
	Validation Summary Template	Control #	IGX-V002
		Version:	1
		Effective Date:	TBD

	APPROVAL SIGNATURE	DATE
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REVISION HISTORY			
Version #	Revised By	Revision Summary	Effective Date

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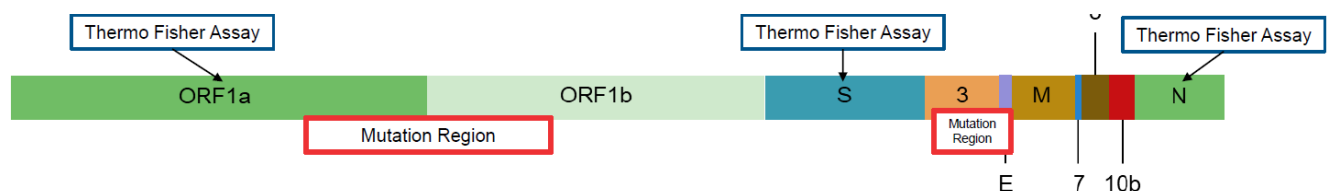
1.0 OVERVIEW OF TEST

1.1 Background

Since late 2019, a novel strain of Coronavirus dubbed SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus-2) has evolved into a global crisis. In response to this global pandemic, molecular diagnostics has been at the forefront of testing, and continues to evolve. The assay to be validated is a modified version of the Emergency Use Authorization (EUA) assay offered by Thermo Fisher Scientific-TaqPath COVID-19 combo kit. The validation will be performed on a Quanstudio 12k, which is not currently covered by an EUA. Brief description of the disease

1.2 Assay Principle

SARS-CoV-2 is a single stranded RNA virus. Viral RNA is manually isolated from nasopharyngeal using the MagMax Viral/Pathogen Nucleic Acid Isolation kit. Extracted material is then assayed using multiplex TaqMan technology in a RT-PCR reaction that qualitatively detects the presence/absence of 3 genes indicative of a SARS-CoV-2 infection; ORF1ab, N Protein, and S Protein. A MS2 phage control spiked in each sample prior to extraction is also assayed as a RNA extraction control. The assay design targets genes specifically to SARS-CoV-2 to avoid the detection of similar viruses, and therefore limit the cause of false positive results.



*Image Obtained from TaqPath COVID-19 Combo Kit reference material

Amplification curves are evaluated within the Quanstudio 12k Software. Resulting is based on the algorithm set by Thermo Fisher Scientific:

ORF1ab	N gene	S gene	MS2	Status	Result	Action
NEG	NEG	NEG	NEG	INVALID	NA	Repeat test. ^[1] If the repeat result remains invalid, consider collecting a new specimen.
NEG	NEG	NEG	POS	VALID	SARS-CoV-2 Not Detected	Report results to healthcare provider. Consider testing for other viruses.
Only one SARS-CoV-2 target = POS			POS or NEG	VALID	SARS-CoV-2 Inconclusive ^[1,2]	Repeat test. ^[1] If the repeat result remains inconclusive, additional confirmation testing should be conducted if clinically indicated.
Two or more SARS-CoV-2 targets = POS			POS or NEG	VALID	Positive SARS-CoV-2	Report results to healthcare provider and appropriate public health authorities.

^[1] Retesting must be performed by re-extracting the original sample and repeating the RT-PCR.

^[2] Samples with a result of SARS-CoV-2 Inconclusive shall be retested one time.

1.3 Clinical Purpose

As the pandemic continues to influence culture at large, increased testing will allow for appropriate quarantine safeguards. Insufficient/inappropriate testing will likely lead to an extension or worsening of the spread of the virus. Early accurate detection of the virus within an individual will allow for appropriate quarantine measures.

2.0 DEFINITIONS

Temperature Definitions

Room temperature or Ambient – Between 15°C to 25°C.

Refrigerated – Between 2°C to 8°C.

Frozen – Between -15°C to -25°C.

Ultra frozen – Colder than or equal to -60°C.

3.0 SUPPLIERS

3.1 REAGENTS

- Follow storage conditions as specified by vendor.
- Follow all reagent labeling and handling, guidelines, and policies as detailed in Molecular Laboratory Practice.

