COVC23190	COVC23193	COVC23197	COVC23198	COVC23201	COVC23206	COVC23255	COVC23263	COVC23264	COVC23267	COVC23268	COVC23282	
COVC23283	COVC23286	COVC23287	COVC23308	COVC23313	COVC23314	COVC23319	COVC23322	COVC23346	COVC23347	COVC23349	COVC23350	
COVC23351	COVC23352	COVC23353	COVC23354	COVC23355	COVC23356	COVC23362	COVC23363	COVC23364	COVC23367	COVC23370	COVC23382	
COVC23384	COVC20803	COVC20807	COVC20811	COVC20817	COVC20819	COVC20822	COVC20823	COVC20824	COVC20826	COVC20827	COVC20828	
COVC20829	COVC20830	COVC20831	COVC20832	COVC20833	COVC20837	COVC20838	COVC20840	COVC20841	COVC20845	COVC20846	COVC20849	
COVC20853	COVC20854	COVC20855	COVC20856	COVC20860	COVC20861	COVC20862	COVC20863	COVC20864	COVC20865	COVC20871	COVC20872	
COVC20877	COVC20879	COVC20881	COVC20884	COVC20886	COVC20887	COVC20889	COVC20890	COVC20891	COVC20892	NC	NC	

Figure 1: TANBead Nucleic acid extractor extraction plan layout.

NB: Wells 95 and 96 are controls whereby one well serves as an extraction negative control and the other is left empty but will be used for the PCR positive control.

REAL-TIME PCR												
Date:						Kit Name	Da An Gene					
Target:	SARS-COV2				Plate Number							
Analyst:							Kit Lot No					
					Samples							
qPCR System							Cycling conditions					
Master Mix:	Full plate	Half	Fewer			cDNA Synthesis	50 °C, 15 min					
Reagents	1X	100	50	20	15	10	Initial denaturation	95 °C, 15 min				
Liquid A	17	1700	850	340	255	170	PCR cycling (45 cycles)	95 °C, 15 sec				
Liquid B	3	300	150	60	45	30		55 °C, 45 sec				
Template	5											
Total	25	2000	1000	400	300	200						
	1	2	3	4	5	6	7	8	9	10	11	12
A	COVC22965	COVC22966	COVC22968	COVC22969	COVC22971	COVC22974	COVC22975	COVC22979	COVC23001	COVC23002	COVC23171	COVC23182
B	COVC23190	COVC23193	COVC23197	COVC23198	COVC23201	COVC23206	COVC23255	COVC23263	COVC23264	COVC23267	COVC23268	COVC23282
C	COVC23283	COVC23286	COVC23287	COVC23308	COVC23313	COVC23314	COVC23319	COVC23322	COVC23346	COVC23347	COVC23349	COVC23350
D	COVC23351	COVC23352	COVC23353	COVC23354	COVC23355	COVC23356	COVC23362	COVC23363	COVC23364	COVC23367	COVC23370	COVC23382
E	COVC23384	COVC20803	COVC20807	COVC20811	COVC20817	COVC20819	COVC20822	COVC20823	COVC20824	COVC20826	COVC20827	COVC20828
F	COVC20829	COVC20830	COVC20831	COVC20832	COVC20833	COVC20837	COVC20838	COVC20840	COVC20841	COVC20845	COVC20846	COVC20849
G	COVC20853	COVC20854	COVC20855	COVC20856	COVC20860	COVC20861	COVC20862	COVC20863	COVC20864	COVC20865	COVC20871	COVC20872
H	COVC20877	COVC20879	COVC20881	COVC20884	COVC20886	COVC20887	COVC20889	COVC20890	COVC20891	COVC20892	NC	PC

Figure 2: Da An Gene plate plan and cycling condition.

