EMERGENCY USE AUTHORIZATION (EUA) SUMMARY
DxTerity SARS-CoV-2 RT-PCR CE Test
(DxTerity Diagnostics, Inc.)

For *in vitro* Diagnostic Use
Rx Only
For Use Under Emergency Use Authorization (EUA) Only

(The DxTerity SARS-CoV-2 RT-PCR CE Test will be performed at the DxTerity Diagnostics Clinical Laboratory, located at 19500 S. Rancho Way, Suite 116 Rancho Dominguez, CA 90220, which is certified under Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a and meets requirements to perform high-complexity tests, as described in the Laboratory Standard Operating Procedure that was reviewed by the FDA under this EUA.)

INTENDED USE

The DxTerity SARS-CoV-2 RT-PCR CE Test is an end point reverse transcription polymerase chain reaction (RT-PCR) test followed by detection with capillary electrophoresis (CE) test intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in saliva specimens collected from any individuals determined to be appropriate for COVID-19 testing by their healthcare provider (HCP), including from individuals without symptoms of COVID-19.

Testing is limited to DxTerity Diagnostics, Inc. located at 19500 S. Rancho Way, Suite 116, Rancho Dominguez, CA 90220, which is certified which is certified under Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, and meets requirements to perform high complexity tests.

DxTerity SARS-CoV-2 RT-PCR CE Test is for use with saliva specimens that are self-collected at home using the DxTerity COVID-19 Test Collection Kit when determined to be appropriate by a HCP.

Results are for the detection and 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 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. Negative results obtained from saliva

specimens from individuals without symptoms should be considered as presumptive and confirmed with a preferred specimen type or different molecular assay validated for testing saliva, if necessary for patient management.

The DxTerity SARS-CoV-2 RT-PCR CE assay is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of PCR assays, capillary electrophoresis and in vitro diagnostic procedures. The DxTerity SARS-CoV-2 RT-PCR CE assay is only for use under the Food and Drug Administration's Emergency Use Authorization.

DEVICE DESCRIPTION AND TESTPRINCIPLE

The DxTerity SARS-CoV-2 RT-PCR CE Test is an end point reverse transcription polymerase chain reaction (RT-PCR) test followed by detection with capillary electrophoresis (CE). The SARS-CoV-2 primers are designed to detect RNA from SARS-CoV-2 in saliva specimens from individuals 18 years old or older as recommended for testing by public health authority guidelines. Children under the age of 18 may use this test, under adult supervision to assist, as needed, with the steps beyond spitting into the collection tube.

Saliva specimens must be self-collected using the DxTerity COVID-19 Test Collection Kit which contains the Spectrum Solutions LLC SDNA-1000 Saliva Collection Device.

DxTerity Ordering Process:
The saliva collection kit will be shipped under a standing ordering prescription from a physician licensed in all 50 states and Washington DC to any individual for COVID-19 testing as determined to be appropriate by the healthcare provider, including individuals without symptoms of COVID-19. The individual first receives the saliva collection kit. When they wish to collect the saliva sample in order to be tested, the individual will login to a secure online portal to complete a required health questionnaire. As determined by the standing ordering prescription, individuals exhibiting severe COVID warning symptoms are not allowed to proceed with kit registration and instead are directed to seek emergency medical care. Individuals deemed to be appropriate for testing are authorized to proceed with collection kit registration and the unsupervised saliva collection process. The collection kit is then returned to DxTerity laboratory for testing; results are returned to the individual via a secure online portal. A statement to the report for positive and invalid results is added instructing the patient to contact their HCP if they have concerns regarding the results. In addition, a link to the fact sheets for both HCP and patient for the test is included in the test report that goes back to the patient via the portal.

The DxTerity COVID-19 Test Collection Kit contains the Spectrum Solutions LLC SDNA-1000 Saliva Collection Device, biohazard specimen bag with absorbent material, pre-labeled return shipping box along with Instructions for Use for shipping the samples on the same day of collection for next day delivery to the laboratory. Saliva specimens must be transported and stored at ambient temperature and tested within 72 hours of collection. Specimens are received at the clinical laboratory for testing with the DxTerity SARS-CoV-2 RT-PCR CE Test.

The test uses primers to detect specific nucleic acid sequences from the genome of the SARS-

CoV-2 from the nucleocapsid (N) gene, envelope gene (E), and ORF1ab region. The human RNase P gene is also a target in the test to serve as an internal and extraction control.

RNA extraction from saliva specimens is performed using Sera-Mag SpeedBeads Carboxyl Magnetic Beads (GE Healthcare) using the Applied Biosystem MagMax 96 Magnetic Particle Processor. The input sample volume is 540µL, the elution volume is 50µL.

Reverse transcription-PCR (RT-PCR) is performed using the ThermoFisher Scientific TaqPath 1-Step Multiplex Master Mix (No ROX) using the DxTerity SARS-CoV-2 RT-PCR CE Test Primer Mix with 10 µL of the extracted sample on the VeritiDx PCR Thermal Cycler. There are four different primer mixes containing primer pairs for N, E, Orf1 ab, and RNase P. Sample testing is performed with only one primer mix per plate. Each sample is run with one of the four primer mixes.

PCR products are then separated by capillary electrophoresis (CE). The amplified PCR products corresponding to each target sequence is identified and quantified based on its characteristic length and dye wavelength.

FSA files generated from the Data Collection Software on the 3500xL Dx Genetic Analyzer are used to determine peak heights in Relative Fluorescent Units (RFU) of each target. The RFUs are then normalized for each injection to the peak heights of the Internal Size Standard (ISS), which are evaluated against target specific thresholds.

DxTerity's custom analysis software, DxTerity Lab API Version 1.2.0, normalizes the Peak height data (RFU) and automatically interprets test results.

INSTRUMENTS FOR USE WITH THE TEST

The DxTerity SARS-CoV-2 RT-PCR CE Test is to be used with the following instruments and software **(Table 1).**

Table 1. Instruments and Software

Instrument	Manufacturer	Model	Software/Version
Automated RNA Extraction Instrument	Applied Biosystem	MagMax 96 Magnetic Particle Processor	software BindIt 4.0
RT-PCR Instrument	ThermoFisher	VeritiDx and Veriti PCR Thermal Cycler 4375786 or 4452300	N/A

Capillary electrophoresis Instrument	ThermoFisher	ABI 3500xL Dx Genetic Analyzer (K191030) Catalog #4404688 (IVD instrument)	Data Collection Software Version 3.2

COLLECTION KITS USED WITH THE TEST

This test must be used with the Spectrum Solutions LLC SDNA-1000 Saliva Collection Device to self-collect saliva specimens at home when determined to be appropriate by an HCP.

REAGENTS AND MATERIALS

Table 2. Reagents and Materials for Use with the DxTerity SARS-CoV-2 RT-PCR CE Test

Reagent	Manufacturer	Catalogue #
DxPure Bead Mix	DxTerity Diagnostics	N/A not commercially available
DxBind II	DxTerity Diagnostics	N/A not commercially available
DxPure Wash Buffer	DxTerity Diagnostics	N/A not commercially available
96 well deep well plates	PerkinElmer	43001-0120
TaqPath™ 1-Step Multiplex Master Mix (No ROX)	ThermoFisher	A28523
DxT SARS-CoV-2 Mix 1 – FAM	DxTerity Diagnostics	N/A not commercially available/Proprietary
DxT SARS-CoV-2 Mix 2 – VIC	DxTerity Diagnostics	N/A not commercially available/ Proprietary
DxT SARS-CoV-2 Mix 3 – NED	DxTerity Diagnostics	N/A not commercially/ Proprietary available
DxT SARS-CoV-2 Mix 4 – PET equivalent Atto-565	DxTerity Diagnostics	N/A not commercially available/ Proprietary
Twist Synthetic SARS-CoV-2 RNA Control	Twist Biosciences	102024
SARS-Related Coronavirus 2, Isolate USA-WA1/2020, Gamma-Irradiated	BEI	NR-52287
Positive Control Dilution Buffer	DxTerity Diagnostics	N/A not commercially available
Amplicon Dilution Buffer	DxTerity Diagnostics	N/A not commercially available

96 well PCR plates	Thermo Fisher Scientific	--
Strip caps	Thermo Fisher Scientific	--

Reagent	**Manufacturer**	**Catalogue #**
Nuclease-free water	--	--
Isopropanol, Molecular Biology Grade, > 99.9%	--	--
Ethanol (96-100%)	--	--
GeneScan 600 LIZ Size Standard v2.0	Thermo Fisher Scientific	4482976
GeneScan Installation Standard DS-33	Thermo Fisher Scientific	4482975
DS-33 Matrix Standard-CG (Dye Set G5	Thermo Fisher Scientific	4482974
Hi-Di Formamide	Thermo Fisher Scientific	4404307
POP-7™ Polymer for 3500xL Dx Genetic Analyzers	Thermo Fisher Scientific	A35219 or A34323
3500xL Dx Genetic Analyzer 24-Capillary Array, 50 cm	Thermo Fisher Scientific	4404688
Cathode Buffer Container for 3500xL Dx Genetic Analyzers	Thermo Fisher Scientific	4408258
Anode Buffer Container for 3500xL Dx Genetic Analyzers	Thermo Fisher Scientific	4393925
MicroAmp Optical 96-Well Reaction Plate with Barcode	Thermo Fisher Scientific	4306737
Plate Septa 96-Well	Thermo Fisher Scientific	4315933

CONTROLS

Assay controls are run concurrently with all test samples. The assay positive and negative controls are prepared fresh for each run.