Table 1: Reagents

Description	Catalog Number	LOT	Vendor	Storage Temp
TAQPATH RTPCR COVID-19-1000RXN EACH	A47814	2005119	Thermo Fisher	Frozen
NUCLEASE-FREE WATER 1000 ML EACH	AM9932	2164719	Thermo Fisher	Room Temp
10ML TP 1STEP MMX NO ROX EA	A28523	2220793	Thermo Fisher	Frozen
MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit	A48383	1912008	Thermo Fisher	Room Temp
ABY™ Dye Spectral Calibration Plate for Multiplex qPCR, Fast 96-well (0.1mL)	A24734	2003049	Thermo Fisher	Frozen
JUN™ Dye Spectral Calibration Plate for Multiplex qPCR, Fast 96-well (0.1mL)	A24735	2004046	Thermo Fisher	Frozen

3.2 ADDITIONAL SUPPLIES & MATERIALS

Table 2: Supplies

Description	Catalog Number	Vendor	Storage Temp
KF 96 KF MICROPLATE (200uL), 48/BOX	97002540	Thermo Fisher	Room Temp
KF 96 TIP COMB FOR DW MAGNETS, 100/BOX	97002534	Thermo Fisher	Room Temp
KF DEEP WELL 96 PLATE, 50/BOX	95040450	Thermo Fisher	Room Temp
MICROAMP CLEAR ADHESIVE FILM, 100 COVERS	4306311(or Equivalent)	Thermo Fisher	Room Temp
NONSTICK RNASE-FREE TUBES 1.5ML, 250 TUBES	AM12450(or Equivalent)	Thermo Fisher	Room Temp

MICROAMP OPTICAL ADHESIVE FILM, 100 PC	4311971(or Equivalent)	Thermo Fisher	Room Temp
MICROAMP FAST OPTICAL 96-WELL PLT W/BARCODE (0.1mL)	4346906 (or Equivalent)	Thermo Fisher	Room Temp

3.3 EQUIPMENT

Table 3: Equipment

Description	Serial Number	Catalog Number	Vendor
Quantstudio 12K	285881621	4470661	Thermo Fisher
Kinfisher Flex	711-82279	5400630	Thermo Fisher
Fast 96 Well block	278014988	4453559	Thermo Fisher
Plate Vortexer	200127006	VXMPDG	Ohaus
Incubator	08191296	H2200-HC	Benchmark

3.4 SOFTWARE

Table 4: Software

Description	Version
Quantstudio 12K Flex Software	1.3
Kingfisher Flex	1.01.0
Bind It	4.0

4.0 SPECIMEN INFORMATION

4.1 Matrix/Specimen

Nasopharyngeal Swabs are obtained by trained collectors and mailed at room temperature to the lab.

Previously extracted viral RNA was evaluated by re-testing RNA that had been stored at frozen temperatures. Testing of RNA samples will be evaluated on a case by case basis by Lab Director.

4.2 Containers/Preservatives

Transport Media, see package insert for details

4.3 Volume

1 swab plus 1.5ml media

4.4 Handling

All specimens should be treated as infectious and handled accordingly with appropriate PPE.

4.5 Sample Stability

Stability was evaluated on QC UTM1 provided by QC medkit, per package insert:

Matrix/Container	Room Temp	Refrigerated
Nasopharyngeal Swab	48 Hrs	48Hrs

However In house stability was done and the following sample stability will be used in house (see Additional Performance Characteristics Section for details).

Matrix/Container	Room Temp	Refrigerated	Frozen
Nasopharyngeal Swab	24 Hrs	8 Days	10 Days

4.6 Unacceptable/Suboptimal specimen criteria

Cracked/Broken, missing swabs, dry swabs, inappropriate storage temperatures, samples that are too old, or labeling issues as defined by internal policies.

4.7 Additional requirements

n/a

5.0 QUALITY CONTROL

5.1 QC Required for Assay

A positive control and negative control (NTC) will be run on each batch. The MS2 Phage control will be spiked into all samples before RNA extraction.

Ct values for all viable runs were tabulated and the Average, Min/Max, CV, and SD were calculated. The acceptable Ct ranges are listed in Tables 6-9, any runs where the controls are outside the ranges below will be evaluated for potential rerun (See Table 6).

