VALIDATION	GINKGO BIOWORKS THE ORGANISM COMPANY	Doc #	VALSUMMARY-GBCL-0001
		Revision	1.8
	Concentric by Ginkgo SARS-CoV-2 NGS Test Performance Summary	Effective	01/18/2021
		Author	Alex Plocik

Concentric by Ginkgo SARS-CoV-2 NGS Test Performance Summary

1. TEST SUMMARY

The Concentric by Ginkgo SARS-CoV-2 NGS test is an end-point multiplex reverse transcription polymerase chain reaction (RT-PCR) assay with internal controls that are quantified by next-generation sequencing (NGS) for the qualitative detection of nucleic acid from SARS-CoV-2 in respiratory specimens. Testing is limited to Ginkgo Bioworks, Boston, MA, or other laboratories designated by Ginkgo Bioworks that are also certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

The assay is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of RT-PCR and in vitro diagnostic procedures.

2. ASSAY PROTOCOL OVERVIEW

The Concentric by Ginkgo SARS-CoV-2 NGS assay is to be used with an RNA extraction procedure using the Thermo Scientific KingFisher Flex Purification System with Applied Biosystems MagMAX Viral/Pathogen Nucleic Acid Isolation Kit. RT-PCR is performed using an Eppendorf Mastercycler x50t for thermocycling. Sequencing libraries are pooled and are then purified using AMpure XP reagent. Quantification is performed using a Quant-iT dsDNA Assay Kit,

Broad Range using Neo 2 Synergy Plate Reader and by a KAPA Library Quantification Kit using a Roche Lightcycler 480 II. Visualization of the library pool fragment size is performed using a DNA 7500 using an Agilent Bioanalyzer. The purified library pools are sequenced using an Illumina NextSeq 500 (software version 2.2.0) or NovaSeq 6000 (software version 1.6.0). Specific library sequencer loading protocols are performed prior to use with each Illumina instrument.

3. REPORTABLE RANGE

The Concentric by Ginkgo SARS-CoV-2 NGS assay is a qualitative test and therefore a reportable range does not apply.

4. REFERENCE RANGE

The reference range will be “SARS-CoV-2 not detected” and will not be validated by a clinical study.

5. PERFORMANCE CHARACTERISTICS

5.1. Accuracy

To assess the test accuracy, we ran our test on 64 and 15, respectively, residual de-identified human nasopharyngeal and human nasal swab specimens, previously tested and provided by ARUP Laboratories. Of this set of 79 specimens, 37 were positive and 42 were negative based on results from the Hologic PCR, Hologic TMA, or ThermoFisher comparator assays (**Table 1**). The reference samples spanned a broad range of Ct values (**Figure 1**) that were collected in a variety of storage buffers, including PBS and VTM (**Table 1**).

Table 1. Reference sample details

	Collection buffer				
Comparator assay	**Other**	**PBS**	**Saline/PBS**	**VTM**	**Grand Total**
Hologic PCR	1	61	1	3	66
Hologic TMA	0	3	0	0	3
ThermoFisher	0	0	10	0	10
Total	1	64	11	3	79

To further demonstrate the test accuracy over an even broader range of viral concentrations, we diluted 14 of the positive clinical samples, by 10- or 100-fold, as was deemed appropriate (**Table 2**). Dilutions were confirmed by an internal assessment by RT-qPCR using the CDC-designed probe for N1 (IDT). **Figure 1** illustrates the correlation between the RT-qPCR Ct values and the NGS s-ratio.

Table 2. Reference sample calls

	Reference call		
Dilution	**Negative**	**Positive**	**Grand Total**
10-fold	0	7	7
100-fold	0	7	7
Undiluted	42	37	79
Total	42	51	93

In total, 49 of 51, positive samples were confirmed positive for a percent positive agreement of 96.1%; 95% CI [86.5%-99.5%]. Additionally, 42 out of 42 negative samples were confirmed negative for a percent negative agreement of 100.0%; 95% CI [91.6%-100%] as shown in **Table 3**.