Negative Control: The negative control is nuclease free water, which is combined with stabilization buffer from the saliva collection device and undergoes RNA extraction prior to PCR. PCR of the Negative Control is conducted using the same PCR mix as used for sample testing in a given batch.

Positive Control: The positive control is prepared by diluting Synthetic SARS-CoV-2 RNA Control (Cat# 102024, concentration 1×10^6 copies/µL) from Twist Biosciences or SARS-Related Coronavirus 2, Isolate USA-WA1/2020, Gamma-Irradiated (BEI, Cat# NR-52287) in positive control dilution buffer to get a final concentration of 2 copies/µL. 10 µL (20 copies of RNA) of this solution is used as positive control for the test. The positive control does not undergo RNA extraction. The Positive Control is PCR amplified with either Primer Mix 1, 2, 3 or 4, depending on the Primer Mix used for sample testing. One PC is used per plate with one primer

mix at the time.

Internal Control: The primer set for RNase P, which is contained in each PCR Mix (1-4) amplifies the human RNase P gene in the saliva sample. The primer sets share a common reverse primer and a fluorescently labeled forward primer.

The controls run with the DxTerity SARS-CoV-2 RT-PCR CE Test are described in **Table 3**.

Table 3. External and Internal Controls of the DxTerity SARS-CoV-2 RT-PCR CE Test

Control Type	Purpose	Frequency of Testing
Negative	To monitor for cross-contamination during RNA extraction and RT-PCR	Once per batch of specimens and Run on every plate
Positive	To monitor the integrity of the RT-PCR reagents and process	Every RT-PCR run
RNAse P (Internal Control)	To monitor the integrity of nucleic acid extraction and PCR for each specimen	Included in each sample

INTERPRETATION OF RESULTS

All test controls must be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted. The results from the controls are interpreted according to the criteria shown in **Table 4.**

Table 4: Expected Results for the Assay External Controls.

Control	**Target**			
	N Gene	**ORF1ab**	**E Gene**	**RNase P[1]**
Negative	– ≤1000	– ≤1000	– ≤1000	– ≤1000
Positive[2]	+ ISS > 1000	+ ISS > 2500	+ ISS > 1000	– ISS ≤1000
All other combinations of Negative Control or Positive Control results yield a Batch Fail and require retest. [1]There is no RNase P in the Positive or Negative Controls [2] Any single sample in the reaction plate (Positive Control or clinical sample) that generates positive results for the 3 viral targets is sufficient to meet the requirements of the Positive Control. In the absence of a passing positive control or positive sample meeting the viral target criteria, the entire plate must be repeated				

The results from testing of patient samples are interpreted according to the criteria described in **Table 5**

Table 5. Result Interpretation for Patient Samples

Step	Sum of ORF1ab + E	N Gene	ORF1ab	E Gene	RNaseP[1]	Result[2]	Result Interpretation/Action
1	N/A	—	—	—	—	INVALID RESULT	Repeat test on original sample. If the repeat result remains invalid, consider collecting a new specimen.
		ISS ≤ 1000	ISS ≤ 1000	ISS ≤ 1000	ISS ≤ 1000 (500 for FAM)		
2	ISS > 3000	+	+	+	±	POSITIVE	SARS-CoV-2 Detected Report results.
		ISS > 1000	ISS > 2500	ISS > 1000			
		—	+	+	±		
		ISS ≤ 1000	ISS > 2500	ISS > 1000			
		+	—	+	±		
		ISS > 1000	ISS ≤ 2500	ISS > 1000			
		+	+	—	±		
		ISS > 1000	ISS > 2500	ISS ≤ 1000			
3	ISS ≤ 3000	±	±	±	+	NEGATIVE	SARS-CoV-2 **Not** Detected. Report results. Consider testing for other respiratory viruses
					ISS > 1000 (500 for FAM)		
4	If Conditions in Step 1, Step 2 and Step 3 are not met					INDETERMINATE	Repeat test on original sample. If the repeat result remains Indeterminate, then report the result as "Indeterminate" and consider collecting a new specimen.

[1] RNase P is not required for a Positive Result

[2] If sum of ISS non-specific signal is greater than 50,000 thresholds, report result as "Invalid", regardless of outcome of steps 1-4.

PERFORMANCE EVALUATION

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

The LoD was evaluated using whole viral genomic RNA obtained from BEI Resources (SARS-CoV-2 isolate USA-WA1/2020, Item NR-52347) diluted in SARS-CoV-2 negative saliva samples prior to mixing with Spectrum Solutions LLC SDNA-1000 Saliva Collection Device stabilization buffer.

Preliminary LoD:
An initial estimate of the LoD of each Primer Mix was determined by testing three (3) RNA extraction replicates at each of the four different target levels, 50, 75, 150, and 300 copies/mL, of the undiluted saliva sample. For each dilution, the PCR amplicon of all four (4) Primer Mixes were combined for analysis on the ABI 3500 ABI 3500xL Dx Genetic Analyzer.

The presumptive LoD (determined as the lowest level at which all three replicates of a Primer Mix were positive for SARS-CoV-2) was **300 copies/mL** for Primer Mix-1, 2, 150copies/mL for Primer Mix-3 and 75 copies/mL for Primer Mix- 4. A single replicate was determined as retest at the 150 copies/mL condition for Primer Mix-1, 2 and 4 colors.

Spiked saliva specimens were tested according to the DxTerity SARS-CoV-2 RT-PCR CE Test protocol.

Confirmation of the LoD:
The estimated LoD was confirmed by testing an additional 20 replicates at 150, 50, 75 and 100 copies/mL.

For all dilutions for the confirmatory LoD testing, the PCR amplicons of all four (4) Primer Mixes were combined for analysis on the ABI 3500xL Dx Genetic Analyzer.

The lowest concentration in the confirmatory LoD testing at which 19 out of the 20 replicates generated SARS-CoV-2 positive results for all Primer Mixes was 150 copies/mL Primer Mix-1, 3 and 4 and 100 copies/mL for Mix-2, so the LoD was therefore confirmed to be **150 copies/mL in undiluted saliva**. **Table 6** below shows the final Limit of detection for the different primer mixes used with the DxTerity SARS-CoV-2 RT PCR Test.

Table 6. Summary of overall results from LoD Confirmation by **Primer Mix.**

Primer Mix 1 FAM						
Transcript Copies/mL (undiluted saliva)	**Number Tested**	**Overall Percent Positive**	**# Detected/ # Tested by Target Genes**			
			N Gene	**ORF1ab**	**E Gene**	**RNase P**
NTC	1	0 (0/1)	0 (0/1)	0 (0/1)	0 (0/1)	0 (0/1)
PTC	1	100 (1/1)	100 (1/1)	100 (1/1)	100 (1/1)	0 (0/1)
50	20	0 (0/20)	15 (3/20)	50 (10/20)	5 (1/20)	100 (20/20)

Primer Mix 1 FAM						
Transcript Copies/mL (undiluted saliva)	**Number Tested**	**Overall Percent Positive**	**# Detected/ # Tested by Target Genes**			
			N Gene	**ORF1ab**	**E Gene**	**RNase P**
75	20	45 (9/20)	35 (7/20)	70 (14/20)	50 (10/20)	100 (20/20)
100	20	60 (12/20)	75 (15/20)	75 (15/20)	35 (7/20)	100 (20/20)
150	**20**	**100 (20/20)**	**100 (20/20)**	**100 (20/20)**	**85 (17/20)**	**100 (20/20)**

Primer Mix 2 VIC						
Transcript Copies/mL (undiluted saliva)	**Number Tested**	**Overall Percent Positive**	**# Detected/ # Tested by Target Genes**			
			N Gene	**ORF1ab**	**E Gene**	**RNase P**
NTC	1	0 (0/1)	0 (0/1)	0 (0/1)	0 (0/1)	0 (0/1)
PTC	1	100 (1/1)	100 (1/1)	100 (1/1)	100 (1/1)	0 (0/1)
50	20	55 (11/20)	75 (15/20)	60 (12/20)	40 (8/20)	90 (18/20)
75	20	80 (16/20)	95 (19/20)	75 (15/20)	20 (4/20)	95 (19/20)
100	20	95 (19/20)	95 (19/20)	90 (18/20)	45 (9/20)	100 (20/20)
150	20	100 (20/20)	100 (20/20)	100 (20/20)	90 (18/20)	100 (20/20)