Table 6: Acceptable Ct Ranges

Material	MS 2	N Gene	ORF1ab	S Gene
Positive Control	>40 (Undetermined)	28.17-33.69	19.74-38.48	27.22-32.74
NTC	16.76-38.06	>40 (Undetermined)	>40 (Undetermined)	>40 (Undetermined)
Clinical Samples	20.09-35.37	17.7-40.0	16.88-40.0	16.89-40.0

The following Ct values were observed for clinical samples. While the majority of positives were below 37 Ct, there were instances where true positives were between the 37-40 Ct ranges. To ensure limitation of false negatives, a conservative range of 3 SD was set as an acceptable range (See Table 7).

Table 7: Clinical Sample Ct Data

	MS 2	N Gene	ORF1ab	S Gene
AVERAGE	27.73	32.14	31.45	32.18
SD	2.55	4.81	4.86	5.10
CV	9%	15%	15%	16%
MIN	23.71	16.29	15.47	15.30
MAX	38.74	39.72	37.75	39.49
3 SD Range	20.09- 35.37	17.7- 40.00	16.88- 40.00	16.89- 40.00

The following Ct values were observed for positive controls. Overall the Positive Control Range was much tighter than the clinical sample range, likely due to the use of the TaqPath COVID-19 Control. A conservative range of 3 SD was set (See Table 8)..

Table 8: Positive Control Ct Data

	MS 2	N Gene	ORF1ab	S Gene
AVERAGE	-	30.93	29.11	29.98
SD	-	0.92	3.12	0.92
CV	-	3.0%	10.7%	3.1%
MIN	-	29.71	19.52	28.24
MAX	-	32.96	31.49	31.30
3 SD Range	-	28.17- 33.69	19.74- 38.48	27.22- 32.74

The following Ct values were observed for the NTC. The only detected target within an NTC should be the MS 2 extraction control. If other templates are present, there was likely contamination and a rerun should be performed. Again 3 SD were used as an acceptable range (See Table 9)..

Table 9: NTC Ct Data

	MS 2	N Gene	ORF1ab	S Gene
AVERAGE	27.41	-	-	-
SD	3.55	-	-	-
CV	13%	-	-	-
MIN	23.38	-	-	-
MAX	35.08	-	-	-
3 SD Range	16.76- 38.06	-	-	-

5.2 Positive Control Material

The positive control is the TaqPath COVID-19 Control (Contained within TaqPath COVID-19 kit). The positive control should show positive amplification for all three of the multiplexed targets (ORF1ab, N Protein, and S Protein). The positive control is added in a well post extraction prior to PCR.

5.3 Negative Control Material

Negative Control (NTC) will consist of Molecular grade Water, and should only have positive amplification for the MS2 phage control.

5.4 Extraction Control Material

The MS2 phage control is included in the COVID Combo kit. It is spiked into all samples and controls before extraction. MS2 phage is a single stranded RNA virus. Observable amplification will be indicative of successful extraction of RNA in each well.

5.5 Preparation/Storage

Material	Preparation	Storage
TaqPath COVID-19 Control	According to package Insert.	Ultra Frozen

6.0 METHOD PERFORMANCE

6.1 Comparative Method

6.1.1 Laboratory and Method

Samples were obtained from Advanced Genomics (CLIA# 45D2092474) and originally tested on the Luminex NxTAG platform (EUA#??). Samples were shipped refrigerated before initial testing and stored frozen after initial testing until run for this validation. All samples were frozen within 3 days of collection.

Comparative method was the EUA approved NxTAG SARS-CoV-2 Panel by Luminex. Differences between these assays are as follows:

- LOD of the comparator assay is set to 5000 copies/ml.
- Assay detects the E-gene instead of the S Gene
- Sample is deemed positive if at least one gene is detected.
- Technology is based on bead count rather than Ct Value.

6.1.2 Type and number of specimens

In total 66 nasopharyngeal swabs (34 Positive and 32 negative)

6.1.3 Duration

July 13th 2020- July 24th 2020

6.2 Result information

6.2.1 Results summary

Summary of the validation results are presented in the table below

Table 10: Results Summary

	Positive Observed	Negative Observed	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value	Concordance
Positive Expected	31	3	91.2%	100.0%	100.0%	92.1%	0.95
Negative Expected	0	35					

6.3 Accuracy

6.3.1 Observed results

In total 66 Nasopharyngeal swabs (34 Positive and 32 negative) were run in a blind accuracy study. During the study there were 5 instances of discrepancy requiring confirmation, all were apparent false negative. Those with sample remaining were confirmed on the Luminex Aries platform (See Table 11).