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Acceptance criteria: > 95% positive agreement and > 95% negative agreement. PASS

Figure 1. Correlation between RT-qPCR Ct value and NGS s-ratio

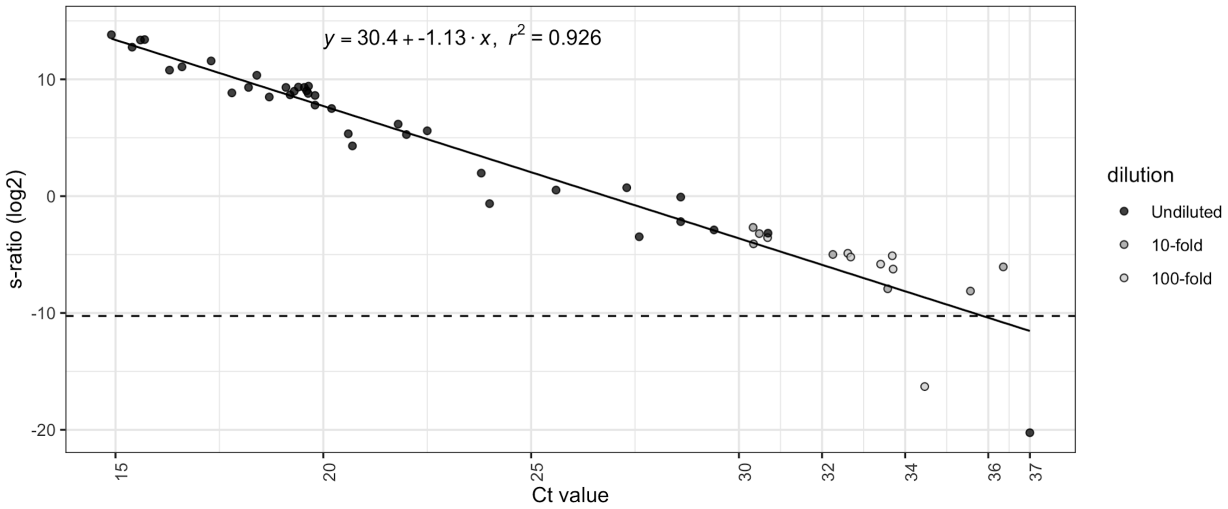

Table 3. Accuracy

		Comparator Assay: Hologic PCR or Hologic TMA or ThermoFisher TaqPath assay		
Nasopharyngeal or Nasal Swabs		**Positive**	**Negative**	**Total**
Concentric by Ginkgo SARS-CoV-2 NGS assay	Positive	49	0	49
	Negative	2*	42	44
	Total	51	42	93
Positive Percent Agreement		49 / 51 = 96.1%; 95% CI [86.5%-99.5%]		
Negative Percent Agreement		42 / 42 = 100.0%; 95% CI [91.6%-100%]		

* Includes a sample with a Ct value of 37 from the comparator assay.

5.2. Precision

5.2.1. Repeatability

To assess the repeatability (within-run precision) of upper respiratory specimens, we performed three runs, each with two replicate measurements of clinical validation samples (described in Accuracy). We then assessed the agreement between the two replicate test results within each run and determined the percent agreement among the within-run replicates. Missing data were not included in the calculation.

In total, results for 164 of 166 patient samples (98.8%; 95% CI [95.7%-99.8%]) were in complete agreement. See **Attachment 1** for detailed run data.

Acceptance criteria: > 95% percent agreement among with-in run replicates. PASS

5.2.2. Reproducibility

To assess the reproducibility (between-run precision) of upper respiratory specimens, we performed two runs, each with two replicate measurements of the clinical validation samples (described in Accuracy) on separate days performed by two different operators. We then assessed the agreement among the four replicate test results and determined the percent agreement. Missing data were not included in the calculation.

In total, results for 71 of 73 patient samples (97.3%; 95% CI [90.4%-99.7%]) were in complete agreement. See **Attachment 1** for detailed run data.

Acceptance criteria: > 95% percent agreement among between-in run replicates. PASS

5.3. Analytical Sensitivity

5.3.1. Limit of Detection

The Limit of Detection (LoD) is defined as the lowest SARS-CoV-2 RNA concentration (measured in genome copy equivalents / mL or GCEs / mL) that is successfully detected with a probability of 95% or greater. Sensitivity standards were prepared by generating contrived samples by spiking

quantified heat-inactivated SARS-CoV-2 virus (ATCC VR-1986HK; lot 70035039; 3.75E5 GCEs / µL) into a negative clinical matrix prior to RNA extraction. Based on development testing, a preliminary LoD between 350 and 1000 GCEs / mL had been established with a small number of replicates. To further refine this range, we performed a 2-fold dilution series ranging in concentration from 125 to 2000 GCEs / mL with 20 replicates performed for each concentration tested. This experiment was performed using a negative clinical matrix of PBS or VTM.