Primer Mix 3 NED						
Transcript Copies/mL (undiluted saliva)	**Number Tested**	**Overall Percent Positive**	**# Detected/ # Tested by Target Genes**			
			N Gene	**ORF1ab**	**E Gene**	**RNase P**
NTC	1	0 (0/1)	0 (0/1)	0 (0/1)	0 (0/1)	0 (0/1)
PTC	1	100 (1/1)	100 (1/1)	100 (1/1)	100 (1/1)	0 (0/1)
50	20	35 (7/20)	60 (12/20)	45 (9/20)	40 8/20	100 (19/20)
75	20	65 (13/20)	80 (16/20)	70 (14/20)	50 (10/20)	100 (20/20)
100	20	85 (17/20)	95 (19/20)	80 (16/20)	75 (15/20)	100 (20/20)
150	**20**	**100 (20/20)**	**100 (20/20)**	**100 (20/20)**	**90 (18/20)**	**100 (20/20)**

Primer Mix 4 PET						
Transcript Copies/mL (undiluted saliva)	**Number Tested**	**Overall Percent Positive**	**# Detected/ # Tested by Target Genes**			
			N Gene	**ORF1ab**	**E Gene**	**RNase P**
NTC	1	0 (0/1)	0 (0/1)	0 (0/1)	0 (0/1)	0 (0/1)
PTC	1	100 (1/1)	100 (1/1)	100 (1/1)	100 (1/1)	0 (0/1)
50	20	40 (8/20)	60 (12/20)	45 (9/20)	40 (8/20)	95 (19/20)
75	20	90 (18/20)	90 (18/20)	85 (17/20)	90 (18/20)	100 (20/20)
100	20	90 (18/20)	85 (17/20)	90 (18/20)	75 (15/20)	100 (20/20)
150	**20**	**95 (19/20)**	**95 (19/20)**	**95 (19/20)**	**90 (18/20)**	**95 (19/20)**

2) Inclusivity (analytical sensitivity):

The DxTerity SARS-CoV-2 RT-PCR CE Test targets specific genomic regions of the SARS-CoV-2 nucleocapsid (N) gene, ORF1ab region, and Envelope (E) gene. Inclusivity was demonstrated by performing BLAST alignment of the primers against the SARS- CoV-2 (taxid:2697049) data set. The resulting alignments were filtered for complete SARS-CoV-2 genomes (sequence length between 29.6 kb to 30.1 kb) available in the GenBank databases as of January 14, 2021. Primer mismatches based on available data in GenBank database as of January 14, 2021 are summarized in **Table 7**. The mismatch frequencies identified for the individual targets are: N = 0.2%, ORF1ab = 0.2 % and E = 0.04%. No SARS-CoV-2 sequence shows mutation(s) in BOTH the N1 and N2 targets. In instances where the Tm of the mismatched primer is higher than the assay annealing temperature of 60°C, the mismatch is unlikely to have a significant impact on sequence detection. Cases where the Tm of the mismatched primer is lower than the assay annealing temperature, for any given strain, mismatches have only been observed in a single primer from a single target, thus there is a low risk of false negative result because loss of all three targets is necessary to generate a negative result.

Table 7: Summary of Primer Mismatches

Primer ID	Primer Length (bp)	Number of Mismatches	Mismatch	Location	No. of GenBank Strains with Mismatch	GenBank Accession Numbers	Exact Tm (°C)	*[1]Mismatch Tm (°C)
N_F	23	1	C-A	3'	4	MT560706, MT509959, MT293178, MW433757	62.3	56
		1	C-T	3'	4	MT786799, MT786800, LR962951, LR962937	62.3	55.2
		1	C-T	3'	1	MT745678	62.3	54.6
		1	G-A	3’	1	MW280179	62.3	59.0
		1	A-G	Middle	1	MT259269	62.3	59.2
		1	G-T	Middle	4	MT259237, MT740431, MW342094, MW332997	62.3	57.9
		1	T-C	Middle	1	MW369388	62.3	60.6
		3	**GAT-CTA (mismatch associated with the B.1.1.7 Variant (UK)**	Middle	12	MW422256, MW423686, MW440433, MW450666, LR991699, LR991698, MW451205, MW422255 MW430966, MW430974, MW430968, MW430967	62.3	59.3
		1	C-A	5’	1	MW326521	62.3	56.8
N_R	19	1	A-G	3'	2	MT520478, MT520290	64.3	60.1
		1	C-T	5'	1	MT259274	64.3	63.1
		1	C-A	5'	4	MT683418, MT683417, MT683416 MW436738	64.3	63.1
		1	A-G	5'	1	MT750164	64.3	58.1
		1	C-A	Middle	2	MT435081, MT435082	64.3	57.8
		1	G-A	Middle	2	MT745654, MW340736	64.3	58.8
		1	C-A	Middle	1	MT628116	64.3	55.4
		1	T-G	Middle	2	MT535500, MW449314	64.3	59.2
		1	C-G	Middle	1	MT759844	64.3	56.8
		1	T-C	Middle	1	MW449515	64.3	56.8

Primer ID	Primer Length (bp)	Number of Mismatches	Mismatch	Location	No. of GenBank Strains with Mismatch	GenBank Accession Numbers	Exact Tm (°C)	*[1]Mismatch Tm (°C)
		1	C-A	3'	2	MT772433, MW321394	64.3	56.5
		1	C-G	3'	3	MT642410, MW369344, MW321360	64.3	57.7
		1	G-R	Middle	1	MT184913	64.3	64.3/59.3
Orf1ab_F	25	1	C-T	5'	2	MT820131, MT806776	66.2	60.5
		1	T-G	Middle	1	MT755896	66.2	61.6
		1	T-C	5’	1	MW341465	66.2	64.8
		1	G-T	Middle	5	MT601281, MT451726, MT773134, MT772297, MT676388	66.2	62.3
		1	C-T	3'	2	MT642225, MW276212	66.2	62.5
		1	G-K	Middle	2	MT614595, MT614347	66.2	66.2/62.3
Orf1ab_R	24	1	C-A	5'	2	MT461651, MT795873	65.5	64.5
		2	G-A, A-G	3'	1	MT641528	65.5	61.8
		1	A-G	3'	18	MT642411, MT642346, MT642259, MT641592, MT641590, MT641582, MT641564, MT641560, MT641555, MT641553, MT641549, MT641538, MT641529, MT641511, MT632979, MT632890, MT632872, MT632841	65.5	62.5
		1	T-A	3’	1	MW321351	65.5	62.3
		1	G-A	3’	1	MW277092	65.5	62.3
		1	C-T	Middle	1	MW369399	65.5	61.7
		1	G-A	Middle	2	MW449310, MW449493	65.5	62
		1	A-G	Middle	1	MW321356	65.5	63

Primer ID	Primer Length (bp)	Number of Mismatches	Mismatch	Location	No. of GenBank Strains with Mismatch	GenBank Accession Numbers	Exact Tm (°C)	*[1]Mismatch Tm (°C)
		2	C-A, G-A	Middle	1	MW321407	65.5	60.2/ 62.3
		1	G-C/T	5’	1	MW321318	65.5	61.3/ 61.8
E_F	24	1	G-C	5'	1	MT632491	62.7	60.1
		1	G-A	Middle	1	MT598173	62.7	58.4
		1	C-T	Middle	1	MT628097	62.7	58.3
		1	G-T	Middle	1	MT731733	62.7	59.7
		1	G-K	5'	7	MT451436, MT451386, MT451358, MT451279, MT451276, MT451269, MT451259	62.7	62.7/57.2
E_R	26	1	C-A	3’	1	MW369373	63.9	58.8
		1	G-A	5’	1	MW321325		60.3
		1	G-T	Middle	1	MW321427		61.4

* Mismatch Tm lower than annealing temperature highlighted in yellow
[1]Mismatch Tm calculated using the Oligo Analyzer tool available through Integrated DNA Technologies (https://www.idtdna.com/calc/Analyzer/Home/Instructions)

3) Cross-reactivity (Analytical Specificity)

The analytical specificity of the DxTerity SARS-CoV-2 RT-PCR CE Assay was demonstrated *in silico* and the analysis included evaluation of the primer homology with the 43 organisms and viruses listed in **Table 8**.

The *in silico* analysis demonstrated that the assay does not cross react with any organisms/strains except some closely related SARS coronavirus and bat coronavirus RaTG13 (Table 9). However, since the prevalence of these strains is very low, and contamination of saliva with bat coronavirus is not expected, the risk of cross-reacting (false positive) is very low.

The analysis also demonstrated a homology of the RNase P gene forward primer with *Candida albicans, Haemophilus influenzae*, and *Moraxella catarrhalis.* However, these species are not expected to produce a detectable PCR product as there is no homology between the RNase P reverse primer and these organisms. There is no risk of reporting SARS-CoV-2 false positive results because of this homology.