REAL-TIME PCR												
Date:						Kit Name	TaqPath COVID-19 CE-IVD RT PCR KIT					
Target:	SARS-COV2				Plate Number							
Analyst:							Kit Lot No					
					Samples							
qPCR System	ABI 7500						Cycling conditions					
Master Mix:	Full plate	Half	Fewer			UNG Incubation	25 °C, 2 min					
Reagents	1X	100	50	20	15	10	cDNA Synthesis	53 °C, 10 min				
Master mix (4X)	6.25	625	312.5	125	93.75	62.5	Initial denaturation	95 °C, 2 min				
Oligos (Purple soln)	1.25	125	62.5	25	18.75	12.5	PCR cycling (40 cycles)	95 °C, 3 sec				
H2O	12.5	1250	625	250	187.5	125		60°C, 30 sec				
Template	5											
Total	25	2000	1000	400	300	200						
	1	2	3	4	5	6	7	8	9	10	11	12
A	COVC22965	COVC22966	COVC22968	COVC22969	COVC22971	COVC22974	COVC22975	COVC22979	COVC23001	COVC23002	COVC23171	COVC23182
B	COVC23190	COVC23193	COVC23197	COVC23198	COVC23201	COVC23206	COVC23255	COVC23263	COVC23264	COVC23267	COVC23268	COVC23282
C	COVC23283	COVC23286	COVC23287	COVC23308	COVC23313	COVC23314	COVC23319	COVC23322	COVC23346	COVC23347	COVC23349	COVC23350
D	COVC23351	COVC23352	COVC23353	COVC23354	COVC23355	COVC23356	COVC23362	COVC23363	COVC23364	COVC23367	COVC23370	COVC23382
E	COVC23384	COVC20803	COVC20807	COVC20811	COVC20817	COVC20819	COVC20822	COVC20823	COVC20824	COVC20826	COVC20827	COVC20828
F	COVC20829	COVC20830	COVC20831	COVC20832	COVC20833	COVC20837	COVC20838	COVC20840	COVC20841	COVC20845	COVC20846	COVC20849
G	COVC20853	COVC20854	COVC20855	COVC20856	COVC20860	COVC20861	COVC20862	COVC20863	COVC20864	COVC20865	COVC20871	COVC20872
H	COVC20877	COVC20879	COVC20881	COVC20884	COVC20886	COVC20887	COVC20889	COVC20890	COVC20891	COVC20892	NC	PC

Figure 3: TaqPath Kit plate PCR layout and cycling conditions.

REAL-TIME PCR										20 X Oligos Preparation		
Date:					Plate Number	1		Component	Concentration	Volume (µL)		
Target Pathogen:	COVID-19				Samples			TE Buffer (Low salt)	1X	150		
Analyst:					Kit Name	KiCqStart MM		Forward Primer	100 p moles	20		
qPCR System					Kit Lot			Reverse Primer	100 p moles	20		
								Probe	100 p moles	10		
								Total	20X	200		
					10							
Master Mix:												
Reagents	1X	10X	15X	20X	25X	30X	40X	50X	60X	80X	100X	
2X Master mix	12.5	125	187.5	250	312.5	375	500	625	750	1000	1250	
ORF1 ab	0.4	4	6	8	10	12	16	20	24	32	40	
N Gene	1.25	12.5	18.75	25	31.25	37.5	50	62.5	75	100	125	
Rnase P	0.8	8	12	16	20	24	32	40	48	64	80	
Nuclease Free H2O	5.05	50.5	75.75	101	126.25	151.5	202	252.5	303	404	505	
Template	5											
Total	25											