The LoD study results are shown in the **Table 4** (VTM negative clinical matrix) and **Table 5** (PBS negative clinical matrix) below. The lowest concentration of SARS-CoV-2 RNA that was successfully detected with at least a 95% detection rate was calculated as 500 GCEs / mL in VTM and PBS negative clinical matrix. Therefore the LoD of the test is established at 500 copies / mL.

Table 4. LoD results (VTM negative clinical matrix spiked with virus)

Virus GCEs / mL	Virus GCEs / rxn	Invalid*	Inconclusive	Negative	Positive	Positive / total valid result	Percent positive
0	0	0	0	20	0	0/20	0%
125	2.5	0	0	11	9	9/20	45%
250	5	0	0	4	16	16/20	80%
500	10	0	0	1	19	19/20	95%
1000	20	0	0	1	19	19/20	95%
2000	40	0	0	0	20	20/20	100%

*INVALID results represent quality control failures and are not included in the percent positive calculation.

GCEs / rxn values are back-calculated based on RNA extraction volumes; these values do not correct for inefficiencies in RNA extraction.

Table 5. LoD results (PBS negative clinical matrix spiked with virus)

Virus GCEs / mL	Virus GCEs / rxn	Invalid*	Inconclusive	Negative	Positive	Positive / total valid result	Percent positive
0	0	0	0	20	0	0/20	0%
125	2.5	1	0	10	9	9/19	47%
250	5	0	0	12	8	8/20	40%
500	10	0	0	1	19	19/20	95%
1000	20	0	0	0	20	20/20	100%
2000	40	0	0	0	20	20/20	100%
*INVALID results represent quality control failures and are not included in the percent positive calculation.							
GCEs / rxn values are back-calculated based on RNA extraction volumes; these values do not correct for inefficiencies in RNA extraction.							

5.3.2. Inclusivity

The Concentric by Ginkgo SARS-CoV-2 NGS assay targets the spike (S) gene region of the SARS-CoV-2 genome. Inclusivity was measured *in silico* by mapping the S gene primers to the 354,002 SARS-CoV-2 genomes from human hosts in GSIAD as of 01/12/2021 to identify strains with low identity (< 100%) to the S gene primers. SARS-CoV-2 genomes available in the GISAID database (https://www.epicov.org/) as of 01/12/2021 were used to conduct an inclusivity analysis. Filtering was carried out to exclude (a) incomplete sequences and (b) low-quality sequences and/or sequences with ambiguous characters:

- As many partial genomes were present, this analysis excluded any sequences that did not have a full-length S gene (i.e., the S gene from the Wuhan-Hu-1 NCBI Reference Sequence [NC_045512.2] was BLASTed against the sequences, and any strains that did not have ≥ 95% query coverage were excluded).
- As many sequences contained stretches of Ns and/or other ambiguous characters, strains with any non-ATGCU characters in the S gene were excluded. This resulted in a high quality dataset of 269,643 sequences.

- 96.22% (259,459/269,643) of the high quality dataset sequences had 100% identity to both S primer sequences. Of the 10,184 sequences that did not have 100% identity to both primer sequences, 10,115 had a single mismatch in either the forward or reverse primer sequences, but not both; <0.03% of the 269,643 high-quality S sequences had >1 mismatch in the primer-binding regions. One individual primer mismatch variant (with one single mutation in the reverse primer-binding region) was present in 5,395 sequences (2% prevalence), and one individual primer mismatch variant (with one single mutation in the forward primer-binding region) was present in 3,525 sequences (1.36% prevalence); all other individual primer mismatch variants were present at < 0.15% prevalence.
- Among the sequences from the high quality, full S dataset, there were no mutations at any of the sites used to differentiate virus from spike-in sequences; thus, all strains in this dataset would be correctly classified as virus by the bioinformatics pipeline.

Of 269,643 genomes with “high-quality” S sequences as of 01/12/2021, 255,098 (94.61%) had an exact match for the full S amplicon, 265,215 (98.36%) had an exact match for the forward primer sequence and 263,842 (97.85%) had an exact match for the reverse primer sequence. Most variants (99.32% of sequences that did not have 100% identity to both primer sequences) had single mismatches in the forward or reverse primer and thus may still be detected by this assay, depending on the type and position of the individual mutations. With the exception of the two single-mutation variants described above (present at 2% and 1.36% prevalence, respectively), all individual variants were present at <0.15% prevalence. Thus, variants that might affect primer binding are rare and may include sequencing artifacts. The risk assessment of these rare variants resulting in a significant loss in reactivity, and false negative result, is low.