Overall Concordance was 95%, these results are deemed acceptable for clinical testing.

6.3.2 Defined acceptance criteria

A concordance of 95% agreement is acceptable clinical performance.

6.3.3 Discrepancies and remediation

Table 11: Discrepancies

Sample ID	Details
ORRP20-07735	Initial Run inconclusive, rerun was negative. No sample remaining for confirmation.
ORRP20-07747	Sample was run 2x, no sample remaining for sendout
PCRRPP20-00202	Sample was repeated at reference laboratory on the Aries Platform. Confirmed Negative.
ORRP20-07857	Initial Run inconclusive, rerun was negative. No sample remaining for confirmation
ORRP20-10536	Sample was repeated at reference laboratory on the Aries Platform. Confirmed Negative.

6.3.4 Deviations from plan

N/A

6.3.5 Supportive documentation

Table 12: Accuracy Study Run Data

Number	Sample ID	Ref Lab Result	N Gene	ORF1ab	S Gene	IGX Result	Concordant
1	ORRP20-07829	Negative	-	-	-	Negative	YES
2	ORRP20-07830	Negative	-	-	-	Negative	YES
3	ORRP20-07831	Negative	-	-	-	Negative	YES
4	ORRP20-07832	Negative	-	-	-	Negative	YES
5	ORRP20-07833	Negative	-	-	-	Negative	YES
6	ORRP20-07834	Negative	-	-	-	Negative	YES
7	ORRP20-07835	Negative	-	-	-	Negative	YES
8	ORRP20-07836	Negative	-	-	-	Negative	YES
9	ORRP20-07838	Negative	-	-	-	Negative	YES
10	ORRP20-07839	Negative	-	-	-	Negative	YES
11	ORRP20-07840	Negative	-	-	-	Negative	YES
12	ORRP20-07841	Negative	-	-	-	Negative	YES
13	ORRP20-07842	Negative	-	-	-	Negative	YES

14	ORRP20-07843	Negative	-	-	-	Negative	YES
15	ORRP20-07844	Negative	-	-	-	Negative	YES
16	ORRP20-07845	Negative	-	-	-	Negative	YES
17	ORRP20-07846	Negative	-	-	-	Negative	YES
18	ORRP20-07848	Negative	-	-	-	Negative	YES
19	ORRP20-07850	Negative	-	-	-	Negative	YES
20	ORRP20-07853	Negative	-	-	-	Negative	YES
21	ORRP20-07856	Negative	-	-	-	Negative	YES
22	ORRP20-07858	Negative	-	-	-	Negative	YES
23	ORRP20-07861	Negative	-	-	-	Negative	YES
24	ORRP20-07862	Negative	-	-	-	Negative	YES
25	ORRP20-07863	Negative	-	-	-	Negative	YES
26	ORRP20-07869	Negative	-	-	-	Negative	YES
27	ORRP20-07872	Negative	-	-	-	Negative	YES
28	ORRP20-07877	Negative	-	-	-	Negative	YES
29	ORRP20-07879	Negative	-	-	-	Negative	YES
30	ORRP20-07885	Negative	-	-	-	Negative	YES
31	ORRP20-07710	Positive	36.76	37.70	-	Positive	YES
32	ORRP20-07735	Positive	39.72	-	-	Inconclusive*	NO
33	ORRP20-07744	Positive	32.18	31.30	31.01	Positive	YES
34	ORRP20-07747	Positive	-	-	-	Negative	NO
35	ORRP20-07750	Positive	30.95	31.15	30.76	Positive	YES
36	ORRP20-07764	Positive	27.60	27.60	27.08	Positive	YES
37	ORRP20-07768	Positive	18.28	17.81	17.61	Positive	YES
38	ORRP20-07792	Positive	18.69	18.02	17.92	Positive	YES
39	ORRP20-07800	Positive	29.57	29.87	29.52	Positive	YES
40	ORRP20-07794	Positive	16.29	15.47	15.30	Positive	YES
41	ORRP20-07805	Positive	32.53	31.92	29.93	Positive	YES
42	ORRP20-07810	Positive	36.42	36.10	38.27	Positive	YES
43	ORRP20-07812	Positive	28.73	28.77	28.82	Positive	YES
44	ORRP20-07819	Positive	23.89	23.61	23.42	Positive	YES
45	ORRP20-07824	Positive	30.62	30.17	29.25	Positive	YES
46	ORRP20-07759	Positive	30.62	30.11	29.55	Positive	YES
47	ORRP20-07767	Positive	32.59	32.57	32.74	Positive	YES
48	ORRP20-07769	Positive	19.18	18.54	18.48	Positive	YES
49	ORRP20-07779	Positive	30.34	29.16	26.84	Positive	YES
50	PCRRPP20-00202	Negative	-	-	-	Negative	YES
51	PCRRPP20-00203	Positive	27.49	26.91	27.08	Positive	YES

