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

(Yale School of Public Health, Department of Epidemiology of Microbial Diseases)

For *In vitro* Diagnostic Use
For use under Emergency Use Authorization (EUA) only

(The SalivaDirect for use with DTC Kits assay will be performed at laboratories designated by the Yale School of Public Health, Department of Epidemiology of Microbial Diseases, that includes the Clinical Molecular Diagnostics Laboratory, Department of Pathology, Yale School of Medicine, located at 310 Cedar St., New Haven, CT 06510, that are also certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a and meet the requirements to perform high complexity tests, as described in the Laboratory Instructions for Use that was reviewed by the FDA under this EUA.)

INTENDED USE

SalivaDirect for use with DTC Kits is a direct to consumer product for testing saliva specimens self-collected at home (which includes in a community-based setting) using the SalivaDirect DTC Saliva Collection Kit when used consistent with its authorization by any individual including individuals without symptoms or other reasons to suspect COVID-19.

Testing is limited to laboratories designated by the Yale School of Public Health, Department of Epidemiology of Microbial Diseases, that includes the Clinical Molecular Diagnostics Laboratory, Department of Pathology, Yale School of Medicine, located at 310 Cedar St., New Haven, CT 06510, that are also certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a and meet the requirements to perform high complexity tests.

Results are for the detection and identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in saliva 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.

Negative test results are provided to the user via text, email and/or online portal. Individuals with positive, or invalid test results will be contacted by a healthcare provider by phone. The direct to consumer home collection system is intended to enable users to access information about their COVID-19 infection status that could aid with determining if self-isolation or quarantine is appropriate and to assist with healthcare decisions after discussion with a healthcare provider.

SalivaDirect for Use with DTC Kits is not a substitute for visits to a healthcare provider. The information provided by this product should not be used to start, stop, or change any course of treatment unless advised by your healthcare provider.

SalivaDirect for use with DTC Kits is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of RT-qPCR and in vitro diagnostic procedures. The assay is only for use under the Food and Drug Administration's Emergency Use Authorization.

DEVICE DESCRIPTION AND TEST PRINCIPLE

SARS-CoV-2 Assay

SalivaDirect for use with DTC Kits is an RNA-extraction free, dualplex RT-qPCR method for SARS-CoV-2 detection. It can be broadly implemented as it (1) does not require saliva collection tubes containing preservatives, (2) does not require specialized equipment for nucleic acid extraction, and (3) is validated for use with products from multiple vendors. Thus, the simplicity and flexibility of SalivaDirect means that it is not as affected by supply chain bottlenecks as some other assays. The method is nucleic acid extraction-free, which enables testing of low volume and minimally processed saliva in dualplex RT-qPCR for SARS-CoV-2 detection. Saliva is first treated with proteinase K followed by a heat inactivation step and is then directly used as input in the dualplex RT-qPCR test using validated primer and probe sets (2019-nCoV_N1 and RP) developed by the US CDC. The human *Ribonuclease P* (RP) probe was modified with a different fluorophore so that the primer/probe set could be combined in a dualplex assay, reducing the number of tests to 1 assay with 2 sets.

The SalivaDirect for use with DTC Kits assay is authorized for use with the SalivaDirect DTC Saliva Collection Kit, which was authorized for use in a separate EUA (EUA210507).

RT-qPCR INSTRUMENTS USED WITH TEST

SalivaDirect for use with DTC Kits should be used with the following RT-qPCR instruments:

Vendor	Instrument	Software
Bio-Rad	CFX96 Touch Real-Time PCR Detection System	Bio-Rad CFX Maestro 1.1 v4.1.2435.1219
Bio-Rad	CFX384 Touch Real-Time PCR Detection System	Bio-Rad CFX Maestro 1.1 v4.1.2435.1219
ThermoFisher Scientific	Applied Biosystems StepOne Real-Time PCR System	StepOne Software v2.3
ThermoFisher Scientific	Applied Biosystems 7500 Fast Real-Time PCR System	7500 Software v2.3
ThermoFisher Scientific	Applied Biosystems 7500 Fast Dx Real-Time PCR System	7500 Fast System SDS software v1.4.1
ThermoFisher Scientific	Applied Biosystems PRISM 7000 Real-Time PCR System	PRISM 7000 Sequence Detection System v1.0
ThermoFisher Scientific	ABI QuantStudio 5 Real-Time PCR system (96 or	QuantStudio Design and Analysis Software

Vendor	Instrument	Software
	384 well format)	v2.4.3
ThermoFisher Scientific	ABI QuantStudio 6 Real-Time PCR system (96 or 384 well format)	QuantStudio Design and Analysis Software v2.4.3
ThermoFisher Scientific	ABI QuantStudio 7 Pro Real-Time PCR system (96 or 384 well format)	QuantStudio Design and Analysis Software v2.4.3
ThermoFisher Scientific	ABI QuantStudio 7 Flex Real-Time PCR system (96 or 384 well format)	QuantStudio Design and Analysis Software v2.4.3
ThermoFisher Scientific	ABI QuantStudio 12K Flex Real-Time PCR system (384 well format)	QuantStudio Design and Analysis Software v2.4.3
ThermoFisher Scientific	ABI QuantStudio Dx Real-Time PCR system (96 well format)	QuantStudio Design and Analysis Software v2.4.3
Ubiquitome	Liberty16	Liberty16 App Version 1.8 (iOS)
Roche	Cobas Z480	User Defined Workflow for cobas z 480
Analytik Jena	qTower	qPCRsoft version 2.2
Agilent	AriaMX Real-Time PCR System	N/A (fully integrated)