5.4. Cross-reactivity (Analytical Specificity)

An *in silico* analysis of the assay primer and probes has already been performed. Primers were compared to common respiratory flora and other viral pathogens was performed to test for cross-reactivity (greater than 80% identity) between the S2 gene primers and any sequence present in the microorganisms listed below.

The pathogens listed in **Attachment 2** were searched *in silico* with the S primer sequences. The only pathogen showing any significant similarity is *Candida albicans*, with 92% identity in the forward primer but mismatches in the 3’ end and no corresponding similarity for the reverse

primer. We therefore conclude that no amplification is expected of *Candida albicans* with the primers used in the assay.

As a second check of specificity, any match found by BLASTN of the forward S2 primer to any of the above genome sequences was evaluated to determine what sequence of amplified material would be generated. For 1962 possible sequences generated, none had fewer than 5 mismatches to either the viral sequence or spike-in sequence generated in the assay, and hence all would be rejected by the current bioinformatics pipeline.

The risk assessment of other pathogens resulting in cross-reactivity, and false positive result, is low.

5.5. Swab pooling

Swab pooling is an approach that minimizes loss of sensitivity of the test during pooling by adding multiple swabs into a small amount of media (similar to that of an individual test). For example, we performed a clinical validation with individual swabs that were collected in 3 mL of media; therefore, swab pooling into 4 to 6 mL of media results in a minimal decrease in sensitivity due to dilution (1.33- to 2-fold, respectively). However, this approach substantially increases the concentration of swab specimens, which could possibly compromise the analytical sensitivity of the test. Thus, to ensure the sensitivity of the test, studies should be performed to demonstrate that the test's limit-of-detection is robust to concentrated amounts of clinical matrix (e.g., mucins or human RNA) and/or analyte (i.e., virus) [1].

5.5.1. Swab pools collected in 4 mL PBS (wet swabs)

To determine the maximum number of swabs that could be collected without compromising the performance of the test, we collected 5-, 10-, and 20- swabs in 4 mL of PBS. In this study, AN swabs (Miraclean 96000E) were collected in a total of 4 mL PBS (Sigma 59321C); from each, 12 RT-PCR replicates were tested. Heat-inactivated virus (ATCC VR-1986HK; lot 70036071; 1.92E5 GCEs / µL stock) was spiked into a each n-swab pooled negative clinical matrix at a final concentration of 1,500 GCEs / mL virus per pool, which is 3x the previously validated limit of detection. For all n-swab pools tested, 100% of pools tested positive (**Table 6**). Negative controls

with clinical matrix from 10 AN swabs and no virus was also performed, which tested negative as expected (**Attachment 1**).

Table 6. Limit-of-detection robustness to swab number

Virus GCEs / mL	Swab number	Invalid*	Inconclusive	Positive	Negative	Positive / total valid test result	Percent positive
	5	0	0	12	0	12/12	100%
	10	0	0	12	0	12/12	100%
1,500	20	0	0	12	0	12/12	100%
		0	0	36	0	36/36	100%

*INVALID results represent quality control failures and are not included in the percent positive calculation.

To confirm that the analytical sensitivity of pooled samples would not be compromised by high amounts of clinical matrix in pools with up to 20 swabs, we performed a bridging study. In this study, 20 AN swabs (Miraclean 96000E) were collected in a total of 4 mL PBS (Teknova P5275); Multiple replicates of 20-swab pools were collected; from each replicate pool, 20 RT-PCR replicates were tested. Heat-inactivated virus (ATCC VR-1986HK; lot 70036071; 1.92E5 GCEs / µL stock) was spiked into a 20-swab pooled negative clinical matrix at a final concentration of 1,500 GCEs / mL virus per pool, which is 3x the previously validated limit of detection. In each pool of 20 AN swabs; 1 of the 20 swabs was spiked with heat-inactivated virus to simulate a single positive swab among 19 negative swabs; results are shown in **Table 7**. As a positive control, a similar experiment was performed using the same concentration of virus, but with swabs lacking a clinical matrix; results are shown in **Table 8**.

Acceptance criteria: The percent positive at 3x the limit-of-detection should be > 95%. PASS

Table 7. 20-swab pool results with clinical matrix

Virus GCEs / mL	20-swab pool replicates	Invalid*	Inconclusive	Positive	Negative	Positive / total valid test result	Percent positive
	1	1	0	19	0	19/19	100%
	2	2	0	18	0	18/18	100%
1,500	3	0	0	20	0	20/20	100%
		3	0	57	0	57/57	100%

*INVALID results represent quality control failures and are not included in the percent positive calculation.

