U.S. FOOD & DRUG ADMINISTRATION

Acknowledgment Letter

9/1/2020

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Dear Yashar Niknafs, Ph.D.:

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Submission Number: EUA202674
Received: 9/1/2020
Applicant: LynxDx Inc.
Device: GenePathDx CoViDx qRT-PCR v2.3 kit test

We will notify you when the review of this document has been completed or if any additional information is required. For information about CDRH review regulations and policies, please refer to http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/default.htm.

Sincerely yours,

Center for Devices and Radiological Health

EMERGENCY USE AUTHORIZATION (EUA) SUBMISSION SARS-CoV-2 RT-PCR Assay

INTENDED USE

The GenePath CoViDx qRT-PCR v2.3 kit is a real time reverse transcription polymerase chain reaction (qRT-PCR) intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in nasopharyngeal (NP), nasal swab, and oropharyngeal (OP) specimens from individuals with signs and symptoms of infection who are suspected of COVID-19 by their healthcare provider.

Testing is limited to Lynx Dx that is 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 nasopharyngeal (NP), oropharyngeal (OP), nasopharyngeal + oropharyngeal (NP+OP), or nasal swab 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 test results to the appropriate public health authorities.

The assay is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures The assay is only for use under the Food and Drug Administration's Emergency Use Authorization.

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 GenePath CoViDx qRT-PCR v2.3 test is to be used with the KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head, the ABI QuantStudio 12K Flex instrument and either Quantstudio 12K Flex Software or Design and Analysis Software.

DEVICE DESCRIPTION AND TEST PRINCIPLE

1) Product Overview/Test Principle:

The assay is a real-time reverse transcription polymerase chain reaction (rRT -PCR) test. The SARS-CoV-2 primer and probe set(s) is designed to detect RNA from the SARS-CoV-2 in respiratory specimens from patients as recommended for testing by public health authority guidelines.

2) Description of Test Steps:

Nucleic acids are isolated and purified from nasopharyngeal (NP), oropharyngeal (OP), nasopharyngeal and oropharyngeal (NP+OP), or nasal swabs specimens using the KingFisher™ Flex

Magnetic Particle Processor with 96 Deep-Well Head and the MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit or MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit with a sample input volume of 200 µL. The RNA is eluted in 50 µL of the elution buffer. Ten (10) microliters (µL) of the purified nucleic acid is reverse transcribed and is then subsequently amplified [the qRT-PCR reaction mix (reaction volume 25 µL) contains a blend of a reverse transcriptase and hotstart DNA polymerase enzymes] in the thermocycler. In the process, the probe anneals to a specific target sequence located between the forward and reverse primers. During the extension phase of the PCR cycle, the 5' nuclease activity of Taq polymerase degrades the probe, causing the reporter dye to separate from the quencher dye, generating a fluorescent signal. With each cycle, additional reporter dye molecules are cleaved from their respective probes, increasing the fluorescence intensity.

Fluorescence intensity is monitored at each PCR cycle by the ABI QuantStudio 12K Flex instrument.

3) Control Material(s) to be Used:

Twist Biosciences Synthetic SARS-CoV-2 RNA for the strain MT007544.1 (SKU 102019) is synthetic RNA (referred to as "Twist RNA" below) that provides full coverage of the full-length RNA from the strain.

The following two controls are to be run along with the samples:

1. Positive Control (PC): Needed to ensure the performance of the RT-PCR master mixes. The positive control used for this evaluation was Twist RNA at 100 copies/reaction. A positive control should be run with every PCR.
2. Negative Control (NTC): This is needed to check for contamination. Collection Medium is used as negative control. It should be run with patient samples at least once per extraction batch and downstream PCR.

In addition, the Human RNaseP target serves as an extraction control, internal control, and specimen collection control for each sample.

Assay results and interpretation

All test controls should be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted.

A) Controls

In order to report patient test results, all controls within that run must be valid and produce the expected results. If any of the controls is determined to be invalid or produces unexpected results, patient results from that run should not be reported.

	Targets				Interpretation
	N-Gene	**RdRP-gene**	**E-gene**	**Human RNaseP**	
NTC	No amplification	No amplification	No amplification	No Amplification (Ct > 35)	Run valid (no contamination)
	Amplification (one or more targets)				Contaminated samples / workspace. Carry out a thorough decontamination and repeat with a freshly prepared reaction set
PC	Amplification (Ct ≤ 40)	Amplification (Ct ≤ 40)	Amplification (Ct ≤ 40)		Run valid
	No amplification (one of more targets)				Run invalid. Repeat the entire assay.

