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
Rx Only
For use under Emergency Use Authorization (EUA) only

(The SalivaDirect 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 is a real-time reverse transcription polymerase chain reaction (rRT-PCR) test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in saliva collected without preservatives in a sterile container in the presence of a trained observer (adult trained on how to collect saliva samples) from individuals suspected of COVID-19 by their healthcare provider. This test is also for use with saliva specimens that are self-collected by individuals 18 years of age or older unsupervised at home, and dropped off at a collection site, using the SalivaDirect Unsupervised Collection Kit when determined to be appropriate by a healthcare provider or unsupervised at home using the SalivaDirect At-Home Collection Kit and mailed to a testing laboratory, when used consistent with its authorization. This test is also intended for use in individuals without symptoms or other epidemiological reasons to suspect COVID-19, when tested at least weekly and with and no more than 168 hours between tests using supervised saliva collection, or unsupervised saliva self-collection with the SalivaDirect Unsupervised Collection Kit or the SalivaDirect At-Home Collection Kit..

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 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 for SARS-CoV-2 RNA from saliva should be confirmed by testing of an alternative specimen type if clinically indicated.

SalivaDirect 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 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 assay is authorized for use with the SalivaDirect At-Home Collection Kit, which was authorized for use in a separate EUA (EUA210243).

SalivaDirect Unsupervised Collection Kit:

The SalivaDirect Unsupervised Collection Kit enables the self-collection of a saliva specimen in a sterile container that will be sent to a laboratory designated by the Yale School of Public Health as authorized to run the SalivaDirect Assay when determined to be appropriate by a healthcare provider. The kit collects viral RNA saliva specimens and can be used for the short-term room temperature storage of a sample. The SalivaDirect Unsupervised Collection Kit is a non-invasive alternative for collection of viral RNA by/from individuals who are suspected of COVID-19 by their healthcare provider.

The self-collection kit consists of one of four different options for obtaining saliva specimens:

- Short straw (5-6 cm in length or of similar dimensions to the Salimetrics Saliva Collection Aid, catalog #5016.02)
- Funnel

- Bulb Transfer Pipette (1 mL)
- Pipette Tip (1000 µl)

The SalivaDirect Unsupervised Collection kit will include the following components:

In a zip-lock bag (or similar):

- Self-collection instructions
- One identifying information form for patients to record their name, date of birth and date and time of sample collection (to be created and provided by the test laboratory)
- One of four different devices for obtaining saliva specimens
- One sterile plastic tube (1 to 5 mL in volume)
- One biohazard bag for specimen transport
- One alcohol wipe.

SALIVADIRECT UNSUPERVISED COLLECTION KIT ORDERING, PROCESSING AND MEDICAL OVERSIGHT

The unsupervised collection of saliva samples for use with the SalivaDirect assay can only occur for patients who have been previously qualified by their healthcare provider as needing SARS-CoV-2 testing. The healthcare provider will submit a prescription for testing to the designated laboratories authorized to run the SalivaDirect assay. The designated laboratories will then be responsible for preparing the collection kits as described in the Instructions for Use and providing the Unsupervised Collection Kit to those individuals for whom testing has been ordered. The Unsupervised Collection Kit will contain one of the four authorized devices for obtaining the saliva specimens, one saliva collection tube, a form to gather identifying information (name, date of birth, date/time of sample collection), the unobserved self-collection instructions, a biohazard bag for specimen transport, and an alcohol wipe for contamination issues. The designated laboratory will also be responsible for informing the individual where to return the sample (i.e., the sample could be dropped off at the lab or a specified collection box for that lab; however, **the sample will not be mailed nor shipped**). Test results will then be communicated back to the ordering physician.

INSTRUMENTS USED WITH TEST

SalivaDirect 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

Vendor	Instrument	Software
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	ABI QuantStudio 5 Real-Time PCR system (96 or 384 well format)	QuantStudio Design and Analysis Software 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 (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
Agilent	AriaMX Real-Time PCR System	N/A (fully integrated)

REAGENTS AND MATERIALS

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
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

Vendor	Item	Catalog number	Quantity	# 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)	12YS-010YST	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
	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
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	
	Nuclease-free water	B1500S	25 mL	

Vendor	Item	Catalog number	Quantity	# Reactions
New England Biolabs		B1500L	100 mL	
Order the following positive control				
Twist Bioscience	Synthetic SARS-CoV-2 RNA Control 2	102024	100 µL	

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
Negative Template Control (NTC)	Nuclease-free water	To monitor for contamination of PCR reagents	Every PCR plate with up to 93 saliva samples
Positive	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
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): The NEC should yield a "not detected" result for both the N1 and RNaseP targets.