Table 8. Organisms and viruses evaluated for potential cross-reaction and/or interference with the DxTerity SARS-CoV-2 RT-PCR CE Assay

Viruses	Bacteria
Adenovirus	*Bacillus anthracis*

Enterovirus	*Bordetella pertussis*
Human coronavirus 229E	*Chlamydophila pneumoniae*
Human coronavirus HKU1	*Chlamydophila psittaci*
Human coronavirus NL63	*Corynebacterium diphtheriae*
Human coronavirus OC43	*Coxiella burnetii*
Human Metapneumovirus (hMPV)	*Haemophilus influenzae*
Influenza A, B and C	*Legionella* (non-pneumophila)
MERS-coronavirus	*Legionella pneumophila*
Parainfluenza 1-4	*Leptospira* sp.
Parechovirus	*Moraxella catarrhalis*
Respiratory Syncytial Virus A and B	*Mycobacterium tuberculosis*
Rhinovirus/Enterovirus	*Mycoplasma pneumoniae*
SARS-coronavirus	*Neisseria elongata* and *Neisseria*
Yeast/Fungus	*Pseudomonas aeruginosa*
Candida albicans	*Staphylococcus aureus*
Pneumocystis jirovecii	*Staphylococcus epidermidis*
	Streptococcus pneumoniae
	Streptococcus pyogenes
	Streptococcus salivarius

Table 9. *Summary of* In Silico *Analytical Exclusivity Analysis*

Gene	Primer	>80% Homology
N	F	SARS coronavirus
	R	SARS coronavirus
E	F	SARS coronavirus
	R	SARS coronavirus
Orf1ab	F	SARS coronavirus
	R	Bat coronavirus RaTG13
RNase P	F	Candida albicans
	R	Haemophilus influenzae, Moraxella catarrhalis

4) ***<u>Competitive Interference</u>***

Lack of competitive interference was demonstrated when combining amplicons from a high positive sample with either a low positive or negative sample. No interference was observed when amplicons from the saliva specimens were combined for analysis on the Genetic Analyzer.

Competitive Interference was evaluated using whole viral genomic RNA obtained from BEI Resources (Genomic RNA from SARS-Related Coronavirus 2, Item NR-52285) diluted in SARS-CoV-2 negative saliva samples collected into the Spectrum Solutions LLC SDNA- 1000 Saliva Collection Device. The pooled negative saliva samples were used to contrive high positive (100x LoD), low positive (3x LoD) and negative samples. The four (4) Primer Mixes were each tested with the low positive (3x LoD), high

positive (100x LoD) and negative samples. Each Primer Mix test combination (**Table 10**), consisted of equal proportions of the four (4) PCR amplicons, one from each Primer Mix generated using a low positive, high positive and negative sample, and combined for analysis on the Genetic Analyzer. Each of these combinations were tested in three (3) PCR replicates. The controls for each Primer Mix, without the PCR amplicon from the interfering high positive sample were used as baseline reference controls. All 3 replicates of the control combination were required to be SARS-CoV-2 positive.

All low positive and high positive samples were detected for all 4 primer mixes for all three replicates. All the expected negative samples were correctly called negative. Thus, no interference was observed when amplicons from the saliva specimens were combined for analysis on the Genetic Analyzer.

Table 10: Summary of Overall results from Competitive Interference Study by Primer Mix

Primer Mix 1 FAM						
Sample Concentration	**Number Tested**	**Overall Percent Positive**	**# Detected / # Tested by Target Genes**			
			N Gene	**ORF1 ab**	**E Gene**	**RNase P**
LP (3X LoD)	12	100 (12/12)	12/12	12/12	12/12	12/12
HP (100X LoD)	9	100 (9/9)	9/9	9/9	9/9	9/9
NEG	27	0 (0/27)	0/27	0/27	0/27	27/27

Primer Mix 2 VIC						
Sample Concentration	**Number Tested**	**Overall Percent Positive**	**# Detected / # Tested by Target Genes**			
			N Gene	**ORF1 ab**	**E Gene**	**RNase P**
LP (3X LoD)	12	100 (12/12)	12/12	12/12	12/12	12/12
HP (100X LoD)	9	100 (9/9)	9/9	9/9	9/9	9/9
NEG	27	0 (0/27)	0/27	0/27	0/27	27/27

Primer Mix 3 NED						
Sample Concentration	**Number Tested**	**Overall Percent Positive**	**# Detected/ # Tested by Target Genes**			
			N Gene	**ORF1 ab**	**E Gene**	**RNase P**
LP (3X LoD)	12	100 (12/12)	12/12	12/12	12/12	12/12
HP(100X LoD)	9	100 (9/9)	9/9	9/9	9/9	9/9

NEG	27	0 (0/27)	0/27	0/27	0/27	27/27

Primer Mix 4 PET						
Sample Concentration	**Number Tested**	**Overall Percent Positive**	**# Detected / # Tested by Target Genes**			
			N Gene	**ORF1 ab**	**E Gene**	**RNase P**
LP (3X LoD)	12	100 (12/12)	12/12	12/12	12/12	12/12
HP (100X LoD)	9	100 (9/9)	9/9	9/9	9/9	9/9
NEG	27	0 (0/27)	0/27	0/27	0/27	27/27

5) Carryover and Cross-Contamination

Carryover and Cross-Contamination from both the extraction as well as the PCR and capillary electrophoresis steps was determined using a checkboard layout of negative and high positive (10x LoD) samples. The high positive sample was contrived using whole viral genomic RNA obtained from BEI Resources (Genomic RNA from SARS-Related Coronavirus 2, Item NR-52285) diluted in SARS-CoV-2 negative saliva samples collected into the Spectrum Solutions LLC SDNA-1000 Saliva Collection Device.

A total of 24 samples (12 high positive and 12 negative) were extracted in an alternating pattern and then the resulting RNA was processed through PCR and capillary electrophoresis in the alternating pattern in a 96-well plate. For each sample replicate (well), the PCR amplicon of all four (4) Primer Mixes were combined for analysis on the ABI 3500 ABI 3500xL Dx Genetic Analyze. A single CE injection consists of 24 samples, thus the 96 well plate evaluates potential carryover from earlier injections.

The results were 100% concordant with the expected results, as shown in **Table 11**. All 48 High positive and 48 negative samples were correctly called, thus there is no carryover or cross-contamination observed from any step of the assay process.

Table 11: Carry Over and Cross-Contamination Study Results

Primer Mix 1 FAM							
Sample Description	**Transcript Copies/mL**	**Number Tested**	**Overall Percent Positive**	**# Detected / # Tested by Target Genes**			
				N Gene	**ORF1ab**	**E Gene**	**RNase P**
Negative	0	48	0 (0 /48)	0 (0/48)	0 (0/48)	0 (0/48)	100 (48/48)
High Positive (10X LoD)	500	48	100 (48/48)	100 (48/48)	100 (48/48)	100 (48/48)	100 (48/48)

Primer Mix 2 VIC							
Sample Description	**Transcript Copies/mL**	**Number Tested**	**Overall Percent Positive**	**# Detected / # Tested by Target Genes**			
				N Gene	**ORF1ab**	**E Gene**	**RNase P**
Negative	0	48	0 (0 /48)	0 (0/48)	0 (0/48)	0 (0/48)	100 (48/48)
High Positive (10X LoD)	500	48	100 (48/48)	100 (48/48)	100 (48/48)	100 (48/48)	100 (48/48)

Primer Mix 3 NED							
Sample Description	**Transcript Copies/mL**	**Number Tested**	**Overall Percent Positive**	**# Detected / # Tested by Target Genes**			
				N Gene	**ORF1ab**	**E Gene**	**RNase P**
Negative	0	48	0 (0 /48)	0 (0/48)	0 (0/48)	0 (0/48)	100 (48/48)

Primer Mix 3 NED							
Sample Description	**Transcript Copies/mL**	**Number Tested**	**Overall Percent Positive**	**# Detected / # Tested by Target Genes**			
				N Gene	**ORF1ab**	**E Gene**	**RNase P**
High Positive (10X LoD)	500	48	100 (48/48)	100 (48/48)	100 (48/48)	100 (48/48)	100 (48/48)

Primer Mix 4 PET							
Sample Description	**Transcript Copies/mL**	**Number Tested**	**Overall Percent Positive**	**# Detected / # Tested by Target Genes**			
				N Gene	**ORF1ab**	**E Gene**	**RNase P**
Negative	0	48	0 (0 /48)	0 (0/48)	0 (0/48)	0 (0/48)	100 (48/48)
High Positive (10X LoD)	500	48	100 (48/48)	100 (48/48)	100 (48/48)	100 (48/48)	100 (48/48)

6) Clinical Evaluation

Contrived Testing:

The clinical performance of the DxTerity SARS-CoV-2 RT-PCR CE Test was first evaluated using negative saliva specimen collected in the Spectrum Solution LLC SDNA-100 Collection device and spiked with quantified SARS-CoV-2 genomic RNA (BEI Resources).

A total of 30 individual negative clinical saliva and 30 positive contrived saliva samples

were tested. Of the 30 contrived positive samples, 10 were prepared at 1.4X LoD (75 copies/mL), 10 samples were spiked at 2X LoD (100 copies/mL), five samples at 3X LoD (150 copies/mL) and 5 samples were spiked at 5X LoD (250 copies/mL) spanning the assay detection range. Each sample was tested with all four Primer Mixes and the PCR amplicons of a sample was combined for analysis on the Genetic Analyzer.

The results of all tested levels for spiked positive in clinical matrix demonstrated 100% agreement with expected results and all negative samples were non-reactive. A summary of the results for each primer mix is provided in **Table 12**

Table 12. Summary of results from the contrived specimen study with Saliva Samples by Primer Mix, stratified by target level and measurand.