	1	2	3	4	5	6	7	8	9	10	11	12
A	COVC22965	COVC22966	COVC22968	COVC22969	COVC22970	COVC22974	COVC22975	COVC22976	COVC22977	COVC22978	COVC22979	COVC22980
B	COVC23190	COVC23193	COVC23197	COVC23198	COVC23200	COVC23206	COVC23207	COVC23208	COVC23209	COVC23210	COVC23211	COVC23212
C	COVC23283	COVC23286	COVC23287	COVC23308	COVC23310	COVC23314	COVC23315	COVC23316	COVC23317	COVC23318	COVC23319	COVC23320
D	COVC23351	COVC23352	COVC23353	COVC23354	COVC23355	COVC23356	COVC23357	COVC23358	COVC23359	COVC23360	COVC23361	COVC23362
E	COVC20825	COVC20830	COVC20831	COVC20832	COVC20833	COVC20837	COVC20838	COVC20839	COVC20840	COVC20841	COVC20842	COVC20843
F	COVC20825	COVC20830	COVC20831	COVC20832	COVC20833	COVC20837	COVC20838	COVC20839	COVC20840	COVC20841	COVC20842	COVC20843
G	COVC20853	COVC20854	COVC20855	COVC20856	COVC20857	COVC20861	COVC20862	COVC20863	COVC20864	COVC20865	COVC20866	COVC20867
H	COVC20877	COVC20879	COVC20881	COVC20884	COVC20886	COVC20887	COVC20888	COVC20889	COVC20890	COVC20891	NC	PC

Cycling conditions	
UDG incubation	50 °C, 10 min
Initial denaturation	94 °C, 1 min
PCR cycling (45 cycles)	94 °C, 3 sec 60°C, 30 sec (data)

Figure 4: ILRI In-House reconstituted kit: Mastermaster mix set-up that includes primer reconstitutions to cocktail stocks of 20x (top right corner) and cycling conditions (bottom).

Deviations

Whereas the probe targeting the N gene is labelled with FAM fluorophore in both the Da An Gene Kit and TaqPath Kit, for In-house reconstituted kit the N gene probe is labelled with ABY. Further, ORF1ab probe for the in-house assay is labelled with the FAM fluorophore while for the Da An Gene kit and TaqPath Kit, it is labelled with the VIC fluorophore. It is not possible to establish whether the two genes, ORF1ab and N gene primer/probe sets are the same sequences since this information is not provided by the different manufacturers.

Results

Table I: Cycle threshold (Ct) quantification for N and ORF1ab SARS-COV-2 gene detection during RT-qPCR testing

Sample Name	N Gene			ORF1ab		
	TaqPath Kit (Ct)	Da An Gene Kit (Ct)	ILRI In-House (Ct)	TaqPath Kit (Ct)	Da An Gene Kit (Ct)	ILRI In-House (Ct)
22965	29.29	34.01	30.96	30.95	0.00	0.00
22966	31.90	36.40	33.73	32.79	0.00	0.00
22968	0.00	38.95	0.00	0.00	0.00	0.00
22969	36.19	37.82	35.27	0.00	0.00	0.00
22971	33.20	36.07	36.96	34.25	38.96	36.67
22974	31.42	35.31	36.03	32.51	0.00	38.05
22975	0.00	37.06	0.00	0.00	0.00	43.89
22979	19.92	23.92	21.81	21.89	28.34	24.59
23001	26.41	27.88	25.57	26.29	29.52	25.48
23002	23.36	25.08	22.61	23.21	26.51	22.27
23171	0.00	0.00	0.00	0.00	0.00	0.00
23182	27.63	32.68	29.94	29.50	37.14	37.54
23190	29.92	34.49	32.75	31.21	0.00	34.73
23193	0.00	38.54	36.67	0.00	0.00	0.00
23197	0.00	0.00	35.04	0.00	0.00	0.00
23198	33.02	36.52	35.35	33.92	0.00	0.00
23201	23.19	25.15	22.67	23.04	27.00	23.25
23206	23.09	24.21	21.62	23.49	26.65	22.51
23255	30.44	30.97	30.12	33.00	39.40	41.20
23263	30.23	33.20	32.12	30.73	37.09	35.60
23264	25.99	30.41	28.92	27.86	33.50	31.19
23267	27.61	30.42	27.85	29.25	36.40	32.84
23268	24.17	25.43	21.60	24.25	27.45	22.61