Table 8. 20-swab pool results without clinical matrix (positive control)

Virus GCEs / mL	20-swab pool replicates	Invalid*	Inconclusive	Positive	Negative	Positive / total valid test result	Percent positive
	1	0	0	16	0	16/16	100%
	2	0	0	16	0	16/16	100%
1,500	3	0	0	16	0	16/16	100%
		0	0	48	0	48/48	100%

*INVALID results represent quality control failures and are not included in the percent positive calculation.

To ensure that the analytical sensitivity of pooled samples would not be compromised by high levels of virus (e.g., multiple positive swabs per pool), we tested 20-swab pools containing high concentrations of heat-inactivated virus. In this study, 20 swabs were collected in a total of 4 mL PBS (Teknova P5275); 2 replicate 20-swab pools were collected and from each replicate pool, 20 RT-PCR replicates were tested. Heat-inactivated virus (ATCC VR-1986HK; lot 70036071; 1.92E5 GCEs / µL stock) was spiked into a 20-swab pooled negative clinical matrix at a final concentration of 75,000 GCEs / mL virus per pool, which is 150x the previously validated limit of

detection. In each pool of 20 AN swabs; 1 of the 20 swabs was spiked with heat-inactivated virus to simulate a single positive swab among 19 negative swabs; results are shown in **Table 9**.

Acceptance criteria: The percent positive at 150x the limit-of-detection should be > 95%. PASS

Table 9. 20-swab pool results with high positive swabs

Virus GCEs / mL	20-swab pool replicates	Invalid*	Inconclusive	Positive	Negative	Positive / total valid test result	Percent positive
	1	0	0	20	0	20/20	100%
75,000	2	0	0	20	0	20/20	100%
		0	0	40	0	40/40	100%

*INVALID results represent quality control failures and are not included in the percent positive calculation.

5.5.2. Dry swab pools reconstituted in 6 mL PBS (dry swabs)

To confirm that the analytical sensitivity of pooled samples would not be compromised by high amounts of clinical matrix in a 35-swab pool, we performed a bridging study as above. In this study, 35 AN swabs (Miraclean 96000E) were collected dry, stored overnight and subsequently reconstituted in a total of 6 mL PBS (Teknova P5275); Multiple replicates of 35-swab pools were collected; from each replicate pool, 20 RT-PCR replicates were tested. Heat-inactivated virus (ATCC VR-1986HK; lot 70036071; 1.92E5 GCEs / µL stock) was spiked into a 35-swab pooled negative clinical matrix at a final concentration of 1,500 GCEs / mL virus per pool, which is 3x the previously validated limit of detection. In each pool of 35 AN swabs; 1 of the 35 swabs was spiked with heat-inactivated virus to simulate a single positive swab among 34 negative swabs; results are shown in **Table 10**. As a positive control, a similar experiment was performed using the same concentration of virus, but with swabs lacking a clinical matrix; results are shown in **Table 11**.

Acceptance criteria: The percent positive at 3x the limit-of-detection should be > 95%. PASS

Table 10. 35-dry swab pool results with low positive swabs in negative clinical matrix (3x LoD)

Virus GCEs / mL	35-swab pool replicate	Invalid*	Inconclusive	Positive	Negative	Positive / total valid test result	Percent positive
	1	0	0	20	0	20/20	100%
	2	1	0	19	0	19/19	100%
1,500	3	0	0	40	0	40/40	100%
		1	0	79	0	79/79	100%
*INVALID results represent quality control failures and are not included in the percent positive calculation.							

Table 11. 35-dry swab pool results without clinical matrix (positive control)

Virus GCEs / mL	35-swab pool replicate	Invalid*	Inconclusive	Positive	Negative	Positive / total valid test result	Percent positive
	1	2	0	18	0	18/18	100%
	2	1	0	19	0	19/19	100%
	3	1	0	19	0	19/19	100%
	4	0	0	20	0	20/20	100%
	5	1	0	19	0	19/19	100%
	6	0	0	20	0	20/20	100%
	7	1	0	19	0	19/19	100%
1,500	8	0	0	20	0	20/20	100%
		6	0	154	0	154/154	100%
*INVALID results represent quality control failures and are not included in the percent positive calculation.							