Primer Mix 1-FAM						
Transcript Copies/mL	**Number Tested**	**Overall Percent Positive**	**# Detected / # Tested by Target Genes**			
			N Gene	**ORF1ab**	**E Gene**	**RNase P**
0	30	0 (0/30)	0 (0/30)	0 (0/30)	0 (0/30)	100 (30/30)
75	10	100 (10/10)	100 (10/10)	100 (10/10)	90 (9/10)	100 (10/10)
100	10	100 (10/10)	80 (8/10)	100 (10/10)	100 (10/10)	100 (10/10)
150	5	100 (5/5)	100 (5/5)	100 (5/5)	100 (5/5)	100 (5/5)
250	5	100 (5/5)	100 (5/5)	100 (5/5)	100 (5/5)	100 (5/5)
All Positives	30	100 (30/30)	100 (30/30)	100 (30/30)	100 (30/30)	100 (30/30)
Percent Positive Agreement:		100% 95% CI: 88.6-100%				
Negative Percent Agreement		100% 95% CI: 88.6-100%				

Primer Mix 2-VIC						
Transcript Copies/mL	**Number Tested**	**Overall Percent Positive**	**# Detected / # Tested by Target Genes**			
			N Gene	**ORF1ab**	**E Gene**	**RNase P**
0	30	0 (0/30)	0 (0/30)	0 (0/30)	0 (0/30)	100 (30/30)
75	10	100 (10/10)	100 (10/10)	100 (10/10)	80 (8/10)	100 (10/10)
100	10	100 (10/10)	90 (9/10)	100 (10/10)	90 (9/10)	100 (10/10)

150	5	100 (5/5)	100 (5/5)	100 (5/5)	100 (5/5)	100 (5/5)
250	5	100 (5/5)	100 (5/5)	100 (5/5)	100 (5/5)	100 (5/5)
All Positives	30	100 (30/30)	100 (30/30)	100 (30/30)	100 (30/30)	100 (30/30)
Percent Positive Agreement:		100% 95% CI: 88.6-100%				

Primer Mix 2-VIC						
Transcript Copies/mL	**Number Tested**	**Overall Percent Positive**	**# Detected / # Tested by Target Genes**			
			N Gene	**ORF1ab**	**E Gene**	**RNase P**
Negative Percent Agreement		100% 95% CI: 88.6-100%				

Primer Mix 3-NED						
Transcript Copies/mL	**Number Tested**	**Overall Percent Positive**	**# Detected / # Tested by Target Genes**			
			N Gene	**ORF1ab**	**E Gene**	**RNase P**
0	30	0 (0/30)	0 (0/30)	0 (0/30)	0 (0/30)	100 (30/30)
75	10	100 (10/10)	100 (10/10)	90 (9/10)	90 (9/10)	100 (10/10)
100	10	100 (10/10)	90 (9/10)	100 (10/10)	90 (9/10)	100 (10/10)
150	5	100 (5/5)	100 (5/5)	100 (5/5)	100 (5/5)	100 (5/5)
250	5	100 (5/5)	100 (5/5)	100 (5/5)	100 (5/5)	100 (5/5)
All Positives	30	100 (30/30)	100 (30/30)	100 (30/30)	100 (30/30)	100 (30/30)
Percent Positive Agreement:		100% 95% CI: 88.6-100%				
Negative Percent Agreement		100% 95% CI: 88.6-100%				

Primer Mix 4-PET						
Transcript Copies/mL	**Number Tested**	**Overall Percent Positive**	**# Detected / # Tested by Target Genes**			
			N Gene	**ORF1ab**	**E Gene**	**RNase P**
0	30	0 (0/30)	0 (0/30)	0 (0/30)	0 (0/30)	100 (30/30)
75	10	100 (10/10)	80 (8/10)	100 (10/10)	80 (8/10)	100 (10/10)
100	10	100 (10/10)	90 (9/10)	100 (10/10)	90 (9/10)	100 (10/10)
150	5	100 (5/5)	100 (5/5)	100 (5/5)	100 (5/5)	100 (5/5)

Primer Mix 4-PET						
Transcript Copies/mL	**Number Tested**	**Overall Percent Positive**	**# Detected / # Tested by Target Genes**			
			N Gene	**ORF1ab**	**E Gene**	**RNase P**
250	5	100 (5/5)	100 (5/5)	100 (5/5)	100 (5/5)	100 (5/5)
All Positives	30	100 (30/30)	100 (30/30)	100 (30/30)	100 (30/30)	100 (30/30)
Percent Positive Agreement:		100% 95% CI: 88.6-100%				
Negative Percent Agreement		100% 95% CI: 88.6-100%				

Paired Saliva and NP Swab Clinical Study

Clinical evaluation was also performed with natural prospectively collected clinical samples to evaluate the use of saliva as a specimen type for detection of SARS-CoV-2 in a population representative of the intended use population from individuals suspected of COVID-19 by their healthcare provider as well as individuals without any symptoms.

Paired NP swab and saliva collections were performed under an IRB approved protocol and informed consent and either purchased from The MT Group (Van Nuys, CA) with samples collected in California and Florida or obtained at “pop-up” testing clinics throughout the greater Los Angeles area and Phoenix, Arizona.

A total of 621 paired NP swab and saliva specimens were collected prospectively at multiple sites. This study included various ethnic groups and ages.

NP swab specimens (Hardy Diagnostics, Cat. No. 972912) were collected by registered nurses, placed into a sterile 0.9% sodium chloride solution (Teknova, Cat. No. S5820)

and shipped on cold packs to Med Fusion Quest Laboratory (Lewisville, TX) for testing with an EUA authorized test.

Following collection of the nasopharyngeal swab, within an hour subjects self-collected a 2 mL saliva sample using the Spectrum Solutions SDNA-1000 Saliva Collection Device. Saliva samples were transported at ambient temperature to DxTerity Diagnostics (Rancho Dominguez, CA) for testing with the DxTerity SARS-CoV-2 RT-PCR CE Test.

A summary of the results of the study is presented in **Table 13C** and **Table 13D** for symptomatic and asymptomatic individuals respectively.

Based on NP swab results, there are 63 positive samples of which 37 positives were from symptomatic individuals and 26 positives from asymptomatic individuals. The median and mean Ct values for symptomatic and asymptomatic were similar as shown in **Table 13A** below.

Table 13A: Ct distribution in Symptomatic and Asymptomatic Populations

FDA authorized comparator assay		Mean Ct Target 1	Mean Ct Target2	Median Ct target1	Median Ct Target2
NP Positive specimens	Symptomatic, (37 positive samples)	28.99	29.50	30.44	31.49
	Asymptomatic (26 positive samples)	28.69	29.74	30.43	31.07

Also, both symptomatic and asymptomatic individuals have similar proportion of specimens with a viral load close to the LoD for NP swabs as determined by Ct values obtained with the FDA comparator assay (**Table 13B**).

Table 13B. Percent of Positive Individuals with Low Viral Load

	Target 1, Ct >31	Target 2, Ct>34
Symptomatic, 37 positive NPS samples	48.6% (18/37)	29.7% (11/37)
Asymptomatic, 26 positive NPS individuals	42.3% (11/26)	34.6% (9/26)
Difference	6.3%, 95%CI: (-17.8%; 29.1%) Not stat. significant	-4.9% 95%CI: (-27.6%; 17.2%) Not stat. significant

Table 13C. Summary of qualitative results obtained from parallel testing of nasopharyngeal swab samples and saliva from individuals suspected of COVID-19 (symptomatic)

DxTerity SARS-CoV-2 RT-PCR CE Test		Nasopharyngeal Swab (FDA EUA))		
		Positive	Negative	Total
Saliva	Positive	36	4*	40
	Negative	1	36	37

	Total	37	40	77
Positive Percent Agreement		97.3% (36/37) [86.2%-99.5%]		
Negative Percent Agreement		90% (36/40) [76.9%-96%]		

*4 of the 4 negative NPS samples as determined by the FDAEUA comparator test were also negative by anotherFDA authorized test
3 of the 4 false positive saliva samples were also positive by an FDA authorized Test

For individuals suspected of COVID-19 by their healthcare provider (symptomatic), there was 97.3% positive percent agreement between the results obtained from testing of saliva and those obtained from nasopharyngeal swabs. There were four symptomatic individuals who tested positive by DxTerity on saliva specimen but were negative by FDA EUA comparator test on NP specimen yielding an NPA of 90%. The negative percent agreement was further evaluated in an FDA-agreed upon post-authorization study (see pages 24 and 25).

Table 13D. Summary of qualitative results obtained from parallel testing of nasopharyngeal and swab samples and saliva from asymptomatic individuals

DxTerity SARS-CoV-2 RT-PCR CE Test		Nasopharyngeal Swab (FDA authorized Test)		
		Positive	Negative	Total
Saliva	Positive	22	5	27
	Negative	4[1]	513	517
	Total	26	518	544
Positive Percent Agreement		84.6% (22/26) [66.5%-93.8%]		
Negative Percent Agreement		99% (513/518) [97.8%-99.6%]		

[1] For the 4 samples which were negative by the DxTerity SARS-CoV-2 RT-PCR Test assay and positive by the NP swab with the EUA comparator test (Ct values are provided in **Table 13E** below). 2 of these 4 samples were negative upon confirmatory testing with the FDA authorized EUA comparator assay, with one of these also negative by another EUA test.