	N Gene			ORFlab		
Sample Name	TaqPath Kit (Ct)	Da An Gene Kit (Ct)	ILRI In-House (Ct)	TaqPath Kit (Ct)	Da An Gene Kit (Ct)	ILRI In-House (Ct)
23282	31.26	33.62	30.94	31.87	36.87	31.74
23283	30.81	34.03	31.73	31.65	39.74	44.67
28286	23.40	25.44	23.34	23.51	27.61	26.55
23287	27.07	29.65	27.03	27.40	32.18	31.96
23308	32.59	34.23	31.71	33.42	37.32	33.00
23313	23.73	24.92	22.50	23.78	26.90	22.68
23314	19.22	20.40	17.72	19.15	21.99	17.78
23319	29.85	31.84	30.59	30.23	34.92	31.17
23322	28.94	31.48	29.15	29.78	35.65	31.81
23346	14.63	16.09	13.05	15.40	19.38	15.74
23347	27.18	28.36	26.25	27.97	31.94	27.76
23349	0.00	0.00	0.00	0.00	0.00	0.00
23350	20.36	21.27	17.77	20.77	24.07	18.97
23351	22.60	26.21	22.63	25.36	33.20	28.33
23352	30.55	32.84	31.10	32.55	36.32	32.46
23353	24.57	28.66	26.90	28.10	34.14	29.83
23354	29.64	33.28	31.55	31.26	35.64	32.16
23355	0.00	0.00	0.00	0.00	0.00	0.00
23356	12.31	12.47	9.36	13.17	16.31	11.85
23362	14.52	14.38	11.13	15.71	18.92	14.45
23363	29.14	30.82	27.81	29.22	32.96	27.97
23364	32.08	33.50	31.67	32.71	36.33	33.92
23367	16.18	16.84	14.70	16.55	20.23	16.07
23370	26.22	29.37	26.37	26.92	33.38	29.15
23382	28.88	29.98	27.63	29.34	33.30	28.30
23384	28.65	29.90	26.75	28.57	32.07	26.97

	N Gene			ORF lab		
Sample Name	TaqPath Kit (Ct)	Da An Gene Kit (Ct)	ILRI In-House (Ct)	TaqPath Kit (Ct)	Da An Gene Kit (Ct)	ILRI In-House (Ct)
20803	33.38	34.45	32.38	33.66	37.07	34.91
20807	28.82	30.39	27.71	28.66	32.38	27.78
20811	32.28	33.25	31.85	32.26	35.84	31.70
20817	21.59	22.80	19.30	21.85	25.24	20.67
20819	27.18	32.23	26.16	27.58	35.00	26.90
20822	24.71	25.87	22.86	24.69	28.15	23.56
20823	21.97	23.38	19.69	22.73	27.62	22.03
20824	24.67	25.57	23.93	25.43	28.70	24.94
20826	30.12	31.50	34.05	30.43	33.15	32.50
20827	23.03	24.29	23.72	23.39	26.23	23.49
20828	29.16	31.08	29.97	29.17	33.06	28.43
20829	23.93	25.30	21.47	23.88	27.46	22.45
20830	13.24	13.75	11.56	14.18	17.64	13.36
20831	30.30	32.13	29.58	30.09	34.27	30.30
20832	19.66	19.96	17.59	20.16	22.86	18.74
20833	30.77	32.21	29.63	31.01	34.86	31.25
20837	22.41	23.88	18.72	22.39	25.58	21.28
20838	25.65	26.62	23.96	25.59	29.17	24.44
20840	29.83	31.22	28.87	29.95	33.77	29.09
20841	13.40	14.25	10.25	13.75	16.81	12.27
20845	22.98	24.82	21.55	23.12	26.80	22.31
20846	24.44	26.10	23.63	24.46	27.99	23.96
20849	20.48	21.44	18.90	20.81	24.40	19.68
20853	29.97	31.60	29.44	29.89	34.03	29.13
20854	18.00	18.94	16.26	17.90	20.81	16.21
20855	24.69	26.85	23.99	24.58	28.33	24.28