B) Samples:

Targets				Interpretation
N-gene	**RdRP-gene**	**E-gene**	**Human Control**	
No amplification	No amplification	No amplification	No amplification	Invalid test: insufficient sample or PCR inhibitors present. **Repeat testing**.
No amplification	No amplification	No amplification	Amplification (Ct ≤ 35)	Negative for SARS-CoV-2
Amplification (Ct ≤ 45) in **all three** viral targets			Any result	Positive for SARS-CoV-2
Amplification (Ct ≤ 45) in **any two** viral targets			Any result	Positive for SARS-CoV-2
Amplification (Ct ≤ 40) of **any one** viral target			No amplification	Ambiguous result. Resample and retest.
Amplification (Ct ≤ 40) of **any one** viral target			Amplification (Ct ≤ 35	Ambiguous result. Retest or resample and retest.

Note: Only a single repeat is suggested for retesting after which a fresh sample/swab should be collected.

PERFORMANCE EVALUATION

1) Limit of Detection (LoD) - Analytical Sensitivity:

a. This test is performed to determine the optimal working range of RNA template in each reaction. The test should identify the lower limit in which the software/assay combination is unable to make a consistent and accurate call.

b. For this test, Verification Test Samples (VTSs) are spiked with Coronavirus 2 (SARS-CoV-2), isolate USA-WA1/2020, Heat Inactivated (NR-52286), acquired from the American Type Culture Collection, at various concentrations [gene copies(gc)/mL] as indicated in the table below. Negative NP-collected and OP-collected samples were pooled with their respective collection/media types. Confirmed negative samples were sourced from the State of Michigan Department of Health and from previous samples that were confirmed negative with the TaqPath™ COVID-19 Combo Kit, a comparator EUA-approved assay. Three identical 9-point dilution series were created from 10,000 gc/mL down to 50 gc/mL, with the 9th dilution being negative.

Verification Test Samples	Replicate number	Final Concentration (gc/mL)
1,10,19	**1-3**	**10,000**
2,11,20	**1-3**	**5,000**
3,12,21	**1-3**	**2,500**
4,13,22	**1-3**	**1,000**
5,14,23	**1-3**	**500**
6,15,24	**1-3**	**250**
7,16,25	**1-3**	**100**
8,17,26	**1-3**	**50**
9,18,27	**1-3**	**0**

c. Each VTS listed in the table was subjected to RNA extraction using the Kingfisher System as described earlier. The RNA was then analyzed in RT-PCR using the QuantStudio 12K Flex Real-Time PCR system and GenePath CoViDx qRT-PCR v.2.3 assay. The results are summarized in Table 1.

(Table 1)

Analytical Sensitivity (LOD) Determination - Nasopharyngeal Swab Collection					
Contrived Viral Load gc/mL	**Target**	**Replicate 1 Ct**	**Replicate 2 Ct**	**Replicate 3 Ct**	**% Clinical Concordance**
10,000	E-Gene	30.71	29.79	30.01	
10,000	RdRP	30.43	29.25	29.74	100%
10,000	N-Gene	30.12	29.60	29.54	
5,000	E-Gene	31.95	31.54	31.20	
5,000	RdRP	31.96	31.67	31.08	100%