For asymptomatic individuals, there was 99.0% negative agreement between the results obtained from testing of saliva and those obtained from nasopharyngeal swabs. There were four asymptomatic individuals which tested negative by the DxTerity SARS-CoV-2 RT-PCR test and positive by the NP swab with the FDA EUA comparator test. The four samples had low viral loads at the limit of detection for the FDA EUA comparator assay.

The Ct values for the 4 NP positive samples that were negative by saliva are provided in **Table 13E**

Table 13E. Ct values of 4 NP Swab Positive/Saliva Negative Specimens:

NP Samples	Target 1Ct	Target 2 Ct
1	45	38.1
2	35.11	38.24

3	32.16	34.71
4	34.13	36.51
Mean Ct	33.80	36.89
Median Ct	34.62	37.305

The DxTerity SARS-CoV2 RT-PCR CE Test was further evaluated in an FDA agreed upon post authorization paired NP and saliva clinical study, which included 201 paired specimens (36 symptomatic, 165 asymptomatic) collected prospectively at multiple sites using the same collection procedure as in the original clinical evaluation study (see page 20 above). Collected NP swab specimens were tested with an FDA authorized comparator, and collected saliva specimens were shipped to Dxterity Diagnostics for testing with the SARS-CoV-2 RT-CR CE Test. Results from this post-authorization study were analyzed as their own cohort (201 samples, see Table 13F) and combined with 34 symptomatic paired specimens from the original clinical study (see page 20 above) from sites at which specimens were not pre-selected (235 specimens, see Table 13G). Negative percent agreement was determined to be 95.8% for the post-authorization specimens and 95.5% for the combined data set, while positive percent agreement was 97.2% and 96.4%, respectively.

Table 13F. Summary of qualitative results obtained from parallel testing of nasopharyngeal swab samples and saliva samples from post-authorization study

DxTerity SARS-CoV-2 RT-PCR CE Test		**Nasopharyngeal Swab (EUA Authorized comparator)**		
		Positive	**Negative**	**Total**
Saliva	**Positive**	35	7[a]	42
	Negative	1[b]	158	159
	Total	36	165	201
Positive Percent Agreement (PPA)		97.2% (35/36) [85.8%-99.5%]		
Negative Percent Agreement (NPA)		95.8% (158/165) [91.5%-97.9%]		

[a]Of the 7 discordant samples (negative by NP swab, positive by saliva), 2 were from symptomatic subjects, one of whom tested positive 4 days later and the other exposed their spouse, who subsequently tested positive. Re-testing of these 2 discordant NP swab samples with another FDA authorized comparator yielded 1 positive and 1 negative result. The 5 additional discordant samples (negative by NP swab, positive by saliva) were from asymptomatic subjects and all the NP swab samples yielded negative results when evaluated by an alternative FDA authorized comparator.

[b]1 discordant sample (negative via saliva, positive via NP swab) was from an asymptomatic individual, who yielded a positive result when their NP swab was evaluated with an alternative FDA authorized comparator.

Table 13G. Summary of qualitative results obtained from parallel testing of nasopharyngeal swab samples and saliva samples from post-authorization study plus non-CRO specimens from original clinical evaluation study

DxTerity SARS-CoV-2 RT-PCR CE Test		Nasopharyngeal Swab (EUA Authorized comparator)		
		Positive	**Negative**	**Total**
Saliva	**Positive**	54	8[b,c]	62
	Negative	2[a]	171	173
	Total	56	179	235
Positive Percent Agreement (PPA)		96.4% (54/56) [87.9%-99%]		
Negative Percent Agreement (NPA)		95.5% (171/179) [91.4%-97.7%]		

[a]1 of 2 discordant NP swab samples (negative by saliva, positive by NP swab) was positive when evaluated with an alternative FDA authorized test. The other discordant NP swab sample was negative when evaluated with the same alternative FDA authorized test.

[b]1 of 8 discordant NP swab samples was negative by the comparator assay, but positive when evaluated with an alternative FDA authorized test.

[c]6/8 discordant saliva samples (negative via NP swab, positive via saliva) were also positive with an alternative FDA authorized assay.

7) Simulated Shipping Study with the SDNA-1000 Saliva Collection Device

To support the stability for samples collected at home using the Spectrum Solutions LLC SDNA-1000 Saliva Collection Device for 72 hours, a Simulated Shipping Study was performed that was designed to evaluate the effect of temperature variation on the stability of SARS-CoV-2 RNA during transport of saliva specimens for an extended duration.

The study was conducted using samples contrived by spiking of inactivated SARS-CoV-2 virus obtained from BEI Resources (Irradiated, Novel Coronavirus, 2019-nCoV/USA-WA1/2020, Item NR-52287) diluted in SARS-CoV-2 negative saliva samples. A separate set of samples were prepared for the summer and winter profiles. Due to the nature of end-point PCR detection used in the DxTerity SARS-CoV-2 RT-PCR CE Test, signals generated from low positive samples may overlap in magnitude with those generated from high positives. Consequently, it is not possible to use signal threshold criteria to categorize clinical samples as low-positive and high-positive. Thus, the SARS-CoV-2 positive specimens were prepared to target Ct values associated with 2X LOD for Low Positives and 5-10X LOD for high positives with the EUA Authorized Test upon initial testing.

Target Range of Ct values based on FDA EUA Comparator				
Sample Description	**# Samples**	**Target 1**	**Target 2**	**S gene**
Low Positive (2x LoD)	20	≥ 31	≥ 33	≥ 32
High Positive (5-10x LoD)	10	28 – 30	29 – 32	29 – 31

The saliva specimens subjected to the thermal profiles outlined in **Table 14D** and **Table 14E** below were intended to simulate the extreme temperature conditions that may be experienced in shipment of specimens during the summer and winter, respectively. After being subjected to each thermal profile (either summer or winter), the samples were tested after 80 hours of total incubation time with the DxTerity's SARS-CoV-2 RT-PCR CE Test the results obtained were compared to initial testing (T0).

A summary of the observed detection for each SARS-CoV-2 specific target gene is provided in **Table 14F.**

Table 14D. Summer Temperature Simulated Shipping Conditions

Temperature (°C)	Cycle Period	Time (hours)	
		Cycle Period	Total Time [1]
40 (39.5-40.70)	1	8	8
22 (20.47-21.83)	2	4	12
40 (39.5-40.70)	3	6	18
30 (27.91 -21.83)	4	56	74
40 (39.5-40.70)	5	6	80

[1]Sum of Cycle Periods

Table 14E. Winter Temperature Simulated Shipping Conditions

Temperature (°C)	Cycle Period	Time (hours)	
		Cycle Period	Total Time [1]
-10 (-15.00-7.61)	1	8	8
18 (16.96-17.51)	2	4	12
-10 (-15.00-7.61)	3	6	18
10 (8.32-10.18)	4	56	74
-10 (-15.00-7.61)	5	6	80

[1]Sum of Cycle Periods

Table 14F. Summary of results from the Simulated Shipping Study with the SDNA-1000 Saliva Collection Device

Sample Group	Test Point	Number Tested	Overall Percent Positive (%)	# Detected / # Tested by Target Genes			
				N Gene	ORF1ab	E Gene	RNase P
NTC	T = 0	1	0 (0/1)	0/1	0/1	0/1	0/1
	Summer	1	0 (0/1)	0/1	0/1	0/1	0/1
	T = 0	1	0 (0/1)	0/1	0/1	0/1	0/1
	Winter	1	0 (0/1)	0/1	0/1	0/1	0/1
PTC	T = 0	1	100 (1/1)	1/1	1/1	1/1	0/1
	Summer	1	100 (1/1)	1/1	1/1	1/1	0/1
	T = 0	1	100 (1/1)	1/1	1/1	1/1	0/1
	Winter	1	100 (1/1)	1/1	1/1	1/1	0/1
Negative	T = 0	10	N/A	0/10	0/10	0/10	10/10
	Summer [1]	10	100 (10/10)	0/10	0/10	0/10	10/10
	T = 0	10	N/A	0/10	0/10	0/10	10/10

	Winter [2]	10	100 (10/10)	0/10	0/10	0/10	10/10
Low Positive	T = 0	20	N/A	20/20	20/20	20/20	20/20
	Summer	20	100 (20/20)	0/20	20/20	20/20	20/20
	T = 0	20	N/A	20/20	20/20	20/20	20/20
	Winter	20	100 (20/20)	15/20	20/20	20/20	20/20
High Positive	T = 0	10	N/A	10/10	10/10	10/10	10/10
	Summer	10	100 (10/10)	0/10	10/10	10/10	10/10
	T = 0	10	N/A	10/10	10/10	10/10	10/10
Sample Group	**Test Point**	**Number Tested**	**Overall Percent Positive (%)**	**# Detected / # Tested by Target Genes**			
				N Gene	**ORF1ab**	**E Gene**	**RNase P**
	Winter	10	100 (10/10)	10/10	10/10	10/10	10/10

N/A: Not Applicable

[1] Testing performed at the conclusion of the thermal excursions described in **Table 14D**

[2] Testing performed at the conclusion of the thermal excursions described in **Table 14E**

Summary of Results (Table 14F):

- For the SARS-CoV-2 negative specimens, 10 /10 (100%) and 10/10 (100%) were reported as Negative after summer and winter temperature excursions, respectively.
- For the SARS-CoV-2 High Positive saliva samples 10/10 (100%) and 10 /10 (100%) were reported as positive after the summer and winter temperature excursions respectively.
- From 20 Low Positive saliva samples exposed to winter shipping profile, 20/20 (100%) were reported positive. From 20 Low Positive saliva samples exposed to the summer shipping profile, 20/20 (100%) were reported positive.