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:

96-Well Format

Bio-Rad CFX96 Touch ABI 7500 Fast ABI 7500 Fast Dx ABI QuantStudio 5		
Result	**Ct value N1**	**Ct value RP**
Positive	<40.0	Any value
Negative	≥40.0	<35.0
*Invalid	≥40.0	≥35.0

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		
Result	**Cq*** 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 Cq value for RP is <30 and the Cq 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.

***Cq values are qualified cycle thresholds in the Agilent AriaMX system and can be interpreted synonymously to Ct values.

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.

SALIVADIRECT UNSUPERVISED COLLECTION KIT SAMPLE ACCESSIONING

In order for the designated laboratory to perform testing, the received samples shall meet the following criteria:

- **Proper return of sample**: sample is present, identifying information form is present and filled out, the sample tube is not broken, sample is not leaking.
- **Verification of patient information**: the patient information on the collection tube matches the information on the identifying information form.
- **Sample acceptability**: sufficient sample volume, sample received within 72 hours from sample collection date and time (as per identifying information form).

PERFORMANCE EVALUATION

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 an additional 96-well format thermocycler, the Agilent AriaMX, and 384-well format thermocycler, CFX384 Touch. 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 to be 6 copies/µL for both thermocyclers by testing 20 replicates at this concentration.

Proteinase K	*RT-qPCR kit*	*RT-qPCR instrument*	*LOD*	*Positive replicates*	*Mean Ct value (SD)*
Thermo	NEB Luna (2x)	Agilent AriaMX	6 copies/µL	100% (20/20)	30.3 (0.4)
Thermo	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 Mix:

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, 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

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

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

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 PCR instrument:

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

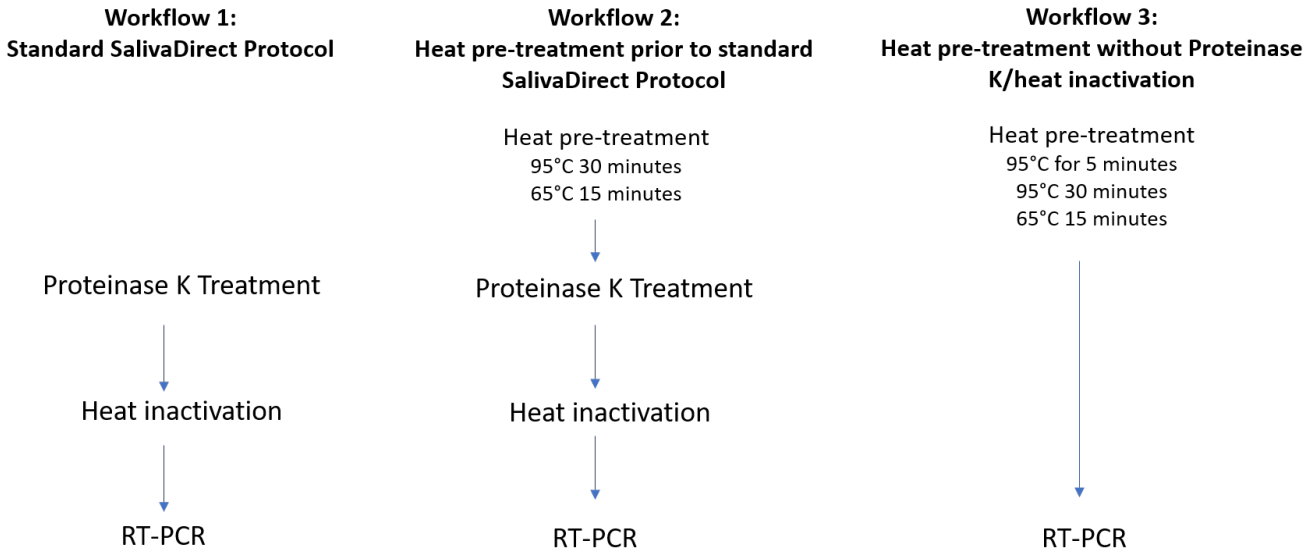
	Concentration (positive replicates)		
	6 copies/µL	3 copies/µL	1.5 copies/µL
65°C for 15 minutes	20/20	20/20	18/20
95°C for 5 minutes	20/20	19/20	18/20
95°C for 30 minutes	20/20	15/20	14/20

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 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)			

4) Human Usability Study for SalivaDirect Unsupervised Collection Kit

A total of 30 participants between the ages of 20 and 80 years who represented a range of racial and educational backgrounds were enrolled in this study. Study demographics are presented below:

Category	**n (%)**
Sex	
Male	11 (37)
Female	19 (63)
Age	
18-29	7 (23)
30-39	16 (53)
40-49	4 (13)
50-59	0 (0)
60-69	1 (3)
70+	2 (7)
Education	
High School/GED	2 (7)
Bachelors	7 (23)
Masters	10 (33)
PhD/MD	11 (37)
Race	
Black/African American	4 (13)
Hispanic/Latino	4 (13)
Asian/South Asian	6 (20)
White	15 (50)
Native American	1 (3)

Individuals who had previously provided a saliva sample, who had relevant, career-level laboratory experience, or who were experiencing symptoms of respiratory infection were excluded from enrollment. Once informed consent was provided, participants received a collection kit containing (1) the four devices for obtaining a saliva specimen, (2) corresponding collection instructions, (3) a biohazard bag, and (4) five alcohol wipes. Participants self-collected four saliva samples consecutively and in a randomized order. Members of the study team observed these collections via a video platform. The observer turned off the camera and audio on their device for the duration of the four collections. Both the observer and the participant completed a survey about their experience following each collection, scoring responses on a scale of 1 (strongly disagree) to 5 (strongly agree). All of the samples (n = 120) were tested for SARS-CoV-2 using SalivaDirect. A laboratory survey assessing the sample quality was completed by the technician during testing.

In 100% of the observed collections, study participants appeared confident in their ability to complete the collection correctly. The majority of participants (93%) understood the importance of following the instructions carefully to avoid incorrect test results, and during only two collections (1.67%), participants appeared to not adequately follow these instructions for proper sample collection. Results for the questions in the observer survey are summarized below:

	Collection device feed-back (1 = strongly disagree, 5 = strongly agree)	**Straw**	**Pipette Tip**	**Funnel**	**Bulb Pipette**
1	Did the study participant read the instructions?	4.93	4.93	5.00	4.97
2	Did the study participant appear confident in their ability to follow the instructions?	4.20	4.30	4.30	4.40
3	Did the study participant properly wash their hands before and after sample collection?	4.20	4.27	4.37	4.60
4	Did the study participant appear to properly follow instructions for sample collection set up?	4.57	4.60	4.63	4.53
5	Did the study participant appear to properly follow instructions for adequate sample collection?	4.63	4.30	4.67	4.43
8	Did the study participant securely fasten the collection tube?	4.90	4.97	4.90	5.00
9	Did the study participant clean down the outside of the sample tube following collection?	4.93	4.97	4.77	4.97
10	Did the study participant properly store their sample in the biohazard bag after collection?	4.07	4.10	4.30	4.13
11	Did the study participant appear to struggle with any particular step? If so, explain which.	1.46	1.79	1.54	1.96

The secondary objective was to compare the quality of samples collected using each device. True saliva, which naturally pools in the mouth, can be easily handled in the laboratory. In contrast, saliva samples that are improperly collected may be problematic. It was found that every sample could be tested for SARS-CoV-2 with SalivaDirect. The internal control, RNaseP was detected in 100% of the samples collected with each of the devices, indicating an adequate specimen was collected. Laboratory survey responses confirmed that 100% of the samples were easy to pipette

and of sufficient volume. Slight discoloration was noted in 18 samples (15%) and food particles were observed in 20 samples (5 participants, 16.7%), but this did not affect test results. No sample tested positive for SARS-CoV-2. Results for the questions in the laboratory survey are summarized below:

Lab questions (1 = strongly disagree, 5 = strongly agree)	Straw	Pipette Tip	Funnel	Bulb Pipette	Average
The sample was of sufficient volume (200-500 ul)	4.97	5	5	5	4.99
The sample was easy to pipette	4.87	4.87	4.87	4.87	4.87
The sample was normal, true saliva	4.87	4.87	4.87	4.87	4.87
The sample was free from food particles	4.76	4.76	4.76	4.76	4.76
The sample was not unusually discolored	4.80	4.87	4.83	4.80	4.83
The sample tested positive for human RNAse P	5.00	5.00	5.00	5.00	5.00
The sample tested positive for SARS-CoV-2	0	0	0	0	0
If the sample tested positive for SARS-CoV-2, this was reported back to the study participant	NA	NA	NA	NA	NA

The results from this study demonstrate that users are able to comprehend the instructions for the four different saliva collection devices as well as collect an adequate specimen for SARS-CoV-2 testing with the SalivaDirect.

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 test has not been FDA cleared or approved, but has been authorized for emergency use by FDA under an Emergency Use Authorization (EUA) for use by authorized laboratories;
- This test 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 test 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.