INSTRUMENTS AND MATERIALS USED WITH SALIVADIRECT FOR USE WITH DTC KITS TEST IN THE AUTOMATED PROTOCOL (INSTRUCTIONS FOR USE, APPENDIX B)

The automated protocol for SalivaDirect for use with DTC Kits as detailed in Appendix B of the Instructions for Use should be used with the following instruments and materials:

Vendor	Item	Catalog #
Hamilton	Vantage 2.0 liquid handling robot equipped with 96-channel head and 8-channel spanner head. The Hamilton Venus 4 software package used for instrument programming and operation via the "Venus on Vantage" software utility.	Custom configuration
Applied Biosystems	384-Well Polypropylene PCR plate	4343814
Hamilton	50 µL filtered pipette tips	235948
ThermoFisher	1 mL sterile-internal threaded tube	3741
Hamilton	96 well PCR FramePlate	814302
Hamilton	LabElite DeCapper SL	193602
Rainin	BenchSmart 96-200 Semi-automated pipette system	BST 96-200

REAGENTS AND MATERIALS

Designated laboratories should refer to the SalivaDirect website for a list of qualified reagent lots.

Vendor	Item	Catalog number	Quantity	# Reactions
Order one of the following Proteinases K				
ThermoFisher Scientific	MagMAX Viral/Pathogen Proteinase K	A42363	10 mL	4,000 reactions

Vendor	Item	Catalog number	Quantity	# Reactions
New England Biolabs	Proteinase K, Molecular Biology Grade	P8107S	2 mL	320 reactions
AmericanBio	Proteinase K	AB00925	100 mg	800 reactions
Order one of the following RT-qPCR kits				
New England Biolabs	Luna Universal Probe One-Step RT-qPCR (2x) Kit	E3006S	2 mL	200 reactions
		E3006L	5 mL	500 reactions
		E3006X	10 mL	1,000 reactions
		E3006E	25 mL	2,500 reactions
New England Biolabs	Luna Probe One-Step RT-qPCR 4x Mix with UDG (for use with 384-well format PCR instruments)	M3019S	1.06 mL	200 reactions
		M3019L	2.5 mL	500 reactions
		M3019X	5 mL	1,000 reactions
		M3019E	10.5 mL	2,500 reactions
Bio-Rad	Reliance One-Step Multiplex RT-qPCR Supermix	12010176	1 mL	200 reactions
		12010220	5 mL	1,000 reactions
		12010221	10 mL	2,000 reactions
ThermoFisher Scientific	TaqPath 1-Step RT-qPCR Master Mix, GC	A15299	5 mL	1,000 reactions
		A15300	10 mL	2,000 reactions
Quantabio	UltraPlex 1-Step ToughMix	95166-100	500 µl	100 reactions
		95166-500	2.5 mL	500 reactions
		95166-01K	5 mL	1,000 reactions
Order one of the following primer and probe sets				
Eurofins Genomics	SalivaDirect primer and probe set (complete set of the 6 primers and probes), Cy5 probe	12YS-010YST	50-100 nmol	12,500 reactions
	SalivaDirect primer and probe set (complete set of the 6 primers and probes), HEX probe	12YS-000YST	50-100 nmol	12,500 reactions
Integrated DNA Technologies	nCOV_N1 Forward Primer Aliquot	10006821	50 nmol	6,250 reactions
		10006830	100 nmol	12,500 reactions
	nCOV_N1 Reverse Primer Aliquot	10006822	50 nmol	6,250 reactions
		10006831	100 nmol	12,500 reactions
	nCOV_N1 Probe Aliquot	10006823	25 nmol	6,250 reactions
		10006832	50 nmol	12,500 reactions
	RNase P Forward Primer Aliquot	10006827	50 nmol	16,600 reactions
		10006836	100 nmol	33,300 reactions
	RNase P Reverse Primer Aliquot	10006828	50 nmol	16,600 reactions
		10006837	100 nmol	33,300 reactions

Vendor	Item	Catalog number	Quantity	# Reactions
	RNase P Probe	Custom order (Cy5)	25 nmol	6,250 reactions
		Custom order (Cy5)	50 nmol	12,500 reactions
		10007061 (ATTO647)	25 nmol	6,250 reactions
		10007062 (ATTO647)	50 nmol	12,500 reactions
LGC Biosearch Technologies	nCOV_N1 Forward Primer	nCoV-N1-F-100	100 nmol	12,500 reactions
		nCoV-N1-F-1000	1000 nmol	125,000 reactions
	nCOV_N1 Reverse Primer	nCoV-N1-R-100	100 nmol	12,500 reactions
		nCoV-N1-R-1000	1000 nmol	125,000 reactions
	nCOV_N1 Probe	nCoV-N1-P-25	25 nmol	6,250 reactions
		nCoV-N1-P-250	250 nmol	62,500 reactions
	RNase P Forward Primer	RNP-F-20	20 nmol	6,660 reactions
		RNP-F-100	100 nmol	33,300 reactions
		RNP-F-1000	1000 nmol	333,300 reactions
	RNase P Reverse Primer	RNP-R-20	20 nmol	6,660 reactions
		RNP-R-100	100 nmol	33,300 reactions
		RNP-R-1000	1000 nmol	333,300 reactions
	RNase P Probe	RNP-PQ670-25	25 mol	6,250 reactions
		RNP-PQ670-250	250 nmol	62,500 reactions
Lighthouse Lab Services	SalivaNow SARS-CoV-2 Assay (Primers and probes come pre-mixed)	9731816-S	-	2,000 reactions
Order one of the following nuclease-free waters				
Integrated DNA Technologies	Nuclease-free water	11-04-02-01	20 mL	
		11-05-01-14	300 mL	
		11-05-01-04	1 L	
New England Biolabs	Nuclease-free water	B1500S	25 mL	
		B1500L	100 mL	
Order one of the following positive controls				
Twist Bioscience	Synthetic SARS-CoV-2 RNA Control 2	102024	100 µL	
Lighthouse Lab Services	Positive CoV-2 Control	9731816PC	80 µL	
Optional negative extraction control (NEC)				
Lighthouse Lab Services	Negative Control	9731816EC	10 mL	