5,000	N-Gene	31.34	31.26	30.86	
2,500	E-Gene	33.05	32.13	33.20	
2,500	RdRP	32.96	31.51	33.36	100%
2,500	N-Gene	32.60	31.44	33.21	
1,000	E-Gene	34.16	34.71	34.12	
1,000	RdRP	35.01	34.53	34.64	100%
1,000	N-Gene	33.54	33.57	33.95	
500	E-Gene	32.64	35.07	34.36	
500	RdRP	32.50	34.63	35.81	100%
500	N-Gene	33.61	33.23	34.67	
250	E-Gene	36.97	36.51	37.92	
250	RdRP	36.52	35.96	37.52	100%
250	N-Gene	36.28	34.88	35.63	
100	E-Gene	37.70	37.83	37.08	
100	RdRP	39.97	35.99	36.54	100%
100	N-Gene	37.31	36.34	35.89	
50	E-Gene	37.23	37.06	37.06	
50	RdRP	35.53	N/A	36.35	100%
50	N-Gene	34.87	36.06	36.94	
0	E-Gene	N/A	N/A	N/A	
0	RdRP	N/A	N/A	N/A	100%
0	N-Gene	N/A	N/A	N/A	
Analytical Sensitivity (LOD) Determination - Oropharyngeal Swab Collection					
Contrived Viral Load gc/mL	**Target**	**Replicate 1 Ct**	**Replicate 2 Ct**	**Replicate 3 Ct**	**% Clinical Concordance**
10,000	E-Gene	30.91	30.54	30.64	
10,000	RdRP	30.45	30.05	30.55	100%
10,000	N-Gene	30.09	29.85	29.90	
5,000	E-Gene	31.71	31.64	31.60	
5,000	RdRP	31.86	31.29	31.54	100%
5,000	N-Gene	31.16	30.67	31.23	
2,500	E-Gene	32.60	32.01	32.20	
2,500	RdRP	32.65	32.57	32.10	100%
2,500	N-Gene	32.10	32.00	30.92	
1,000	E-Gene	34.60	33.89	33.71	
1,000	RdRP	33.90	33.63	34.57	100%
1,000	N-Gene	33.69	33.75	33.55	

500	E-Gene	34.38	35.26	35.76	
500	RdRP	34.31	34.26	34.90	100%
500	N-Gene	32.88	33.87	33.78	
250	E-Gene	35.97	36.64	36.69	
250	RdRP	36.54	35.60	37.50	100%
250	N-Gene	35.48	37.64	35.09	
100	E-Gene	35.98	38.26	34.84	
100	RdRP	36.79	N/A	36.59	100%
100	N-Gene	35.46	36.97	36.88	
50	E-Gene	N/A	N/A	N/A	
50	RdRP	38.44	35.86	N/A	66%
50	N-Gene	N/A	37.58	37.70	
0	E-Gene	N/A	N/A	N/A	
0	RdRP	N/A	N/A	N/A	100%
0	N-Gene	N/A	N/A	N/A	

c. The tentative LOD was the lowest concentration that was detected 100% of the time.

d. The tentative LOD for this assay was determined to be 50 gc/mL with contrived NP-collected specimens and 100 gc/mL with contrived OP-collected specimens.

e. We created 20 replicates of samples at our tentative LoD and one step above our tentative LoD for our contrived NP-collected specimens. At our final LoD, determined to be 100 gc/mL, we found 95% (19 out of 20) detection at 100 gc/ml, with 5% (1 out of 20) prompting a re-run. Results are summarized in Table 2. For comparison, as evident from the data at one step below this final LoD (Table 2), detection was not found 100 % of the time in the analytical determination.

Results - CT values were calculated by setting a baseline threshold of 100,000 for all genes. This was set above all noise detected in NTCs and samples with a negative clinical outcome, in order to prevent false positives. Below are the CT values for targets in samples spiked-in with the LOD concentration (Table 2)

(Table 2)

Sample Type	Replicate #	RNaseP	E-Gene	RdRp	N-Gene	Clinical Outcome
100 gc/ml	1	25.24	37.01	N/A	35.61	**Positive**
	2	25.51	N/A	38.45	37.59	**Positive**
	3	25.45	N/A	35.83	37.43	**Positive**

	4	24.72	36.17	37.14	34.55	**Positive**
	5	25.64	37.98	35.70	36.35	**Positive**
	6	25.89	36.69	36.52	36.66	**Positive**
	7	25.41	37.86	N/A	36.11	**Positive**
	8	25.44	N/A	N/A	36.87	**Ambiguous**
	9	26.10	36.27	36.01	37.57	**Positive**
	10	26.43	37.49	36.44	36.32	**Positive**
	11	25.83	36.96	38.13	36.72	**Positive**
	12	25.97	38.18	36.54	37.04	**Positive**
	13	25.81	36.85	36.68	37.01	**Positive**
	14	25.83	36.57	35.64	34.95	**Positive**
	15	25.43	36.92	37.23	35.11	**Positive**
	16	25.52	37.95	37.32	36.34	**Positive**
	17	25.94	37.25	37.71	35.67	**Positive**
	18	25.95	37.40	39.81	37.27	**Positive**
	19	25.80	38.19	36.45	36.31	**Positive**
	20	25.66	37.22	36.74	35.90	**Positive**
Average CT		25.68	37.23	36.96	36.37	
Clinical Concurrence		95-100%				