52	ORRP20-07790	Positive	36.32	35.47	36.01	Positive	YES
53	ORRP20-07804	Positive	21.61	20.74	20.68	Positive	YES
54	ORRP20-07813	Positive	34.03	35.88	33.97	Positive	YES
55	ORRP20-07857	Positive	-	36.88	-	Inconclusive*	NO
56	ORRP20-07870	Positive	24.28	22.27	22.38	Positive	YES
57	ORRP20-07888	Positive	19.34	18.22	18.30	Positive	YES
58	ORRP20-07849	Positive	36.72	36.54	-	Positive	YES
59	ORRP20-07889	Positive	29.72	29.55	29.28	Positive	YES
60	ORRP20-07891	Positive	34.55	34.89	33.62	Positive	YES
61	ORRP20-10573	Positive	20.87	19.26	20.52	Positive	YES
62	ORRP20-10585	Positive	32.11	30.20	32.31	Positive	YES
63	ORRP20-10536	Negative	-	-	-	Negative	YES
64	ORRP20-10600	Positive	37.77	-	39.11	Positive	YES
65	ORRP20-10627	Positive	30.13	28.76	29.65	Positive	YES
66	ORRP20-10631	Positive	35.25	37.01	39.49	Positive	YES

*Inconclusives Yielded Negative Results upon repeat Testing.

6.4 Precision

6.4.1 Observed results

Four clinical samples (3 positive one negative) were extracted on three separate days to establish inter-precision. On each day, the extracted nucleic acid was run in triplicate to establish intra-precision.

All targets showed excellent correlation with a CV under 4% for all except one sample (ORRP20-07819) which had a CT difference of 10 between the first and second extraction. This change brought the CV up to 15%.

These results are acceptable for clinical use.

6.4.2 Defined acceptance criteria

100% Correlation should be observed within each precision study.

6.4.3 Discrepancies and remediation

n/a

6.4.4 Deviations from plan

n/a

6.4.5 Supportive documentation

Table 13: Precision Study

Sample ID	Target	7/21/2020			7/22/2020			7/23/2020		
		Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
<i>ORRP20-07819</i>	MS 2	31.85	31.38	31.70	29.28	29.39	29.09	27.66	28.24	28.43
	N Gene	23.98	23.95	24.06	33.42	32.72	32.86	32.68	33.20	33.34
	ORF1ab	23.65	23.79	23.83	32.95	33.52	32.96	31.29	32.25	32.00
	S Gene	24.01	24.15	24.14	33.29	32.88	33.67	33.16	33.18	33.52
<i>ORRP20-07767</i>	MS 2	29.60	29.55	29.86	28.51	28.66	29.21	27.30	27.14	26.96
	N Gene	33.37	32.97	33.19	33.20	34.27	33.43	32.98	32.97	34.02
	ORF1ab	32.94	34.71	33.11	33.09	32.93	33.89	31.88	32.99	31.18
	S Gene	34.12	33.49	34.92	34.18	33.37	34.15	33.33	33.37	32.32
<i>PCRRPP20-00203</i>	MS 2	30.85	31.22	31.25	29.00	29.30	29.15	28.45	28.19	28.47
	N Gene	27.36	27.42	27.56	28.56	28.50	28.43	28.16	27.87	27.97
	ORF1ab	26.77	27.15	27.20	28.31	28.37	28.21	26.54	25.72	26.27
	S Gene	27.41	27.90	27.68	28.64	28.67	28.67	27.93	27.62	27.73
<i>ORRP20-07872</i>	MS 2	30.98	31.03	30.93	28.20	27.92	28.04	27.50	27.69	27.52
	N Gene	-	-	-	-	-	-	-	-	-
	ORF1ab	-	-	-	-	-	-	-	-	-
	S Gene	-	-	-	-	-	-	-	-	-