CONTROLS RUN WITH THE COVID-19 RT-PCR

The following controls are run with the SalivaDirect assay:

Control	Description	Purpose	Frequency
Negative Extraction Control (NEC)	Nuclease-free water	To monitor for contamination during saliva processing	Every batch of up to 93 saliva samples
	Lighthouse Labs Negative Control (synthetic RNAse P control)	To monitor for effective proteinase K treatment and contamination during saliva processing	
Negative Template Control (NTC)	Nuclease-free water	To monitor for contamination of PCR reagents	Every PCR plate with up to 93 saliva samples
Positive Control	Twist Synthetic SARS-CoV-2 RNA control. (**Dilute to 100 copies/µL**)	To monitor functioning of RT-qPCR reagents	Every PCR plate with up to 93 saliva samples
	Lighthouse Lab Services Positive CoV-2 Control (synthetic SARS-CoV-2 RNA control, 100 copies/µl)		
Internal Process Control	Primer/Probe set detecting RNaseP	To ensure that saliva of a sufficient quantity and quality was tested	Every sample

INTERPRETATION OF RESULTS

1) SARS-CoV-2 RT-PCR test Controls – Positive, Negative, and Internal:

Positive control: The positive control should yield a "detected" result for the N1 target and "not detected" for the RNaseP control.

Negative Extraction Control (NEC): If using nuclease-free water, the NEC should yield a "not detected" result for both the N1 and RNaseP targets. If using the Lighthouse Lab Services Negative Control, the NEC should yield a "not detected" result for the N1 target and a Ct value <30 Ct for the RNaseP target.

Negative Template Control: The NTC should yield a "not detected" result for both the N1 and RNaseP targets.

Internal Control: Detection of RNaseP below a specified cut-off (see tables below) indicates that saliva of sufficient quantity and quality were tested. Detection of RNaseP is required to report a negative SARS-CoV-2 result.

2) Examination and Interpretation of Patient Specimen Results:

All test controls should be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted. Results will be interpreted according to the tables below:

16-Well and 96-Well Formats

Bio-Rad CFX96 Touch ABI 7500 Fast ABI 7500 Fast Dx ABI PRISM 7000ABI QuantStudio Dx ABI QuantStudio 5 ABI QuantStudio 7 Flex Analytik Jena qTower Ubiquitome Liberty16		
Result	**Ct value N1**	**Ct value RP**
Positive	<40.0	Any value
Negative	≥40.0	<35.0
*Invalid	≥40.0	≥35.0

ABI StepOne ABI QuantStudio 6 ABI QuantStudio 7 Pro		
Result	**Ct value N1**	**Ct value RP**
Positive	<37.0	Any value
Negative	≥37.0	<35.0
*Invalid	≥37.0	≥35.0

Agilent AriaMX Roche Cobas Z480		
Result	**Ct*** value N1**	**Ct value RP**
Positive	<34.0	Any value
Negative	≥36.0	<30.0
**Inconclusive	≥34.0 - <36.0	<30.0
*Invalid	≥34.0	≥30.0

*Invalid test results will be repeated by retesting the primary specimen from the beginning of the protocol. Results from retested samples will follow the same interpretation as listed in the table above.

**When the Ct value for RP is <30 and the Ct is in the range of ≥34.0 - <36.0 for N1, the sample will be retested from the beginning of the protocol to potentially resolve an inconclusive result to a confirmed negative or positive, if desired by the requesting healthcare provider. Results from retested samples will follow the same interpretation as listed in the table above.

384-Well Format

CFX384 Touch ABI QuantStudio 5 ABI QuantStudio 6 ABI QuantStudio 7 Pro ABI QuantStudio 7 Flex ABI QuantStudio 12K Flex		
Result	**Ct value N1**	**Ct value RP**
Positive	<40.0	Any value
Negative	≥40.0	<35.0

*Invalid	≥40.0	≥35.0

*Invalid test results will be repeated by retesting the primary specimen from the beginning of the protocol. Results from retested samples will follow the same interpretation as listed in the table above.

PERFORMANCE EVALUATION

(The performance of SalivaDirect for use with DTC kits (EUA210506) is the same data used to support the previous authorization of SalivaDirect (EUA202097) – which represent the same real-time RT-PCR test used for different indications of use.)

1) Analytical Sensitivity:

Limit of Detection (LoD):

A positive saliva specimen from a confirmed COVID-19 healthcare worker with a known virus concentration (3.7×10^4 copies/µL) was spiked into saliva collected from healthcare workers who tested negative for SARS-CoV-2 using the CDC assay. The following 2-fold dilution series was tested in triplicate to determine the preliminary limit of detections: 400, 200, 100, 50, 25, 12, 6, 3, and 1.5 copies/µL. Spiked saliva specimens were tested according to the SalivaDirect protocol. In total, three different proteinase K reagents, three different RT-qPCR kits, and three different RT-qPCR thermocyclers were validated with the assay. Input volumes, matrices and RT-qPCR programs were the same for each combination of proteinase K, RT-qPCR kit, and RT-qPCR instrument. The preliminary limit of detection was then confirmed with 20 additional replicates. The table below shows the final limit of detection for the different reagents/instruments used with SalivaDirect.