	N Gene			ORF1ab		
Sample Name	TaqPath Kit (Ct)	Da An Gene Kit (Ct)	ILRI In-House (Ct)	TaqPath Kit (Ct)	Da An Gene Kit (Ct)	ILRI In-House (Ct)
20856	29.37	31.38	28.92	29.15	33.51	28.98
20860	25.01	25.96	23.18	25.39	28.80	24.33
20861	36.10	35.44	34.54	36.24	37.99	34.70
20862	13.30	13.57	9.95	14.50	17.96	13.25
20863	28.34	29.91	27.79	28.53	32.09	28.08
20864	20.94	21.88	19.34	21.14	24.10	19.60
20865	22.39	23.62	21.32	22.42	26.11	21.48
20871	31.00	32.71	30.71	31.03	35.13	30.20
20872	32.17	33.41	31.18	31.99	36.07	31.80
20877	19.88	21.07	18.36	19.86	23.21	18.69
20879	28.71	30.56	28.83	28.75	32.97	28.60
20881	25.26	26.93	23.88	25.27	29.32	24.65
20884	28.93	30.31	28.79	29.17	32.58	28.18
20886	19.86	21.09	17.54	19.86	23.19	18.70
20887	32.72	35.18	31.71	32.85	36.80	32.52
20889	27.06	28.60	26.24	26.83	30.26	25.94
20890	0.00	36.65	33.92	34.60	39.41	40.63
20891	28.07	29.56	27.44	27.94	31.54	27.48
20892	23.31	24.38	21.94	23.19	26.07	21.83
NC	0.00	0.00	0.00	0.00	0.00	0.00
PC	19.57	31.26	17.95	19.28	31.96	17.90

Table 2: Interpretation of results for SARS-COV-2 tests on Table 1 above.

Sample Name	N gene Interpretation			ORFlab Interpretation			Overall Interpretation		
	Da An Gene	TaqPath	ILRI In-House	Da An Gene	TaqPath	ILRI In-House	Da An Gene	TaqPath	ILRI In-House
22965	Positive	Positive	Positive	Negative	Positive	Negative	Negative	Positive	Negative
22966	Positive	Positive	Positive	Negative	Positive	Negative	Negative	Positive	Negative
22968	Positive	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative
22969	Positive	Positive	Positive	Negative	Positive	Negative	Negative	Positive	Negative
22971	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
22974	Positive	Positive	Positive	Negative	Positive	Positive	Negative	Positive	Positive
22975	Positive	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Negative
22979	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
23001	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
23002	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
23171	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
23182	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
23190	Positive	Positive	Positive	Negative	Positive	Positive	Negative	Positive	Positive
23193	Positive	Negative	Positive	Negative	Negative	Negative	Negative	Negative	Negative
23197	Negative	Positive	Positive	Negative	Negative	Negative	Negative	Negative	Negative
23198	Positive	Positive	Positive	Negative	Positive	Negative	Negative	Positive	Negative
23201	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
23206	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
23255	Positive	Positive	Positive	Positive	Positive	Negative	Positive	Positive	Negative
23263	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
23264	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
23267	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
23268	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
23282	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
23283	Positive	Positive	Positive	Positive	Positive	Negative	Positive	Positive	Negative

	N gene Interpretation			ORF lab Interpretation			Overall Interpretation		
Sample Name	Da An Gene	TaqPath	ILRI In-House	Da An Gene	TaqPath	ILRI In-House	Da An Gene	TaqPath	ILRI In-House
28286	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
23287	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
23308	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
23313	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
23314	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
23319	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
23322	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
23346	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
23347	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
23349	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
23350	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
23351	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
23352	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
23353	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
23354	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
23355	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
23356	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
23362	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
23363	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
23364	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
23367	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
23370	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
23382	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
23384	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
20803	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
20807	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive

	N gene Interpretation			ORF lab Interpretation			Overall Interpretation		
Sample Name	Da An Gene	TaqPath	ILRI In-House	Da An Gene	TaqPath	ILRI In-House	Da An Gene	TaqPath	ILRI In-House
20861	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
20862	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
20863	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
20864	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
20865	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
20871	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
20872	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
20877	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
20879	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
20881	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
20884	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
20886	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
20887	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
20889	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
20890	Positive	Negative	Positive	Positive	Positive	Negative	Positive	Negative	Negative
20891	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
20892	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
NC	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
PC	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive

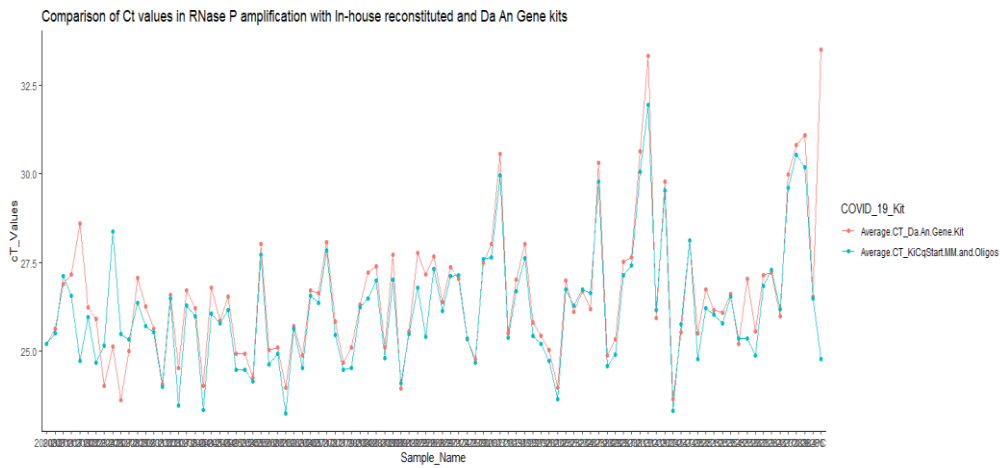


Figure 5: Comparison of RNase P gene cycles of quantification for Dan An Gene Kit and ILRI In-House master mix, respectively.

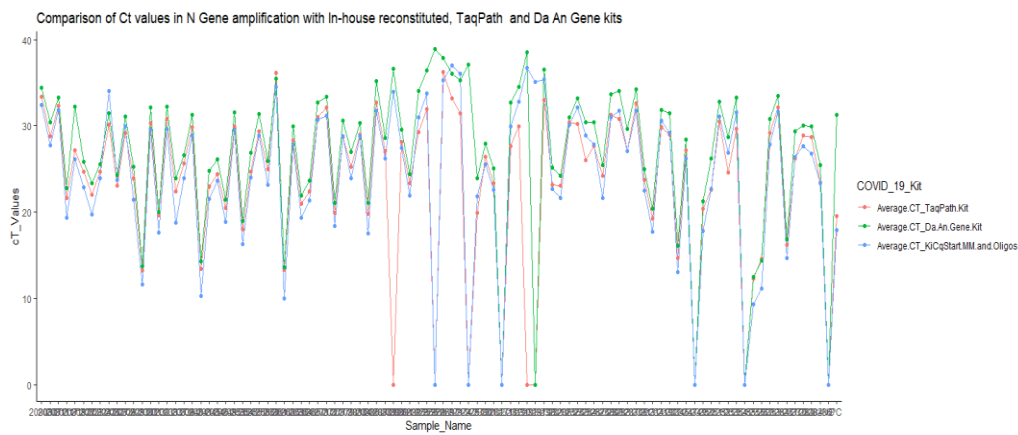


Figure 6: Comparison of COVID-19 N gene cycles of quantification for TaqPath Kit, Dan An Gene Kit and ILRI In-House, respectively.