To ensure that the analytical sensitivity of pooled samples would not be compromised by high levels of virus (e.g., multiple positive swabs per pool), we tested 35-swab pools containing high concentrations of virus. In one study, 35 swabs were collected dry, stored overnight and

reconstituted in a total of 6 mL PBS (Teknova P5275); 2 replicate 35-swab pools were collected and from each replicate pool, 20 RT-PCR replicates were tested. Heat-inactivated virus (ATCC VR-1986HK; lot 70036071; 1.92E5 GCEs / µL stock) was spiked into a 35-swab pooled negative clinical matrix at a final concentration of 75,000 GCEs / mL virus per pool, which is 150x LoD. In each pool of 35 AN swabs; 1 of the 35 swabs was spiked with heat-inactivated virus to simulate a single positive swab among 34 negative swabs; results are shown in **Table 12**.

Table 12. 35-swab pool results with high positive swabs in negative clinical matrix (150x LoD)

Virus GCEs / mL	35-swab pool replicate	Invalid*	Inconclusive	Positive	Negative	Positive / total valid test result	Percent positive
75,000	1	0	0	20	0	20/20	100%
	2	0	0	20	0	20/20	100%
		0	0	40	0	40/40	100%

In a second experiment using high positive swabs, we followed the same procedure as above, except that we further increased the amount of virus by using a more concentrated stock of virus. Gamma-irradiated virus (BEI NR-52287; lot 70035888; 1.75E9 GCEs / µL stock) was spiked into a 35-swab pooled negative clinical matrix at a final concentration of 1,000,000 GCEs / mL virus per pool, which is 2,000x LoD. Results are shown in **Table 13**.

Table 13. 35-swab pool results with high positive swabs in negative clinical matrix (2,000x LoD)

Virus GCEs / mL	35-swab pool replicate	Invalid*	Inconclusive	Positive	Negative	Positive / total valid test result	Percent positive
1,000,000	1	0	0	20	0	20/20	100%
*INVALID results represent quality control failures and are not included in the percent positive calculation.							

Acceptance criteria: The percent positive high virus swabs detected should be > 95%. PASS

6. QUALITY CONTROL AND VALIDATION EXCLUSIONS

The controls used in the clinical validation study are listed in **Table 14** and described below. Controls were evaluated using read count or read count ratio thresholds for detection.

RNA Extraction Negative Control: For each 96-well RNA extraction plate, one well containing a human specimen control of 4.5E5 HEK293T cells is included to monitor the RNA extraction reagents and process for contamination.

RNA Extraction Positive Control: For each 96-well RNA extraction plate, one well containing 4.5E5 HEK293T cells spiked with 1.5E4 GCEs / mL synthetic SARS-CoV-2 RNA (Twist #102019) is included to monitor the integrity of the RNA extraction reagents and process.

RT-PCR Positive Control: For each 384-well RT-PCR plate, one well containing 96 GCEs of synthetic SARS-CoV-2 RNA [Twist Bioscience, PN: 102019] will be included to monitor the integrity of the RT-PCR reagents and process.

RT-PCR Negative Control (No Template Control): For each 384-well RT-PCR plate, DNase/RNase-free distilled water [Invitrogen, PN: 10977-023] will be included to monitor the RT-PCR reagents and process for contamination.

S Spike-in Internal Control: For each specimen in a 384-well RT-PCR plate, 200 GCEs of S Synthetic Control RNA Spike is used to monitor the integrity of the RT-PCR reagents and process. This control is also used to normalize the amount of viral RNA detected as a ratio of viral RNA to spike-in.

Table 14. Controls used in the assay

Control Type	Purpose	Frequency of Testing
RNA Extraction Negative Control	To monitor RNA extraction reagents and process for contamination	At least once per 96-well plate batch
RNA Extraction Positive Control [Twist Bioscience, PN:102019]	To monitor the integrity of the RNA extraction reagents and process	At least once per 96-well plate batch

RT-PCR Positive Control [Twist Bioscience, PN:102019]	To monitor the integrity of the RT-PCR reagents and process	At least once per 384-well plate of specimens
RT-PCR Negative Control (No Template Control)	To monitor for cross-contamination during RT-PCR	At least once per 384-well plate of specimens
S Spike-in Internal Control [custom preparation]	To normalize the amount of viral RNA detected as a ratio of viral RNA to spike-in	Added to each specimen and control during RT-PCR

The results from the controls were interpreted according to the criteria shown in **Table 15**.