Sample Type	**Replicate #**	**RNaseP**	**E-Gene**	**RdRp**	**N-Gene**	**Clinical Outcome**
50 gc/ml	1	25.56	38.62	37.29	35.96	**Positive**
	2	25.59	37.89	36.61	37.58	**Positive**
	3	25.67	N/A	37.85	35.96	**Positive**
	4	25.62	38.08	N/A	37.75	**Positive**
	5	25.43	N/A	38.46	35.86	**Positive**

	6	25.35	35.97	N/A	37.68	**Positive**
	7	25.49	35.97	37.39	N/A	**Positive**
	8	25.74	37.37	N/A	N/A	**Ambiguous**
	9	26.29	36.42	37.10	36.99	**Positive**
	10	25.99	N/A	N/A	N/A	**Negative**
	11	25.43	37.66	37.21	36.25	**Positive**
	12	25.40	N/A	38.98	N/A	**Ambiguous**
	13	25.82	N/A	N/A	37.77	**Ambiguous**
	14	25.68	38.11	N/A	37.76	**Positive**
	15	25.49	38.13	N/A	N/A	**Ambiguous**
	16	25.56	N/A	N/A	37.90	**Ambiguous**
	17	25.67	N/A	N/A	N/A	**Negative**
	18	26.49	38.56	38.84	36.97	**Positive**
	19	25.45	37.99	36.11	34.91	**Positive**
	20	24.83	37.22	N/A	36.11	**Positive**
	21	24.96	37.45	36.79	36.99	**Positive**
Average CT		25.60	37.53	37.51	36.83	
Clinical Concurrence		75-90%				

2) Inclusivity (analytical sensitivity):

In-silico analysis was done on 3811 genomes of SARS-CoV-2 (Taxonomy ID:2697049) available in GenBank as of June 13, 2020. There was 100% homology for at least two of three viral targets for all genomes. In 3736/3811 genomes (98.03%) genomes, there was 100% homology for all three viral targets. The assay requires two of three viral targets to amplify for a positive indication which should happen for all the strains. The following table shows the Genbank sequences without 100% homology for one of the three viral targets:

Viral Target	GenBank IDs without 100% homology
E-gene	Total of 3 sequences

	Probe: 3 sequences MT451456.1, MT350246.1, MT039890.1
N-gene	Total of 59 sequences: Forward primer: 48 sequences MT601285.1, MT601286.1, MT601287.1 MT479223.1, MT582454.1, MT520307.1 MT560705.1, MT536964.1, MT536972.1 MT472624.1, MT461626.1, MT450949.1 MT450973.1, MT450980.1, MT451007.1 MT451158.1, MT451168.1, MT451186.1 MT451194.1, MT451204.1, MT451375.1 MT451436.1, MT451447.1, MT451476.1 MT451590.1, MT451616.1, MT451712.1 MT451713.1, MT451754.1, MT451771.1 MT447177.1, MT365028.1, MT428551.1 MT374102.1, MT374103.1, MT374104.1 MT374106.1, MT374107.1, MT370516.1 MT370518.1, MT370904.1, MT371047.1 MT114414.1, MT114415.1, MT358693.1 MT326035.1, MT326184.1, MT327745.1 Reverse primer: 3 sequences MT513758.1, MT467254.1, MT370971.1 Probe: 8 sequences MT520390.1, MT509497.1, MT506905.1 MT370849.1, MT334533.1, MT334534.1 MT322420.1, MT263430.1
RdRp-gene	Total of 13 sequences Forward primer: 2 sequences MT451733.1, MT451748.1 Probe: 11 sequences MT535500.1, MT536180.1, MT528600.1 MT358640.1, MT451172.1, MT451231.1 MT375437.1, MT370869.1, MT334541.1 MT291831.1, MT263452.1

3) Cross-reactivity (Analytical Specificity)

Recommended List of Organisms to be Analyzed in silico and by Wet Testing*

Other high priority pathogens from the same genetic family	High priority organisms likely present in a respiratory specimen.
Human coronavirus 229E	**Adenovirus (e.g. C1 Ad. 71)**
Human coronavirus OC43	**Human Metapneumovirus (hMPV)**
Human coronavirus HKU1	**Parainfluenza virus 1-4**
Human coronavirus NL63	**Influenza A & B**
SARS-coronavirus	**Enterovirus (e.g. EV68)**
MERS-coronavirus	**Respiratory syncytial virus**
	Rhinovirus
	Chlamydia pneumoniae
	Haemophilus influenzae
	Legionella pneumophila
	Mycobacterium tuberculosis
	Streptococcus pneumoniae
	Streptococcus pyogenes
	Bordetella pertussis
	Mycoplasma pneumoniae
	***Pneumocystis jirovecii* (PJP)**
	Pooled human nasal wash – *to represent diverse microbial flora in the human respiratory tract*
	Candida albicans
	Pseudomonas aeruginosa
	Staphylococcus epidermis
	Streptococcus salivarius