Table 14: Precision Study Statistics

Sample ID	Target	Statistics		
		SD	Average	%CV
<i>ORRP20-07819</i>	MS 2	1.49	29.67	0.05
	N Gene	4.27	30.02	0.14
	ORF1ab	4.16	29.58	0.14
	S Gene	4.33	30.22	0.14
<i>ORRP20-07767</i>	MS 2	1.07	28.53	0.04
	N Gene	0.44	33.38	0.01
	ORF1ab	0.96	32.97	0.03
	S Gene	0.70	33.70	0.02

<i>PCRRPP20-00203</i>	MS 2	1.16	29.54	0.04
	N Gene	0.44	27.98	0.02
	ORF1ab	0.90	27.17	0.03
	S Gene	0.47	28.03	0.02
<i>ORRP20-07872</i>	MS 2	1.51	28.87	0.05
	N Gene	-	-	-
	ORF1ab	-	-	-
	S Gene	-	-	-

6.5 Analytical Sensitivity

6.5.1 Observed results

Using Zepetmetrix NATSARS (COV2)-ST and UTM with a preliminary dilution was made at 1000, 500, 200, 100, and 50 copies/ml. These were extracted and run in triplicate to establish a preliminary LOD (See Table 15).

Initially 200 copies/ml was set as preliminary, and run at 20 replicates. These failed to provide the 95% detection rate required to set the LOD. Therefore the next level up of 500 copies/ml was tested over 20 replicates with a 100% detection rate (See Table 16).

6.5.2 Defined acceptance criteria

Lowest level with 95% detection at 20 replicates will be set as LOD

6.5.3 Discrepancies and remediation

N/A

6.5.4 Deviations from plan

N/A

6.5.5 Supportive documentation

Table 15: Preliminary LOD Study Data

Sample	Target	Rep 1	Rep 2	Rep 3	SD	Average	%CV
1000 Copies/ml	MS 2	27.46	27.40	27.58	0.07	27.48	0.3%
	N Gene	32.11	32.30	32.36	0.11	32.26	0.3%
	ORF1ab	31.96	32.38	31.92	0.21	32.09	0.6%
	S Gene	34.06	35.48	38.38	1.80	35.97	5.0%
500 Copies/ml	MS 2	28.74	27.40	28.43	0.57	28.19	2.0%
	N Gene	33.94	35.05	33.96	0.52	34.32	1.5%
	ORF1ab	35.33	33.79	33.90	0.70	34.34	2.0%
	S Gene	36.19	38.96	34.29	1.92	36.48	5.3%
200 Copies/ml	MS 2	27.28	26.92	27.12	0.15	27.11	0.5%
	N Gene	36.12	36.89	34.79	0.87	35.93	2.4%
	ORF1ab	35.70	37.21	-	0.75	36.45	2.1%
	S Gene	-	-	-	-	-	-
100 Copies/ml	MS 2	25.88	27.26	26.80	0.57	26.65	2.2%
	N Gene	-	35.46	-	0.00	35.46	0.0%
	ORF1ab	-	-	-	-	-	-
	S Gene	-	-	-	-	-	-
50 Copies/ml	MS 2	24.86694	25.16828	25.2117	0.15	25.08	0.6%
	N Gene	35.6684	-	-	0.00	35.67	0.0%
	ORF1ab	-	37.42629	36.06173	0.68	36.74	1.9%
	S Gene	-	-	-	-	-	-

Table 16: LOD Confirmation

	MS 2	N Gene	ORF1ab	S Gene
	25.43	35.10	32.55	34.45
	25.28	36.56	33.91	34.85
	25.42	35.29	34.74	35.28
	25.38	35.47	32.42	34.21
	25.43	34.49	33.62	33.43
	25.65	34.96	33.55	36.06
	25.48	34.89	33.29	33.95
	25.58	34.34	32.74	35.22
	25.70	38.01	35.22	34.56
	25.94	34.78	33.98	34.18
	25.54	34.98	33.96	34.01
	25.50	35.06	34.43	34.24
	25.45	37.27	34.23	33.14
	25.30	35.16	33.67	34.84
	25.34	35.80	34.17	32.88
	25.92	35.06	32.96	34.89
	25.89	35.75	33.20	33.50
	25.81	34.55	33.08	33.06
	25.82	34.16	33.25	34.71
	25.88	36.20	34.55	33.88
Average	25.59	35.39	33.68	34.27
SD	0.21	0.95	0.73	0.80
%CV	1%	3%	2%	2%

6.6 Analytical Specificity/Selectivity

6.6.1 Observed results

Thermo Fisher was contacted to provide a Right to Reference-Letter of Authorization for the Specificity studies of the assay. Please see attached Package Insert and reference letter in related Documents section.