Proteinase K					
Proteinase K	*RT-qPCR kit*	*RT-qPCR instrument*	*LOD*	*Positive replicates*	*Mean Ct value (SD)*
Thermo	NEB Luna (2x)	Bio-Rad CFX96 Touch	6 copies/µL	100% (20/20)	36.7 (1.0)
NEB	NEB Luna (2x)	Bio-Rad CFX96 Touch	3 copies/µL	100% (20/20)	36.6 (1.0)
AmericanBio	NEB Luna (2x)	Bio-Rad CFX96 Touch	3copies/µL	100% (20/20)	33.51 (0.4)
RT-qPCR kit					
Proteinase K	*RT-qPCR kit*	*RT-qPCR instrument*	*LOD*	*Positive replicates*	*Mean Ct value (SD)*
Thermo	Bio-Rad Reliance	Bio-Rad CFX96 Touch	6 copies/µL	100% (20/20)	36.4 (0.6)
Thermo	Thermo TaqPath	Bio-Rad CFX96 Touch	12 copies/µL	100% (20/20)	35.9 (1.2)
RT-qPCR instrument					
Proteinase K	*RT-qPCR kit*	*RT-qPCR instrument*	*LOD*	*Positive replicates*	*Mean Ct value (SD)*
Thermo	Thermo TaqPath	ABI 7500 Fast	12 copies/µL	95% (19/20)	36.8 (1.2)

Thermo	Thermo TaqPath	ABI 7500 Fast Dx	6 copies/µL	95% (19/20)	32.4 (0.9)

Additional LoD studies were conducted to validate the Agilent AriaMX 96-well format thermocycler, the Liberty16 16-well format thermocycler, and the CFX384 Touch 384-well format thermocycler. Samples were prepared by spiking saliva from a confirmed positive patient into negative clinical matrix. The following dilutions were tested in triplicate in the range finding study: 100, 50, 25, 12, 6, 3, and 1.5 copies/µL. The LoD was then confirmed by testing 20 replicates and determined to be 6 copies/µL for the Agilent AriaMx and the CFX384 Touch thermocyclers, and 12 copies/µL for the Liberty16.

Proteinase K	*Primer/Probe*	*RT-qPCR kit*	*RT-qPCR instrument*	*LOD*	*Positive replicates*	*Mean Ct value (SD)*
Thermo	IDT	NEB Luna (2x)	Agilent AriaMX	6 copies/µL	100% (20/20)	30.3 (0.4)
Thermo	Eurofins	NEB Luna (2x)	Liberty16	12 copies/µL	100% (20/20)	35.18 (0.7)
Thermo	IDT	NEB Luna (2x)	CFX384 Touch	6 copies/µL	100% (20/20)	36.25 (0.4)

In addition, 22 weak positive clinical samples were tested in both the CFX96 Touch and CFX384 Touch PCR instruments with the NEB Luna 2x RT-PCR kit, with 100% concordance. Additionally, 9 clinical samples were tested on both the CFX96 Touch and QuantStudio 5 (384) PCR instruments with NEB Luna 2x RT-PCR kit, with 100% concordance. These results demonstrate similar detection in clinical samples when using either the 96 or 384 well formats Results are summarized below:

Thermocycler	*Positive Replicate*	*Mean Ct Value*
CFX96 Touch	100% (22/22)	35.78
CFX384 Touch	100% (22/22)	36.68

Thermocycler	*Positive Replicates*	*Mean Ct Value*
CFX96 Touch	100% (9/9)	28.62
QuantStudio 5 (384)	100% (9/9)	27.76

Additional RT-PCR Mixes:

In addition to the 2x NEB Luna RT-PCR mixture validated above, a 4x concentration was also validated via an LoD study on the CFX384 Touch using the Thermo Proteinase K. The LoD of 6 copies/mL previously confirmed for the NEB Luna 2x was confirmed on the CFX384 Touch, as shown below:

	6 copies/ul		3 copies/ul	
	Positive Replicates	Mean Ct	Positive Replicates	Mean Ct
NEB Luna (4x)	100% (20/20)	35.77	85% (17/20)	36.57

The Quantabio UltraPlex 1-Step ToughMix PCR mixture was also validated via an LoD study on the CFX96 Touch using the Thermo Proteinase K and was found to have a confirmed LoD of 3 copies/mL, as shown below:

	6 copies/ul		3 copies/ul	
	Positive Replicates	Mean Ct	Positive Replicates	Mean Ct
UltraPlex 1-Step ToughMix	100% (20/20)	36.42	95% (19/20)	37.45

Additional Primer/Probe mix:

The SalivaNow assay consists of a pre-mixed, ready to use mixture of the CDC-N1 and RNaseP primers and probes. For bridging of the SalivaNow SARS-CoV-2 assay, samples were prepared by spiking positive saliva from a confirmed COVID-19 healthcare worker with a known concentration (3.7×10^4 copies/µL) into saliva collected from healthcare workers who tested negative for SARS-CoV-2. The following concentrations were tested: 100, 50, 25, 12, 6, 3, and 1.5 copies/µL. All samples were tested in the standard SalivaDirect assay using the Thermo Proteinase K then in RT-qPCR with both the NEB Luna 2x RT-qPCR kit and the TaqPath One Step kit in the CFX96 Touch. Results were compared to the standard SalivaDirect assay using the Eurofins primer/probe sequences, the Thermo Proteinase K and the NEB Luna 2x RT-qPCR kit also in the CFX 96 Touch. The table below lists the positivity rates for each concentration when tested using validated and new primer/probe vendors:

		Concentration (positive replicates)							
Primer/Probes	RT-PCR mix	100 copies/µL	50 copies/µL	25 copies/µL	12 copies/µL	6 copies/µL	3 copies/µL	1.5 copies/µL	0 copies/µL
Eurofins	**NEB Luna 2x**	**3/3**	**3/3**	**3/3**	**3/3**	**3/3**	**3/3**	**2/3**	**0/3**
SalivaNow	NEB Luna 2x	3/3	3/3	3/3	3/3	3/3	3/3	2/3	0/3
SalivaNow	TaqPath One Step	3/3	3/3	3/3	3/3	3/3	2/3	1/3	0/3

Additional RNAseP probe:

For the SalivaDirect RT-qPCR assay to be compatible with the ABI PRISM 7000 and ABI StepOne, the Cy5 fluorophore on the RNAseP probe had to be exchanged to a HEX fluorophore. For this bridging study to validate the use of a HEX fluorophore on the RNAseP probe, samples were prepared by spiking positive saliva from a confirmed COVID-19 healthcare worker with a known concentration (3.7×10^4 copies/µL) into saliva collected from healthcare workers who tested negative for SARS-CoV-2. The following concentrations were tested: 100, 50, 25, 12, 6, 3, and 1.5 copies/µL. All samples were tested using the Thermo Proteinase K with the NEB Luna 2x RT-qPCR kit. The samples on the previously validated CFX96 Touch were tested with the RNAseP probe labelled with Cy5 and the samples on the ABI PRISM 7000 and ABI StepOne were tested with the RNAse probe labelled with HEX. The table below lists the positivity rates for each concentration when tested using validated and new thermocyclers:

	Concentration (positive replicates)							
	100 copies/µL	50 copies/µL	25 copies/µL	12 copies/µL	6 copies/µL	3 copies/µL	1.5 copies/µL	0 copies/µL
CFX96 Touch RP-Cy5	3/3	3/3	3/3	3/3	3/3	3/3	2/3	0/3
ABI PRISM 7000 RP-HEX	3/3	3/3	3/3	3/3	2/3	3/3	2/3	0/3
ABI StepOne, RP-HEX	3/3	3/3	3/3	3/3	3/3	2/3	1/3	0/3

Bridging Studies for Additional Instruments

Bridging studies were performed to validate additional thermocyclers. A 2-fold dilution series was tested in triplicate with each new thermocycler in parallel with a previously validated thermocycler to establish equivalent performance. The previously validated thermocycler is highlighted in bold for each study. Samples were prepared by spiking positive saliva from a confirmed COVID-19 healthcare worker with a known concentration (3.7×10^4 copies/µL) into saliva collected from healthcare workers who tested negative for SARS-CoV-2. The following concentrations were tested: 100, 50, 25, 12, 6, 3, and 1.5 copies/µL. All samples were tested using the Thermo Proteinase K with the NEB Luna RT-qPCR kit. The previously validated thermocyclers were tested with the 2x NEB Luna RT-PCR mix, while the new thermocyclers were tested with either the 2x (for 96-well and 384-well instruments) or 4x (for 384-well instruments) RT-PCR mix. The table below lists the positivity rates for each concentration when tested using validated and new thermocyclers:

	Concentration (positive replicates)							
	100 copies/µL	50 copies/µL	25 copies/µL	12 copies/µL	6 copies/µL	3 copies/µL	1.5 copies/µL	0 copies/µL
Bridging Study 1								
ABI 7500 Dx Fast	**3/3**	**3/3**	**3/3**	**3/3**	**3/3**	**3/3**	**2/3**	**0/3**
ABI QuantStudio 5	3/3	3/3	3/3	3/3	3/3	3/3	1/3	0/3
Bridging Study 2								
Bio-Rad CFX96 Touch	**3/3**	**3/3**	**3/3**	**3/3**	**3/3**	**3/3**	**2/3**	**0/3**
ABI QuantStudio 6	3/3	3/3	3/3	3/3	3/3	2/3	1/3	0/3
Bridging Study 3								
Bio-Rad CFX96 Touch	**3/3**	**3/3**	**3/3**	**3/3**	**3/3**	**3/3**	**2/3**	**0/3**
ABI QuantStudio 7	3/3	3/3	3/3	3/3	3/3	2/3	1/3	0/3
Bridging study 4								
Bio-Rad CFX96 Touch	**3/3**	**3/3**	**3/3**	**3/3**	**3/3**	**3/3**	**2/3**	**0/3**
ABI QuantStudio 5, 384 well (NEB Luna 2x)	3/3	3/3	3/3	3/3	3/3	3/3	0/3	0/3
ABI QuantStudio 5, 384 well (NEB Luna 4x)	3/3	3/3	3/3	3/3	3/3	2/3	0/3	0/3
Bridging study 5								
Bio-Rad CFX96 Touch	**3/3**	**3/3**	**3/3**	**3/3**	**3/3**	**3/3**	**2/3**	**0/3**