***** For wet testing, concentrations of 106 CFU/ml or higher for bacteria and 105 pfu/ml or higher for viruses is recommended.

In-silico analysis for cross-reactivity was done by a blast (BLASTn) search of publicly available sequences at NCBI (National Center for Biotechnology Information, U.S. National Library of Medicine). The database search parameters were as follows: The nucleotide collection consists of GenBank+EMBL+DDBJ+PDB+RefSeq sequences, but excludes EST, STS, GSS, WGS, TSA, patent sequences as well as phase 0, 1, and 2 HTGS sequences and sequences longer than 100Mb; SARS-CoV-2 sequences were excluded. The database is non-redundant. Identical sequences have been merged into one entry, while preserving the accession, GI, title and taxonomy information for each entry; Database was updated on 2020/06/13. The search parameters automatically adjust for short input sequences and the expected threshold is 1000; The match and mismatch scores are 1 and -3, respectively. The penalty to create and extend a gap in an alignment is 5 and 2 respectively. The word size to initiate an alignment was 7 bases.

In a real-time PCR assay, high homology for the forward primer, reverse primer and the probe is required to generate a signal. Further, the primers and probes have to be in proximity (within 1000 bases). The search results were therefore filtered by (a) constraining >80% homology for the primers and probes and (b) (subject) distance between primers and probe alignments to be <1000 bases.

The RdRp-gene and N-gene targets are very specific to SARS-CoV-2. The only cross-reactivities observed were from the putative origins of SARS-CoV-2, isolated from pangolin and bats. Twelve (12) sequences for coronavirus isolates from pangolin and bat were found to have homology for the RdRp-gene target. Similarly thirty four (34) sequences of coronavirus isolates from pangolin and bat were found to have homology for the N-gene target. As the origin of SARS-CoV-2 has been tied to both pangolin and bat, this is to be expected (e.g. Liu P, Jiang JZ, Wan XF, et al. Are pangolins the intermediate host of the 2019 novel coronavirus (SARS-CoV-2)?. PLoS Pathog. 2020;16(5):e1008421. Published 2020 May 14. doi:10.1371/journal.ppat.1008421).

The E gene primer/probe set is specific to the Sarbecovirus group [a clade of betacoronaviruses known to be associated with (mainly) rhinolophid bats across the Palearctic, including the SARS-related coronaviruses]. As expected, the blast search revealed homology to bat, pangolin and SARS related coronaviruses.

The assay specificity comes from the requirement that two of three primer/probe sets have to be amplified. The specificity of RdRP and N gene targets to the SARS-CoV-2 results in a very specific assay.

4) Clinical Evaluation

Confirmed positive specimens for this evaluation were obtained from the State of Michigan Department of Health and were taken from routine testing with the TaqPath™ COVID-19 Combo Kit. Confirmed negative specimens were sourced from previous samples that were confirmed negative during routine testing with the TaqPath™ COVID-19 Combo Kit. In the first run (Table 3), consisting only of samples taken from routine testing, the samples consisted of 2 unique COVID positive and 88 unique COVID negative samples, all confirmed orthogonally by the TaqPath™ Combo Kit. In the second run (Table 4), 46 confirmed positive samples were used for clinical evaluation. Among these 46 were 24 samples explicitly certified as weak or extremely weak positives from the State of Michigan.

Specimens were subject to RNA extraction and downstream PCR analysis as previously described. Samples from the State of Michigan Department of Health had been previously verified as positive with the CDC 2019-nCoV RT-PCR Diagnostic Panel.

As previously stated, samples sourced from routine testing were verified with the TaqPath™ COVID-19 Combo Kit (Table 3 and Table 4).

Samples were randomized and labelled with randomly-assigned codes for blinded testing. The code designations were revealed after analysis in order to confirm results.

The acceptance criteria was 100% agreement with the samples for positive and negative. Results are shown below (Table 3 and Table 4).