6.6.2 Defined acceptance criteria

In the case of cross-reactivity or inhibition occurrence, further investigation will be performed, and if necessary, it will be detailed in assay limitations.

6.7 Reportable Range

Based on the number of targets detected (N-Gene, S-Gene, and ORF1ab) the results range from Negative (no genes detected), inconclusive (one of 3) or Positive (2 or 3 of the genes detected).

6.8 Reference Intervals

Expected Result is Not Detected “Negative” for SARS-CoV-2

6.9 Additional Performance Characteristics

6.9.1 Observed results

Room Temp Stability

Three clinical samples (2 positive and one negative) were stored at room temperatures and tested after 24 hours. Results were concordant, with an average ΔCt of 1.35 indicating robustness for up to 24 hours stored at room temperature (See Table 17).

Refrigerated Stability

Three clinical samples (2 positive and one negative) were stored at refrigerated temperatures and tested at 8 days. Results were concordant, with an average ΔCt of 2.67 indicating robustness for up to 8 days at refrigerated temperatures (See Table 17).

Frozen Stability

All samples from accuracy study were frozen for 7 Days before testing in house. In addition, precision study samples were stored for an additional 4 days with consisting of at least 3 freeze-thaw cycles. From these observations, we can infer that samples are stable for at least 10 days

at frozen temperatures, with a robustness that can handle up to 3 freeze thaws (See Precision study).

Excursion Study

To adequately simulate the transit of specimens from collectors to the laboratory. A set of samples were shipped out of the lab and back again. Samples were left at room temp for 6 hours before being shipped via UPS to arrive back at the lab the following day 24 hours later.

Samples consisted of 3 Negative Clinical Samples, 10 low positives used during the sensitivity study set to the assay LOD (500 copies/ml), 3 positive clinical specimens run in triplicate, and 2 positive specimens set to 1000 copies/ml from the sensitivity study (See Table 18).

All specimens

6.9.2 Defined acceptance criteria

Conditions that show consistent results throughout storage conditions will be considered acceptable for this sample type.

6.9.3 Discrepancies and remediation

N/A

6.9.4 Deviations from plan

N/A

6.9.5 Supportive documentation

Table 17: Stability Study

Sample	Target	Initial	24 Hrs RT	ΔCt
Room Temp 01	N Gene	19.1291	19.92658	0.80
	ORF1ab	19.48603	19.18227	0.30
	S Gene	20.92133	20.45201	0.47
Room Temp 02	N Gene	21.73944	21.25285	0.49
	ORF1ab	22.77728	20.15585	2.62
	S Gene	24.55581	21.1401	3.42
Room Temp 03	N Gene	-	-	-
	ORF1ab	-	-	-

		S Gene	-	-	-
					Average: 1.35
Sample	Target	Initial	8 Days RF	ΔCt	
Refrigerated 01	N Gene	25.72989	30.17462	4.44	
	ORF1ab	26.67359	26.29605	0.38	
	S Gene	28.69693	26.75708	1.94	
Refrigerated 02	N Gene	24.70601	29.06635	4.36	
	ORF1ab	25.10206	25.45631	0.35	
	S Gene	30.44315	25.92073	4.52	
Refrigerated 03	N Gene	-	-	-	
	ORF1ab	-	-	-	
	S Gene	-	-	-	
					Average: 2.67