ABI QuantStudio 6, 384 well (NEB Luna 2x)	3/3	3/3	3/3	3/3	3/3	3/3	1/3	0/3
Bridging study 6								
Bio-Rad CFX96 Touch	**3/3**	**3/3**	**3/3**	**3/3**	**3/3**	**3/3**	**3/3**	**0/3**
ABI QuantStudio 7 Pro, 384 well (NEB Luna 2x)	3/3	3/3	3/3	3/3	3/3	3/3	1/3	0/3
ABI QuantStudio 7 Pro, 384 well (NEB Luna 4x)	3/3	3/3	3/3	3/3	3/3	3/3	3/3	0/3
Bridging study 7								
Bio-Rad CFX96 Touch	**3/3**	**3/3**	**3/3**	**3/3**	**3/3**	**3/3**	**2/3**	**0/3**
ABI QuantStudio 7 Flex, 384 well (NEB Luna 4x)	3/3	3/3	3/3	3/3	3/3	2/3	1/3	0/3
Bridging study 8								
Bio-Rad CFX96 Touch	**3/3**	**3/3**	**3/3**	**3/3**	**3/3**	**3/3**	**3/3**	**0/3**
ABI QuantStudio 12K Flex, 384 well (NEB Luna 4x)	3/3	3/3	3/3	3/3	3/3	3/3	1/3	0/3
Bridging study 9								
ABI 7500 Dx Fast	**3/3**	**3/3**	**3/3**	**3/3**	**3/3**	**3/3**	**3/3**	**0/3**
ABI QuantStudio Dx, 96 well	3/3	3/3	3/3	3/3	3/3	3/3	2/3	0/3
Bridging study 10								
Bio-Rad CFX96 Touch	3/3	3/3	3/3	3/3	3/3	3/3	2/3	0/3
Roche Cobas Z480	3/3	3/3	3/3	3/3	3/3	3/3	2/3	0/3
Bridging study 11								
Bio-Rad CFX96 Touch	3/3	3/3	3/3	3/3	3/3	3/3	2/3	0/3
ABI PRISM 7000	3/3	3/3	3/3	3/3	3/3	2/3	2/3	0/3
Bridging study 12								
Bio-Rad CFX96 Touch	3/3	3/3	3/3	3/3	3/3	3/3	2/3	0/3
ABI StepOne	3/3	3/3	3/3	3/3	3/3	2/3	1/3	0/3
Bridging study 13								
Bio-Rad CFX96 Touch	3/3	3/3	3/3	3/3	3/3	3/3	2/3	0/3
ABI QuantStudio 7 Flex	3/3	3/3	3/3	3/3	3/3	3/3	2/3	0/3
Bridging study 14								
Bio-Rad CFX96 Touch	3/3	3/3	3/3	3/3	3/3	3/3	2/3	0/3
Analytik Jena qTower	3/3	3/3	3/3	3/3	3/3	3/3	2/3	0/3

The lowest concentration at which 100% of replicates were positive for the new thermocyclers was within 2X of the validated thermocycler when tested side-by-side, indicating comparable analytical performance.

The bridging studies for the QuantStudio 5 (384) and QuantStudio 7 (384) thermocyclers also included testing with the Bio-Rad Reliance and TaqPath One Step RT-PCR reaction mixtures previously validated for the 96-well thermocyclers. These results also demonstrated comparable analytical performance for these reaction mixes when used on the 384-well instruments compared to the previously validated thermocycler (highlighted in bold):

		Concentration (positive replicates)							
	RT-PCR Mix	100 copies/µL	50 copies/µL	25 copies/µL	12 copies/µL	6 copies/µL	3 copies/µL	1.5 copies/µL	0 copies/µL
Bio-Rad CFX96 Touch	**NEB Luna 2x**	**3/3**	**3/3**	**3/3**	**3/3**	**3/3**	**3/3**	**2/3**	**0/3**
ABI QuantStudio 5, 384 well	Bio-Rad Reliance	3/3	3/3	3/3	3/3	3/3	3/3	2/3	0/3
Bio-Rad CFX96 Touch	**NEB Luna 2x**	**3/3**	**3/3**	**3/3**	**3/3**	**3/3**	**3/3**	**3/3**	**0/3**
ABI QuantStudio 7 Pro, 384 well	Bio-Rad Reliance	3/3	3/3	3/3	3/3	3/3	3/3	2/3	0/3
ABI QuantStudio 7 Pro, 384 well	TaqPath One Step	3/3	3/3	3/3	3/3	3/3	3/3	1/3	0/3

Validation of automated protocol (Appendix B in the Instructions for Use)

An LoD finding study was conducted by testing gamma irradiated SARS-CoV-2 virus (BEI) spiked into saliva negative for SARS-CoV-2 at concentrations of 100, 50, 25, 12, 6, 3 and 1.5 copies/µl. Samples were tested in triplicate following Workflow Three (heat pre-treatment of 95°C for 30 minutes) followed by RT-qPCR testing in the 384-well format QuantStudio 5 with the NEB Luna 2x RT-PCR mix and the Cy5 labelled RP probe. Following, 20 replicates at 0.5x, 1x, and 2x of the preliminary LoD (6 copies/µl) were also tested in the same workflow. Results for the automated protocol are summarized below:

	Concentration (positive replicates)		
	12 copies/µL	6 copies/µL	3 copies/µL
Automated protocol	20/20	19/20	16/20

The LoD of the SalivaDirect Assay using the automated protocol was confirmed to be 6 copies/µl.