Results:

We found 100% positive/negative agreement with samples tested under the GenePath CoViDx qRT-PCR v2.3 assay. Analysis with GenePath's assay was done with baseline thresholding and a threshold of 100,000.

(Table 3)

Sample No.	Sample Code	GenePath CT Values			GenePath Status	True Status
		E Gene	RdRp	N Gene		
1	S28245	34.13	32.92	33.76	**Positive**	**Positive**
2	S19808	N/A	N/A	N/A	**Negative**	**Negative**
3	S19886	N/A	N/A	N/A	**Negative**	**Negative**
4	S17116	N/A	N/A	N/A	**Negative**	**Negative**
5	S17219	N/A	N/A	N/A	**Negative**	**Negative**
6	S17222	N/A	N/A	N/A	**Negative**	**Negative**
7	S17175	N/A	N/A	N/A	**Negative**	**Negative**
8	S19560	N/A	N/A	N/A	**Negative**	**Negative**
9	S19540	N/A	N/A	N/A	**Negative**	**Negative**
10	S19572	N/A	N/A	N/A	**Negative**	**Negative**
11	S19580	N/A	N/A	N/A	**Negative**	**Negative**
12	S28256	N/A	N/A	N/A	**Negative**	**Negative**
13	S20117	N/A	N/A	N/A	**Negative**	**Negative**
14	S19658	N/A	N/A	N/A	**Negative**	**Negative**
15	S19823	N/A	N/A	N/A	**Negative**	**Negative**
16	S17115	N/A	N/A	N/A	**Negative**	**Negative**
17	S17163	N/A	N/A	N/A	**Negative**	**Negative**
18	S17204	N/A	N/A	N/A	**Negative**	**Negative**
19	S17235	N/A	N/A	N/A	**Negative**	**Negative**
20	S19551	N/A	N/A	N/A	**Negative**	**Negative**
21	S19568	N/A	N/A	N/A	**Negative**	**Negative**
22	S19649	N/A	N/A	N/A	**Negative**	**Negative**
23	S19608	N/A	N/A	N/A	**Negative**	**Negative**
24	S28255	N/A	N/A	N/A	**Negative**	**Negative**
25	S19715	25.02	25.96	25.25	**Positive**	**Positive**
26	S19769	N/A	N/A	N/A	**Negative**	**Negative**

27	S19783	N/A	N/A	N/A	**Negative**	**Negative**
28	S17209	N/A	N/A	N/A	**Negative**	**Negative**
29	S17249	N/A	N/A	N/A	**Negative**	**Negative**
30	S17260	N/A	N/A	N/A	**Negative**	**Negative**
31	S17180	N/A	N/A	N/A	**Negative**	**Negative**
32	S19624	N/A	N/A	N/A	**Negative**	**Negative**
33	S19531	N/A	N/A	N/A	**Negative**	**Negative**
34	S19630	N/A	N/A	N/A	**Negative**	**Negative**
35	S28258	N/A	N/A	N/A	**Negative**	**Negative**
36	S28252	N/A	N/A	N/A	**Negative**	**Negative**
37	S28246	N/A	N/A	N/A	**Negative**	**Negative**
38	S19907	N/A	N/A	N/A	**Negative**	**Negative**
39	S19909	N/A	N/A	N/A	**Negative**	**Negative**
40	S17226	N/A	N/A	N/A	**Negative**	**Negative**
41	S17156	N/A	N/A	N/A	**Negative**	**Negative**
42	S17117	N/A	N/A	N/A	**Negative**	**Negative**
43	S17169	N/A	N/A	N/A	**Negative**	**Negative**
44	S19636	N/A	N/A	N/A	**Negative**	**Negative**
45	S19549	N/A	N/A	N/A	**Negative**	**Negative**
46	S19556	N/A	N/A	N/A	**Negative**	**Negative**
47	S28253	N/A	N/A	N/A	**Negative**	**Negative**
48	S28260	N/A	N/A	N/A	**Negative**	**Negative**
49	S19776	N/A	N/A	N/A	**Negative**	**Negative**
50	S19895	N/A	N/A	N/A	**Negative**	**Negative**
51	S19838	N/A	N/A	N/A	**Negative**	**Negative**
52	S17103	N/A	N/A	N/A	**Negative**	**Negative**
53	S17248	N/A	N/A	N/A	**Negative**	**Negative**
54	S17206	N/A	N/A	N/A	**Negative**	**Negative**
55	S19578	N/A	N/A	N/A	**Negative**	**Negative**
56	S19576	N/A	N/A	N/A	**Negative**	**Negative**
57	S19565	N/A	N/A	N/A	**Negative**	**Negative**
58	S19638	N/A	N/A	N/A	**Negative**	**Negative**
59	S28254	N/A	N/A	N/A	**Negative**	**Negative**
60	S28263	N/A	N/A	N/A	**Negative**	**Negative**
61	S19869	N/A	N/A	N/A	**Negative**	**Negative**
62	S20077	N/A	N/A	N/A	**Negative**	**Negative**
63	S17162	N/A	N/A	N/A	**Negative**	**Negative**
64	S17111	N/A	N/A	N/A	**Negative**	**Negative**
65	S17225	N/A	N/A	N/A	**Negative**	**Negative**