Table 15. Read counts and count ratios that must be observed to obtain valid results

Control	**S** (S)	**S** (S / S spike-in)[1]	**RPP30** (RPP30 / S + S spike-in)[1]	**Internal control** (S + S spike-in)[1]
RNA Extraction Negative Control	Any	< 0.00082 count ratio	≥ 0.00025 count ratio	≥ 4000 counts
RNA Extraction Positive Control	≥ 10 counts	≥ 0.00082 count ratio	Any	≥ 4000 counts
RT-PCR Positive Control	≥ 10 counts	≥ 0.00082 count ratio	Any	≥ 4000 counts
RT-PCR Negative Control (No Template Control)	Any	< 0.00082 count ratio	Any	≥ 4000 counts

[1]A pseudocount read is added to the numerator and denominator of each ratio calculation.

In addition to RNA extraction and RT-PCR positive and negative sample controls, the following quality controls are in place to ensure sequencing reagent integrity and instrument performance.

Runs that do not meet the criteria listed in **Table 15** will result in all patient samples reported as INVALID or retested if sufficient material remains.

7. ASSAY RESULTS AND INTERPRETATION

Results were interpreted according to the following sample classification scheme illustrated in **Figure 2** and described in **Table 16**.

Figure 2. Decision tree of result classifications for patient samples

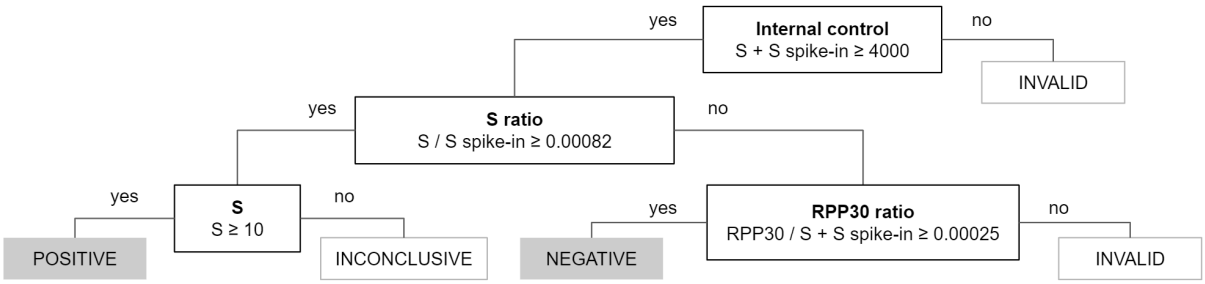

Table 16. Result interpretation for patient samples

Internal control S + S spike-in ≥ 4000 counts	**S ratio** S / S spike-in ≥ 0.00082 ratio	**S** S ≥ 10 counts	**RPP30 ratio** RPP30 / S + S spike-in ≥ 0.00025 ratio	**Report Result**	**Result Interpretation**
+	+	+	±	POSITIVE	SARS-CoV-2 detected
+	-	±	+	NEGATIVE	SARS-CoV-2 not detected
+	+	-	±	INCONCLUSIVE	Inconclusive Result

±	-	±	-	INVALID	Invalid Result
-	±	±	±	INVALID	Invalid Result

[1]A pseudocount read is added to the numerator and denominator of each ratio calculation.

The following quality control measures will be in place to ensure reagent integrity and instrument performance. Runs that do not meet the below criteria will be excluded from the validation or retested if sufficient material remains.

Table 17. Quality control criteria that must be observed to obtain valid results

Control Type	Purpose	Value
% Q30 score	To monitor the integrity of the sequencing reagents and process	≥ 85%
Library pool concentration	To monitor the integrity of the library pooling reagents and process	≥ 4 nM

8. REFERENCES

8.1. FDA. Molecular Diagnostic Template for Laboratories, updated 28 July 2020, https://www.fda.gov/media/135658/download.