Table 18: Excursion Study Data

Sample	Target	Day 0	After Shipping	ΔCt
Clinical Sample 1	N Gene	Undetermined	Undetermined	-
	ORF1ab	Undetermined	Undetermined	-
	S Gene	Undetermined	Undetermined	-
Clinical Sample 2	N Gene	Undetermined	Undetermined	-
	ORF1ab	Undetermined	Undetermined	-
	S Gene	Undetermined	Undetermined	-
Clinical Sample 3	N Gene	Undetermined	Undetermined	-
	ORF1ab	Undetermined	Undetermined	-
	S Gene	Undetermined	Undetermined	-
Low Pos 1	N Gene	33.27	36.03	2.75
	ORF1ab	32.59	35.90	3.31
	S Gene	34.00	34.43	0.44
Low Pos 2	N Gene	33.75	35.63	1.88
	ORF1ab	33.34	33.76	0.41
	S Gene	36.52	35.67	0.85
Low Pos 3	N Gene	33.94	35.24	1.30
	ORF1ab	35.33	34.87	0.46
	S Gene	36.19	37.64	1.45
Low Pos 4	N Gene	33.74	35.55	1.80

	ORF1ab	33.50	33.92	0.42
	S Gene	34.34	35.25	0.91
Low Pos 5	N Gene	33.36	37.60	4.25
	ORF1ab	34.19	35.40	1.21
	S Gene	34.89	36.06	1.17
Low Pos 6	N Gene	35.68	34.69	0.99
	ORF1ab	33.61	35.32	1.71
	S Gene	34.92	35.66	0.74
Low Pos 7	N Gene	33.96	35.96	1.99
	ORF1ab	33.90	36.40	2.49
	S Gene	34.29	38.57	4.29
Low Pos 8	N Gene	34.37	36.73	2.35
	ORF1ab	34.31	35.02	0.71
	S Gene	36.08	36.38	0.30
Low Pos 9	N Gene	33.71	35.01	1.31
	ORF1ab	32.40	33.54	1.14
	S Gene	35.24	36.33	1.08
Low Pos 10	N Gene	33.42	35.98	2.57
	ORF1ab	33.02	34.45	1.43
	S Gene	34.45	35.41	0.96
Stab 1a	N Gene	18.28	18.97	0.69
	ORF1ab	17.81	18.08	0.27
	S Gene	17.61	18.23	0.62
Stab 2a	N Gene	18.69	19.77	1.08
	ORF1ab	18.02	18.88	0.86
	S Gene	17.92	19.14	1.23
Stab 3a	N Gene	19.18	21.42	2.24
	ORF1ab	18.54	20.17	1.64
	S Gene	18.48	20.33	1.85
Stab 1b	N Gene	18.28	19.34	1.06
	ORF1ab	17.81	18.39	0.58
	S Gene	17.61	18.69	1.08
Stab 2b	N Gene	18.69	19.83	1.13
	ORF1ab	18.02	18.94	0.92
	S Gene	17.92	18.95	1.04
Stab 3b	N Gene	19.18	21.45	2.27
	ORF1ab	18.54	20.01	1.47
	S Gene	18.48	20.24	1.76

Stab 1c	N Gene	18.28	19.35	1.07
	ORF1ab	17.81	18.47	0.66
	S Gene	17.61	18.65	1.04
Stab 2c	N Gene	18.69	19.94	1.24
	ORF1ab	18.02	19.00	0.98
	S Gene	17.92	19.19	1.28
Stab 3c	N Gene	19.18	21.48	2.30
	ORF1ab	18.54	20.09	1.55
	S Gene	18.48	20.24	1.76
High Pos1	N Gene	32.88	35.71	2.84
	ORF1ab	32.26	32.97	0.71
	S Gene	37.73	34.27	3.46
High Pos2	N Gene	31.76	33.37	1.61
	ORF1ab	32.00	34.87	2.87
	S Gene	35.97	36.03	0.07
			Average	1.46

7.0 INTERFACES

No Automatic Interface

7.1 LIMS Validation

The LIMS was validated using 20 – digital only, test patients. Ct values across the range established during the validation study were used to confirm positives were interpreted appropriately. All samples yielded appropriate results.

7.2 Workbook Validation

During validation Workbook IGX-W001 was built to handle lot tracking, sample locations, and sample ID management between systems. See IGX-V003 for full validation details.

8.0 CRITICAL/ALERT VALUES

State laws will be followed regarding positivity rate reporting.

9.0 CONTINGENCY PLAN

In the event of assay irregularity, materials shortage, or other contingencies, an external reference laboratory using an EUA approved SARS-CoV2 test system will be utilized.

10.0 ATTACHMENTS

- Swab Package Insert
- Thermo Fisher - Right to Reference-Letter of Authorization