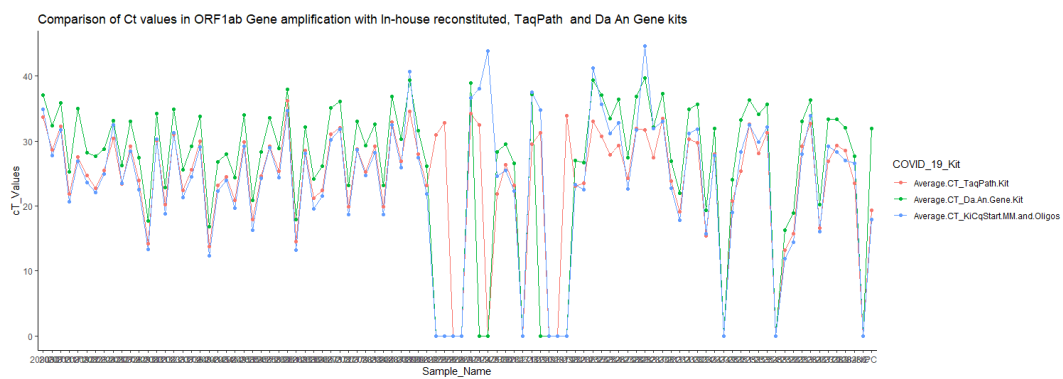


Figure 7. comparison of COVID-19 ORF1ab gene cycles of quantification for TaqPath Kit, Dan An Gene Kit and ILRI In-House master mix, respectively.

Table 3. Comparison of total agreement levels using Da An Gene Kit as the gold standard Kit.

Kit	Result	qPCR Data		Total agreement (%)	Kappa* \pm SE	
		Neg	Pos			
Da An Gene	Neg	14	0	96	(100.00)	N/A
	Pos	0	82			
TaqPath	Neg	9	0	91	(94.79)	0.7546 \pm 0.203
	Pos	5	82			
ILRI In-House	Neg	14	1	95	(98.96)	0.9594 \pm 0.079
	Pos	0	81			

Neg =negative; Pos= positive; qPCR= quantitative PCR

*Kappa agreement level: K<0.2, poor; 0.21 - 0.40, fair; 0.41 - 0.60, moderate; 0.61 - 0.80, good; 0.81 - 1.00, very good.

Discussion

From the observed results, there was 98.96% concordance between the ILRI In-House and Da An Gene Kit with a discordance in only one sample. The TaqPath kit had 94.79% concordance with the Da An Gene kit. The difference in Ct Value and analytical specificity varied slightly between the ILRI In-House and the Da An Gene Kit. The differences in the Ct Value between ILRI In-House and Da An Gene (ILRI In-House – Da An Gene) for the N and ORF1ab gene was -2.21 and -3.23 respectively. The trend was also observed in the Human Rnase P gene which had a difference of -0.57. On average ILRI In-House has shown slightly lower Ct values compared to Da An Gene which may indicate that ILRI In-House is slightly more sensitive than the Da An Gene Kit for high viral load samples.

The ILRI In-House master mix contains a fast Taq enzyme thus all assays end within 60 minutes as compared to Da An Gene kit which takes 110 minutes to complete a run. This will help reduce the turnaround time for COVID testing.

Conclusion

Based on the results obtained, there is high concordance between the ILRI In-house reconstituted kit and the commercial kits (Da An Gene and TaqPath). The validation assay has demonstrated that the ILRI In-House reconstituted kit can be used as an alternative to the commercial assay kits for the RT-qPCR diagnosis of SARS-CoV-2.