These results demonstrate that SARS-CoV-2 positive saliva specimens are stable in the SDNA-1000 Saliva Collection Device when exposed to a broad range of temperature conditions. These data support the use of the SDNA-1000 Saliva Collection Device for transport and storage of specimens for 72 hours following home collection of saliva.

8) <u>Saliva Sample Volume Tolerance Study:</u>

A study was conducted to evaluate the effect of over or under filling of the Spectrum Solutions LLC SDNA-1000 Saliva Collection Device by the user. The over fill and under fill of 25, 50 and 75% were evaluated and compared to intended (standard - STD) saliva collection volume of 2.0 mL.

The effect of over or under filling the Spectrum Solutions LLC SDNA-1000 Saliva Collection Device was evaluated using a total of 4 contrived positive and 2 negative specimens. Presumptive negative saliva collected into the Spectrum Solutions LLC SDNA-1000 Saliva Collection Device were pooled and spiked with SARS-CoV-2 genomic RNA (BEI Resources) at 2x LoD and 200x LoD to create low and high positive samples. Negative clinical samples without spiked-in viral genomic RNA were used as negative samples.

A summary of the results is provided in **Table 15.**

Table 15. Summary of results from Saliva Sample Volume Tolerance Study by Primer Mix, stratified by condition and measurand

Primer Mix 1 FAM							
Sample	**Filling Condition**	**Replicates Tested**	**Overall Percent Agreement**	**# Detected / #Tested by Target Gene**			
				N Gene	**ORF1ab**	**E Gene**	**RNase P**
Negative	-75	2	100 (2/2)	0/2	0/2	0/2	2/2
	-50	2	100 (2/2)	0/2	0/2	0/2	2/2
	-25	2	100 (2/2)	0/2	0/2	0/2	2/2
	STD	2	100 (2/2)	0/2	0/2	0/2	2/2
	+25	2	100 (2/2)	0/2	0/2	0/2	2/2
	+50	2	0 (0/2)	0/2	0/2	0/2	0/2
	+75	2	0 (0/2)	0/2	0/2	0/2	0/2
2x LoD Low Positive	-75	2	100 (2/2)	1/2	2/2	1/2	2/2
	-50	2	100 (2/2)	1/2	2/2	2/2	2/2
	-25	2	100 (2/2)	1/2	2/2	2/2	2/2
	STD	2	100 (2/2)	2/2	2/2	2/2	2/2
	25	2	100 (2/2)	2/2	2/2	1/2	2/2
	50	2	100 (2/2)	2/2	2/2	2/2	2/2
	75	2	100 (2/2)	1/2	2/2	2/2	2/2
200x LoD High Positive	-75	2	100 (2/2)	2/2	2/2	2/2	2/2
	-50	2	100 (2/2)	2/2	2/2	2/2	2/2
	-25	2	100 (2/2)	2/2	2/2	2/2	2/2
	STD	2	100 (2/2)	2/2	2/2	2/2	2/2
	+25	2	100 (2/2)	2/2	2/2	2/2	2/2
	+50	2	100 (2/2)	2/2	2/2	1/2	1/2
	+75	2	100 (2/2)	2/2	2/2	1/2	0/2

Primer Mix 2 VIC							
Sample	**Filling Condition**	**Replicates Tested**	**Overall Percent Agreement**	**# Detected / #Tested by Target Gene**			
				N Gene	**ORF1ab**	**E Gene**	**RNase P**
Negative	-75	2	100 (2/2)	0/2	0/2	0/2	2/2
	-50	2	50 (1/2)	0/2	0/2	0/2	1/2
	-25	2	100 (2/2)	0/2	0/2	0/2	2/2
	STD	2	100 (2/2)	0/2	0/2	0/2	2/2
	+25	2	100 (2/2)	0/2	0/2	0/2	2/2
	+50	2	0 (0/2)	0/2	0/2	0/2	0/2
	+75	2	50 (1/2)	0/2	0/2	0/2	1/2
2x LoD Low	-75	2	100 (2/2)	2/2	2/2	1/2	2/2
	-50	2	100 (2/2)	1/2	2/2	2/2	1/2

Primer Mix 2 VIC							
Sample	**Filling Condition**	**Replicates Tested**	**Overall Percent Agreement**	**# Detected / #Tested by Target Gene**			
				N Gene	**ORF1ab**	**E Gene**	**RNase P**
Positive	-25	2	50 (1/2)	0/2	2/2	1/2	2/2
	STD	2	100 (2/2)	2/2	2/2	1/2	2/2
	25	2	100 (2/2)	2/2	2/2	2/2	2/2
	50	2	100 (2/2)	2/2	2/2	1/2	2/2
	75	2	100 (2/2)	2/2	2/2	2/2	2/2
	-75	2	100 (2/2)	2/2	2/2	2/2	2/2

Primer Mix 3 NED							
Sample	Filling Condition	Replicates Tested	Overall Percent Agreement	# Detected / #Tested by Target Gene			
				N Gene	ORF1ab	E Gene	RNase P
Negative	-75	2	100 (2/2)	0/2	0/2	0/2	2/2
	-50	2	100 (2/2)	0/2	0/2	0/2	2/2
	-25	2	100 (2/2)	0/2	0/2	0/2	2/2
	STD	2	100 (2/2)	0/2	0/2	0/2	2/2
	+25	2	100 (2/2)	0/2	0/2	0/2	2/2
	+50	2	0 (0/2)	0/2	0/2	0/2	0/2
	+75	2	0 (0/2)	0/2	0/2	0/2	0/2
2x LoD Low Positive	-75	2	100 (2/2)	2/2	2/2	1/2	2/2
	-50	2	100 (2/2)	2/2	2/2	1/2	2/2
	-25	2	100 (2/2)	2/2	2/2	0/2	2/2
	STD	2	100 (2/2)	2/2	2/2	0/2	2/2
	25	2	100 (2/2)	2/2	2/2	1/2	2/2
	50	2	100 (2/2)	2/2	2/2	1/2	2/2
	75	2	100 (2/2)	2/2	2/2	2/2	2/2
200x LoD High Positive	-75	2	100 (2/2)	2/2	2/2	2/2	2/2
	-50	2	100 (2/2)	2/2	2/2	2/2	2/2
	-25	2	100 (2/2)	2/2	2/2	2/2	2/2
	STD	2	100 (2/2)	2/2	2/2	2/2	2/2
	+25	2	100 (2/2)	2/2	2/2	2/2	2/2
	+50	2	100 (2/2)	2/2	2/2	1/2	1/2
	+75	2	100 (2/2)	2/2	2/2	1/2	1/2
200x LoD High Positive	-50	2	100 (2/2)	2/2	2/2	2/2	2/2
	-25	2	100 (2/2)	2/2	2/2	2/2	2/2
	STD	2	100 (2/2)	2/2	2/2	2/2	2/2
	+25	2	100 (2/2)	2/2	2/2	2/2	2/2
	+50	2	100 (2/2)	2/2	2/2	2/2	2/2
	+75	2	100 (2/2)	2/2	2/2	2/2	2/2

Primer Mix 4 PET							
Sample	Filling Condition	Replicates Tested	Overall Percent Agreement	# Detected / #Tested by Target Gene			
				N Gene	ORF1ab	E Gene	RNase P
Negative	-75	2	100 (2/2)	0/2	0/2	0/2	2/2
	-50	2	100 (2/2)	0/2	0/2	0/2	2/2
	-25	2	100 (2/2)	0/2	0/2	0/2	2/2
	STD	2	100 (2/2)	0/2	0/2	0/2	2/2
	+25	2	100 (2/2)	0/2	0/2	0/2	2/2
	+50	2	0 (0/2)	0/2	0/2	0/2	0/2
	+75	2	0 (0/2)	0/2	0/2	0/2	0/2
	-75	2	100 (2/2)	2/2	2/2	2/2	2/2
	-50	2	100 (2/2)	2/2	2/2	2/2	2/2

2x LoD Low Positive	-25	2	100 (2/2)	1/2	2/2	2/2	2/2
	STD	2	100 (2/2)	1/2	2/2	2/2	2/2
	25	2	100 (2/2)	2/2	2/2	2/2	2/2
	50	2	100 (2/2)	2/2	2/2	2/2	2/2
	75	2	100 (2/2)	2/2	2/2	2/2	2/2
200x LoD High Positive	-75	2	100 (2/2)	2/2	2/2	2/2	2/2
	-50	2	100 (2/2)	2/2	2/2	2/2	2/2
	-25	2	100 (2/2)	2/2	2/2	2/2	2/2
	STD	2	100 (2/2)	2/2	2/2	2/2	2/2
	+25	2	100 (2/2)	2/2	2/2	2/2	2/2
	+50	2	100 (2/2)	2/2	2/2	2/2	2/2
	+75	2	100 (2/2)	2/2	2/2	2/2	1/2

These results demonstrate that positive saliva samples were unaffected by overfilling the Spectrum saliva collection device up to 50% and 75% over-fill for all four Primer Mixes. However, negative samples may be invalid and require retest, if the internal control (RNaseP) is below the threshold. Thus, the RNAse P internal control serves as an effective control for ensuring that saliva samples are not overfilled. The Spectrum Solutions LLC SDNA-1000 Saliva Collection Device is tolerant to saliva collection volume range of up to 75% under-fill to 25% over-fill. The collection device instructions for use emphasize the need to not overfill.