In addition, a trial of the automated protocol was also conducted using 10 negative and 10 contrived positive samples with 10^6 copies/mL of gamma-irradiated SARS-CoV-2 virus, loaded next to each other in a Matrix tube rack in alternating positions. This was to simulate a worst-case scenario for potential sample cross-contamination. All negative samples remained negative for SARS-CoV-2 N1 RNA.

Bridging Studies for Pre-Treatment Heat step

An LoD confirmation study was performed to validate pre-treatment heat steps. Samples were prepared by spiking positive saliva from a confirmed COVID-19 healthcare worker with a known

concentration (3.7×10^4 copies/µL) into saliva collected from healthcare workers who tested negative for SARS-CoV-2. The following concentrations were tested: 6, 3, and 1.5 copies/µL, each with 20 individual replicates. All samples were tested with or without the Thermo Proteinase K and heat inactivation step. Following, all lysates were tested by the standard SalivaDirect RT-qPCR protocol with the NEB Luna kit on the CFX96 Touch PCR instrument:

Pre-Treatment Heat step prior to SalivaDirect protocol without the addition of Proteinase K and heat inactivation step

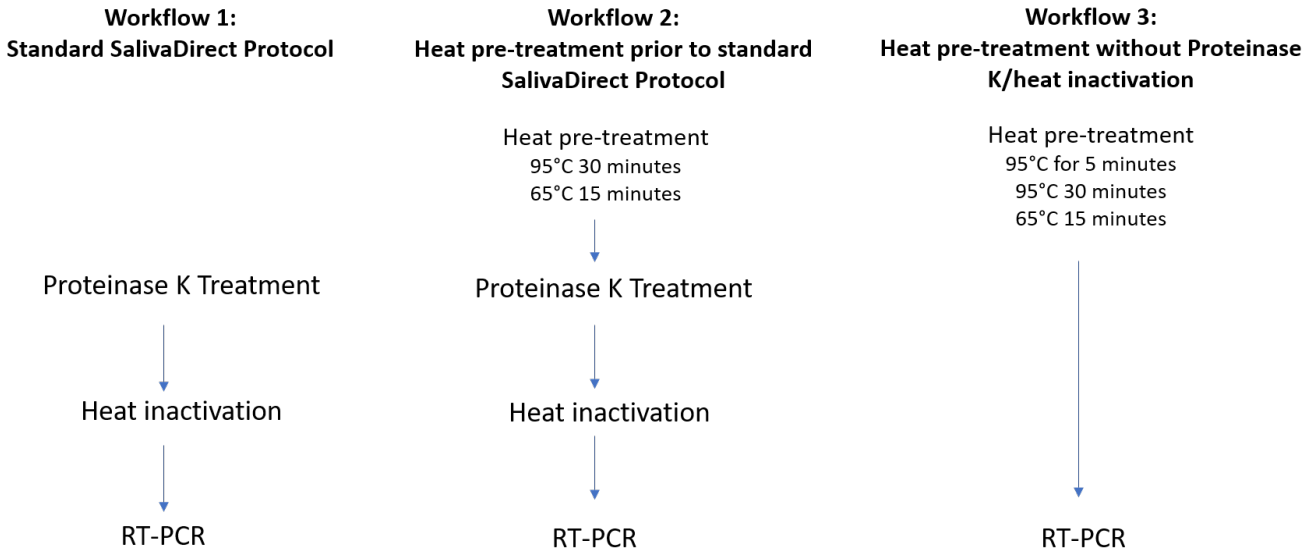
	Concentration (positive replicates)		
	6 copies/µL	3 copies/µL	1.5 copies/µL
65°C for 15 minutes	20/20	20/20	18/20
95°C for 5 minutes	20/20	19/20	18/20
95°C for 30 minutes	20/20	15/20	14/20

The LoD when utilizing a Pre-treatment heat step at the above conditions without the Proteinase K and heat inactivation step confirms to be 3-6 copies/µl, which is comparable to the standard SalivaDirect protocol.

Pre-Treatment Heat step prior to standard SalivaDirect protocol with Proteinase K and heat inactivation step

	Concentration (positive replicates)		
	6 copies/µL	3 copies/µL	1.5 copies/µL
65°C for 15 minutes	20/20	17/20	15/20
95°C for 30 minutes	20/20	16/20	19/20

The LoD when utilizing a Pre-treatment heat step at the above conditions prior to the standard SalivaDirect protocol with the Proteinase K and heat inactivation confirms to be 6 copies/µl, which is comparable to the standard SalivaDirect protocol. Below is an illustrative summary of the workflows including the heat pre-treatment steps:



2) ***Analytical Inclusivity/Cross Reactivity***

The sequences for the N1 primers and probe used in this assay are identical to the primer/probe sequences used in the FDA authorized CDC SARS-CoV-2 assay. Please refer to EUA200001/A004 for an updated in silico analysis of the primers/probes used with the CDC assay.

In addition, SalivaDirect was tested on 52 saliva specimens collected from adults during the 2018/2019 and 2019/2020 (pre-COVID19) autumn/winter influenza seasons. Out of the 52 specimens tested, 51 resulted as negative, and one resulted as invalid (both N1 and RP were not detected).

3) ***Clinical Evaluation:***

Performance in a population suspected of COVID-19:

Performance of SalivaDirect was compared to the authorized ThermoFisher Scientific TaqPath RT-PCR COVID-19 combo kit by testing paired nasopharyngeal and saliva samples. Nasopharyngeal swabs and saliva were collected from inpatients and healthcare workers in the Yale-New Haven Hospital. Saliva was collected in sterile urine cups or 5 mL tubes without addition of any preservatives.