9. APPENDIX

Appendix 1. *In silico* S primer cross-reactivity

Pathogen	GenBank Accession #	% Homology Test Forward Primer	% Homology Test Reverse Primer
SARS-coronavirus	NC_004718.3	S: no significant homology	S: no significant homology
MERS-coronavirus	NC_019843.3	S: no significant homology	S: no significant homology
Human coronavirus OC43	MN306053.1	S: no significant homology	S: no significant homology
Human coronavirus NL63	MG428704.1	S: no significant homology	S: no significant homology

Human coronavirus 229E	AF304460.1	S: no significant homology	S: no significant homology
Human coronavirus HKU1	DQ415901.1	S: no significant homology	S: no significant homology
Human adenovirus type 1	AC_000017	S: no significant homology	S: no significant homology
Adenovirus (e.g. C1 Ad. 71)	J01917.1	S: no significant homology	S: no significant homology
Human Metapneumovirus (hMPV)	NC_039199.1	S: no significant homology	S: no significant homology
Parainfluenza virus 1	NC_003461.1	S: no significant homology	S: no significant homology
Parainfluenza virus 2	NC_003443.1	S: no significant homology	S: no significant homology
Parainfluenza virus 3	EU326526.1	S: no significant homology	S: no significant homology
Parainfluenza virus 4	NC_021928	S: no significant homology	S: no significant homology
Influenza A	NC_007373.1 NC_007372.1 NC_007371.1 NC_007366.1 NC_007369.1 NC_007368.1 NC_007367.1 NC_007370.1	S: no significant homology	S: no significant homology
Influenza B	NC_002204.1 NC_002205.1 NC_002206.1 NC_002207.1 NC_002208.1 NC_002209.1 NC_002210.1 NC_002211.1	S: no significant homology	S: no significant homology
Enterovirus D	KX351801.1	S: no significant homology	S: no significant homology
Respiratory syncytial virus	NC_001803.1	S: no significant homology	S: no significant homology
Human Rhinovirus A39	KM362429.1	S: no significant homology	S: no significant homology
Chlamydia pneumoniae	NC_000922.1	S: no significant homology	S: no significant homology
Haemophilus influenzae	NC_000907.1	S: no significant homology	S: no significant homology
Legionella pneumophila	NC_002942.5	S: no significant homology	S: no significant homology
Mycobacterium tuberculosis	NC_000962.3	S: no significant homology	S: no significant homology
Streptococcus pneumoniae	NC_003098.1	S: no significant homology	S: no significant homology

Streptococcus pyogenes	NC_002737.2	S: no significant homology	S: no significant homology
Bordetella pertussis	NC_002929.2	S: no significant homology	S: no significant homology
Mycoplasma pneumoniae	NC_000912.1	S: no significant homology	S: no significant homology
Pneumocystis jirovecii (PJP)	NW_017264775.1 NW_017264784.1 NW_017264785.1 NW_017264786.1 NW_017264787.1 NW_017264788.1 NW_017264789.1 NW_017264790.1 NW_017264791.1 NW_017264792.1 NW_017264793.1 NW_017264776.1 NW_017264794.1 NW_017264795.1 NW_017264796.1 NW_017264797.1 NW_017264798.1 NW_017264799.1 NW_017264800.1 NW_017264801.1 NW_017264802.1 NW_017264803.1 NW_017264777.1 NW_017264804.1 NW_017264805.1 NW_017264806.1 NW_017264807.1 NW_017264808.1 NW_017264809.1 NW_017264810.1 NW_017264811.1 NW_017264812.1 NW_017264813.1 NW_017264778.1 NW_017264814.1 NW_017264815.1 NW_017264816.1 NW_017264817.1 NW_017264818.1 NW_017264819.1 NW_017264820.1 NW_017264821.1 NW_017264822.1 NW_017264823.1 NW_017264779.1 NW_017264824.1 NW_017264825.1 NW_017264826.1 NW_017264827.1 NW_017264828.1 NW_017264829.1 NW_017264830.1 NW_017264831.1	S: no significant homology	S: no significant homology

	NW_017264832.1 NW_017264833.1 NW_017264780.1 NW_017264834.1 NW_017264835.1 NW_017264836.1 NW_017264837.1 NW_017264838.1 NW_017264839.1 NW_017264840.1 NW_017264841.1 NW_017264842.1 NW_017264843.1 NW_017264781.1 NW_017264844.1 NW_017264782.1 NW_017264783.1		
Candida albicans	NC_032089.1 NC_032090.1 NC_032091.1 NC_032092.1 NC_032093.1 NC_032094.1 NC_032095.1 NC_032096.1	S: 92% identity but mismatched for 3'-most nucleotide	S: no significant homology
Pseudomonas aeruginosa	NC_002516.2	S: no significant homology	S: no significant homology
Staphylococcus epidermidis	NC_004461.1 NC_005008.1 NC_005007.1 NC_005006.1 NC_005005.1 NC_005004.1 NC_005003.1	S: no significant homology	S: no significant homology
Staphylococcus salivarius	FR873481.1	S: no significant homology	S: no significant homology