66	S17192	N/A	N/A	N/A	**Negative**	**Negative**
67	S19625	N/A	N/A	N/A	**Negative**	**Negative**
68	S19542	N/A	N/A	N/A	**Negative**	**Negative**
69	S19562	N/A	N/A	N/A	**Negative**	**Negative**
70	S19604	N/A	N/A	N/A	**Negative**	**Negative**
71	S28262	N/A	N/A	N/A	**Negative**	**Negative**
72	S28264	N/A	N/A	N/A	**Negative**	**Negative**
73	S19824	N/A	N/A	N/A	**Negative**	**Negative**
74	S19896	N/A	N/A	N/A	**Negative**	**Negative**
75	S17104	N/A	N/A	N/A	**Negative**	**Negative**
76	S17142	N/A	N/A	N/A	**Negative**	**Negative**
77	S17159	N/A	N/A	N/A	**Negative**	**Negative**
78	S17252	N/A	N/A	N/A	**Negative**	**Negative**
79	S19566	N/A	N/A	N/A	**Negative**	**Negative**
80	S19579	N/A	N/A	N/A	**Negative**	**Negative**
81	S19614	N/A	N/A	N/A	**Negative**	**Negative**
82	S19567	N/A	N/A	N/A	**Negative**	**Negative**
83	S28261	N/A	N/A	N/A	**Negative**	**Negative**
84	S28265	N/A	N/A	N/A	**Negative**	**Negative**
85	S19671	N/A	N/A	N/A	**Negative**	**Negative**
86	S19839	N/A	N/A	N/A	**Negative**	**Negative**
87	S17214	N/A	N/A	N/A	**Negative**	**Negative**
88	S17212	N/A	N/A	N/A	**Negative**	**Negative**
89	S17134	N/A	N/A	N/A	**Negative**	**Negative**
90	S17221	N/A	N/A	N/A	**Negative**	**Negative**

(Table 4)

Sample No.	**Sample Code**	**E Gene**	**RdRp**	**N Gene**	**Clinical Outcome**
1	438	22.23	21.71	21.48	**Positive**
2	S01230	33.59	33.07	33.77	**Positive**
3	474	38.10	38.13	37.98	**Positive**
4	337	30.82	30.08	29.96	**Positive**
5	398	30.63	29.79	30.72	**Positive**
6	475	35.83	34.69	35.61	**Positive**
7	433	32.46	31.49	32.41	**Positive**
8	406	20.90	20.41	20.65	**Positive**
9	335	20.52	19.62	20.54	**Positive**
10	477	37.21	37.32	37.95	**Positive**