For the preliminary selection of specimens, specimens were tested with a modified version of the US CDC assay. Based on these results, a total of 67 NP/saliva pairs were tested for the current study, with 37 being NP positive and 30 being NP negative by the modified CDC assay. These NP and saliva specimens were subsequently tested in parallel with the EUA-authorized TaqPath COVID-19 combo kit (on NP specimens) and SalivaDirect (on saliva specimens). The ThermoFisher Scientific TaqPath COVID-19 combo kit combines RNA extraction using the MagMax Viral/Pathogen Nucleic Acid Isolation Kit with a multiplex RT-PCR diagnostic assay targeting 3 regions of the SARS-CoV-2 genome. For SalivaDirect testing, the ThermoFisher

Scientific proteinase K, ThermoFisher Scientific TaqPath RT-PCR kit, and Bio-Rad CFX96 Touch instrument were utilized.

Out of the 37 NP specimens that originally tested positive by the modified CDC assay, 34 tested positive with the TaqPath COVID-19 Combo Kit and three tested negative. The TaqPath results from these 34 specimens were used as the comparator for the SalivaDirect when evaluating positive percent agreement (PPA). All 30 NP specimens that were negative by the original modified CDC assay also tested negative by the TaqPath assay. The results from these 30 specimens plus the three TaqPath negative NP specimens described above were used as the comparator for the SalivaDirect when evaluating negative percent agreement (NPA). The results from this paired study are described below:

Qualitative outcome of parallel testing of paired nasopharyngeal swabs and saliva with SalivaDirect and the ThermoFisher Scientific TaqPath COVID-19 combo kit.

		TaqPath RT-PCR COVID-19	
		Nasopharyngeal swab	
		Positive	Negative
SalivaDirect Saliva	Positive	32	3
	Negative	2	30
Total		34	33
Positive agreement = 94.1% (32/34) Negative agreement = 90.9% (30/33)			

Out of the 34 individuals with nasopharyngeal swab specimens that tested positive by the TaqPath COVID-19 kit, 32 had saliva specimens that were positive by the SalivaDirect, yielding a PPA of 94.1%. Out of the 33 individuals with negative NP swab specimens by the TaqPath assay, 30 had saliva specimens that were negative by SalivaDirect, generating an NPA of 90.9%. There were three individuals who tested positive by SalivaDirect on saliva specimens but negative by TaqPath on NP specimens. It should be noted that these 3 individuals previously tested weakly positive with the modified CDC assay.

As an additional analysis, the results from the SalivaDirect on saliva specimens were compared to the results from the modified CDC assay on the paired NP specimens. This modified CDC assay used the 2019-nCoV_N1, 2019-nCoV_N2, and RP primer-probe sets with the NEB Luna Universal Probe One-Step RT-qPCR kit on the Bio-Rad CFX96 Touch. The SalivaDirect results were concordant with 94.6% (35/37) of the NP positive results and 100% of the NP negative results, as shown below:

		Modified CDC RT-PCR	
		Nasopharyngeal swab	
		Positive	Negative
SalivaDirect Saliva	Positive	35	0
	Negative	2	30

Total	37	30
Positive agreement = 94.6% (35/37) Negative agreement = 100% (30/30)		

Performance in an Asymptomatic Screening Population:

To validate the SalivaDirect test for SARS-CoV-2 detection in a screening population, paired nasopharyngeal and saliva samples were collected from asymptomatic individuals enrolled in a routine SARS-CoV-2 testing program. Paired nasopharyngeal and saliva samples were collected at the same sampling moment from 20 consecutive individuals who tested positive and 100 consecutive individuals who tested negative. Paired samples were collected on the same day as initial sample collection and diagnosis while all individuals were still asymptomatic. Saliva samples were tested using the SalivaDirect test with the Thermo Proteinase K and the NEB Luna 2x RT-qPCR kit on the QuantStudio 7 Pro PCR instrument while nasopharyngeal swab specimens were tested using an FDA EUA-authorized high sensitivity testing platform for SARS-CoV-2 detection. A total of 45% of the nasopharyngeal swab specimens tested were low positives according to the EUA authorized comparator assay. Results between the two sample types were 100% concordant:

		EUA-authorized comparator	
		Nasopharyngeal swab	
		Positive	Negative
SalivaDirect Saliva	Positive	20	0
	Negative	0	100
Total		20	100
Positive agreement = 100% (20/20) (95% CI: 83.89%, 100%) Negative agreement = 100% (100/100) (95% CI: 96.3%, 100%)			

These results indicate acceptable performance of the SalivaDirect assay in an asymptomatic screening population.

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. For the study, the ThermoFisher Scientific proteinase K, ThermoFisher Scientific TaqPath RT-PCR kit, and Bio-Rad CFX96 Touch instrument were utilized. The results are summarized in the following Table.

Summary of LoD Confirmation Result using the FDA SARS-CoV-2 Reference Panel

Reference Materials Provided by FDA	Specimen Type	Product LoD	Cross- Reactivity
SARS-CoV-2	Saliva	$1.8x10^4$ NDU/mL	N/A

MERS-CoV		N/A	ND

NDU/mL = RNA NAAT detectable units/mL
N/A: Not applicable
ND: Not detected

LIMITATIONS:

- Negative results for SARS-CoV-2 RNA from saliva should be confirmed by testing of an alternative specimen type if clinically indicated.
- The clinical performance 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:

- This product has not been FDA cleared or approved, but has been authorized for emergency use by FDA under an EUA for use by authorized laboratories;
- This product has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens; and
- 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 Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.