9) Human Usability Study: for home-collection and mailing the sample to a CLIA-certified lab for testing:

To support home use of the DxTerity Home Collection Kit, using the Spectrum Solutions LLC SDNA-1000 Saliva Collection Device, a Human Usability Study was conducted to evaluate the entire workflow including kit registration, sample collection, packaging of the sample, and mailing to the laboratory with pre-paid label.

Testing included 41 participants representing varying education levels and ages and took place in user's homes. Two (2) participants with prior self-collection experience were excluded from the study.

After enrollment in the study, the collection kit was shipped to each participant for next day delivery. Upon receipt of the kit and on the day/time of the scheduled video conference call set up to monitor self-collection, each participant logged onto an electronic portal to consent for the study and to provide basic demographic data and information on risk factors or symptoms of COVID-19.

Each participant collected the saliva sample while under observation by a DxTerity staff via video conference, who recorded any difficulties with the registration and the sample collection process.

After the entire process was completed, the user was given a questionnaire to indicate the ease of use of the kit and sample collection as well as understanding the consequences if steps are not performed correctly.

Two out of the 39 participants had no responses to any questions. Overall, 89% (33/37) of the respondents rated the entire process as easy to use. The difficulties experienced by the users related to saliva collection (getting enough saliva and cap closure) and packaging are listed as common feedback in **Table 16A** below and will be addressed by revising the instructions for use as appropriate.

Table 16A. Summary of participant feedback from Human Usability Study

Questions in the Participant Questionnaire with a YES or NO Response			
Questions in the Participant Questionnaire	**Yes**	**No**	**Common Feedback**
Were the sample collection kit instructions easy to follow?	29	8	1) Instructions on sealing the box could be clearer 2) Include instructions on which box contents to keep or discard 3) Instructions on which barcode to use-the box or the tube was not clear 4) Clarify that no eating and drinking prior to collection also includes not drinking water
Did you encounter any problem with the saliva collection process?	6	31	1) Hard to gather enough saliva 2) Cap is hard to close
Did you have any concerns with packaging and shipping the sample back to us?	3	34	Include instructions on which box contents to keep or discard.
Are you able to easily perform self-collection using this kit and the materials we provided?	34	3	1) Confused on which instructions to use (those included with kit vs collection device) and therefore had difficulty with packaging and barcode registration 2) Difficulty in closing the tube

Questions in the Participant Questionnaire with Response Rated on a Scale of 1 through 5		
Questions in the Participant Questionnaire	**Rating of 1-3 (Very Easy to Neutral)/ Total Responses**	**Rating of 4-5 (Difficult or very Difficult)/ Total Responses**
How would you rate your experience with the registration process?	21/37	6/37
How would you rate your experience with the sample collection process?	36/37	1/37

How would you rate your overall experience with the entire process (from registration to collection to shipping)?	33/37	4/37
2 out of the 39 participants had no responses to any of these questions		

The sample was packed with the provided shipping materials and shipped via FedEx to the laboratory with the provided pre-paid return envelope. 35 out of the 39 samples were shipped and received at DxTerity within 24 hours of saliva collection. Of the 4 samples received after 24 hours, 2 were received after 48 hours and the other 2 were received after 72 hours.

Upon receipt, laboratory personnel inspected the packaging and recorded any packaging errors and noted acceptability of the sample for testing. Two out of the 39 samples were processed by the laboratory and not inspected and thus excluded from further testing. A total of 37 samples were inspected upon receipt for packaging errors and were tested using the DxTerity SARS- CoV-2 RT-PCR CE test (Primer Mix-3 NED), that detects RNase P for specimen adequacy.

The packaging error (4/37) was mainly failure to seal the sample box. The users did not peel the adhesive cover strip prior to closing the box. All 37 samples were deemed acceptable for testing. The major observations with the samples were green colored samples (24/37), less than standard fill (13/37) and greater than standard fill (6/37) saliva sample. For samples where greater than standard fill was observed, the over fill volumes were less than 50% overfill. Based on the sample volume tolerance study, no failure in RNase P detection was expected in the under fill or over fill samples. This was confirmed, as these 37 samples plus 4 samples received later than 24 hours after collection were valid with RNase P was detected.

Table 16B. Summary of Human Usability Study

Participant Summary	N	%
Participants Enrolled	41	100% (41/41)
Participants with No Prior Medical or Laboratory Training	39	95% (39/41)
Kit Summary		
Kits Distributed	41	100% (41/41)
Kits Received for Testing within 24 hrs of Saliva Collection	37	95% (37/39)
Kits Received for Testing after 48 hrs of Saliva Collection	1	2.5% (1/39)

Kits Received for Testing after 72 hrs of Saliva Collection	1	2.5% (1/39)
Errors Noted at Sample Accessioning		
Kits Received for Testing	39	100% (39/39)
Kits Not Inspected Prior to Processing, Thus Excluded from Testing	2	5% (2/39)
Participant Responses		
Participants who Provided Feedback	37	95% (37/39)
Participants who Rated the Entire Process as Easy to Use	33	85% (33/39)
Packaging Errors		
Package Intact	37	100% (37/37)
Package Seal	31	84% (31/37)
Absorbent Sheet Present	37	100% (37/37)
No Visible Signs of Sample Leakage	37	100% (37/37)
Observations at Sample Accessioning		
Green Color Observed[1]	25	67.5% (25/37)
Less Than Standard Fill Observed	13	35% (13/37)
Greater Than Standard Fill Observed	6	16% (6/37)
Sample Validity / Adequacy		
Acceptable for Testing	37	100% (37/37)
Unacceptable for Testing	0	0% (0/37)

[1]The Spectrum buffer solution is blue, upon mixture with saliva (which can be clear or yellow), the resulting solution is expected to be blue or green. Only dark colored such as brown are rejected.

FDA SARS-CoV-2 Reference Panel Testing

The evaluation of sensitivity and MERS-CoV cross-reactivity was performed using reference material (T1), blinded samples and a standard protocol provided by the FDA. The study included a range finding study and a confirmatory study for LoD. Blinded sample testing was used to establish specificity and to confirm the LoD. The extraction method used was Sera-Mag SpeedBeads Carboxyl Magnetic Beads (GE Healthcare) using the Applied Biosystem MagMax 96 Magnetic Particle Processor (software BindIt 4.0). The DxTerity SARS-CoV-2

RT-PCR CE Test was run with the VeritiDx PCR Thermal Cycler. The results are summarized in the following Table.

Table 17. Summary of LoD Confirmation Result Using the FDA SARS-CoV-2 Reference Panel

Reference Materials Provide d by FDA	Specimen Type	Product LoD	Cross-Reactivity
SARS-CoV-2	Saliva	$3.6x10^3$ NDU/mL	N/A
MERS-CoV		N/A	ND

NDU/mL: RNA NAAT detectable units/mL
N/A: Not Applicable
ND: Not Detected

LIMITATIONS:

- For asymptomatic individuals, negative results obtained using saliva should be considered as presumptive and confirmed with a preferred specimen type or different molecular assay validated for testing saliva, if necessary, for patient management.
- Testing of saliva for individuals with or without symptoms of COVID-19 should be prescribed by healthcare provider.
- Based on the in-silico analysis, other SARS-like coronaviruses in the same subgenus (Sarbecovirus) as SARS-CoV-2 may cross-react with the DxTerity SARS-CoV-2 RT-PCR. Other SARS-like coronaviruses in the same subgenus (Sarbecovirus) as SARS-CoV-2 are not known to be currently circulating in the human population, therefore, are highly unlikely to be present in individual specimens.
- The clinical performance of this test has not been established in all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.

WARNINGS

- Caution: Federal Law restricts this device to sale by or on the order of a licensed practitioner.
- For *in vitro* diagnostic use.
- This product has not been FDA cleared or approved but has been authorized by FDA under an EUA for use by DxTerity Diagnostics, located at 19500 S. Rancho Way Suite 116, Rancho Dominguez, CA 90220 USA which is certified under CLIA and meets the requirements to perform high-complexity tests.
- This product has been authorized only for the detection of nucleic acid from SARSCoV-2, not for any other viruses or pathogens.
- The emergency use of this product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug and Cosmetics Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.