11	473	32.31	31.75	31.84	**Positive**
12	383	25.13	24.70	24.73	**Positive**
13	315	20.47	19.96	20.02	**Positive**
14	S01453	38.62	36.35	37.13	**Positive**
15	480	37.43	38.24	37.61	**Positive**
16	374	24.96	24.67	24.37	**Positive**
17	372	28.11	27.72	27.81	**Positive**
18	1235	20.11	19.10	19.99	**Positive**
19	478	33.45	32.13	33.39	**Positive**
20	436	29.08	28.55	29.46	**Positive**
21	339	26.36	25.56	26.32	**Positive**
22	1234	31.93	31.74	31.78	**Positive**
23	476	35.14	33.86	34.09	**Positive**
24	494	34.93	34.50	34.30	**Positive**
25	491	36.65	35.50	36.62	**Positive**
26	490	31.46	30.83	31.35	**Positive**
27	407	20.43	19.82	20.15	**Positive**
28	431	27.84	27.59	27.46	**Positive**
29	5257	39.75	39.59	N/A	**Positive**
30	481	40.17	37.11	38.96	**Positive**
31	484	38.37	36.33	38.99	**Positive**
32	485	32.09	30.48	31.62	**Positive**
33	492	37.49	35.77	36.82	**Positive**
34	495	33.48	32.65	33.03	**Positive**
35	493	31.83	31.49	31.52	**Positive**
36	496	36.84	35.99	36.75	**Positive**
37	373	27.46	26.81	26.89	**Positive**
38	432	29.68	28.97	28.98	**Positive**
39	S00516	35.12	34.25	34.98	**Positive**
40	479	35.88	35.03	35.25	**Positive**
41	488	38.02	37.14	38.28	**Positive**
42	489	35.54	34.94	35.08	**Positive**
43	486	32.85	31.60	31.88	**Positive**
44	487	36.30	34.35	36.57	**Positive**
45	482	36.27	36.00	34.57	**Positive**
46	483	40.17	36.53	38.27	**Positive**

APPROVED/CLEARED ALTERNATIVE PRODUCTS

Currently no methods for the detection of the SARS-CoV-2 have been approved/ cleared by FDA.

INSTRUCTIONS FOR USE/ PROPOSED LABELING/PACKAGE INSERT:

Automated RNA extraction is performed using the KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head and the MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit or MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit with a sample input volume of 200 µL. Alternative RNA extraction methods will need to be validated with this assay by the individual labs that choose to use them.

Detailed PCR Protocol:

Control Formation:

Positive and negative controls should be included in every run, such as Twist Biosciences Synthetic SARS-CoV-2 RNA for the strain MT007544.1 (SKU 102019) as the positive control directly added to the PCR plate. As an example of a negative control, collection media should be included in the Kingfisher run, and extracted RNA from that step will be the negative control.

Pre-PCR Steps:

1. Thaw all reagents before use and briefly pipette-mix.

Preparation of Premix:

1. If 'n' is the number of clinical samples and controls being tested, then prepare sufficient premix for 20% overage.
2. The total reaction volume per tube is 25 µL (15 µL prepared premix + 10 µL of RNA sample).
3. Prepare the PCR premix as outlined in Table 2 ahead.

Table 2: Preparation of RT-qPCR Premix for One Sample

Reagents	**Amount for Premix (uL)**
GPDx Master Mix	13
GPDx Enzyme Mix	1
GPDx Primer/Probe Mix	1
Total	**15**

4. After preparing the premix, return all the enzymes and reagents to the freezer at -10°C to - 20°C.
5. Add 15 µL of the freshly prepared RT-qPCR premix to the labelled tubes compatible with the real-time PCR equipment being used.
6. Add 10 µL of each RNA sample/NTC/PC in their respectively labelled tubes or microtiter plate wells.

7. Firmly seal wells with optical covering (plates) or tube caps (tubes)
8. Thoroughly vortex the entire plate for 5-10 seconds.
9. Centrifuge the tubes/plate for at least 1 minute at 3,000 rpm.
10. Place the tubes/plate in the thermal cycler and set the thermal cycling conditions per Tables 3 and 4 below.

Table 3: RT-qPCR Cycling Conditions

Step	Stage	Temperature	Time
UNG activation	Hold	30°C	5 min
Reverse transcription and UNG deactivation	Hold	52°C	7 min
RT inactivation	Hold	95°C	3.5 min
PCR	Cycling	95°C	5 sec
		58°C	35 sec (Acquire fluorescence at Green/Yellow/Orange/Red channels)

Table 4: Required Settings for Real Time PCR Instruments

Target	Channel (Dye)	Fluorophore Excitation Wavelength (nm)	Fluorophore Emission Wavelength (nm)
RdRP-gene	FAM	495	520
Human RNaseP	HEX	535	560
N-gene	CalRed 610	590	610
E-gene	Quasar 670	645	670

M. RECORD KEEPING AND REPORTING INFORMATION TO FDA:

The laboratory will track adverse events and report to FDA under 21 CFR Part 803. A website is available to report on adverse events, and this website is referenced in the Fact Sheet for Health Care providers. The laboratory will maintain information on the performance of the test, and report to FDA any suspected

change in performance of which they become aware. The laboratory will maintain records associated with this EUA and ensure these records are maintained until notified by FDA. Such records will be made available to FDA for inspection upon request.