ACCELERATED 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 from individuals suspected of COVID-19 by their healthcare provider. 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 and trained 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

SalivaDirect is an RNA-extraction free, dualplex RT-qPCR method for SARS-CoV-2 detection (Fig. 1). 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.

INSTRUMENTS USED WITH TEST

SalivaDirect should be used with the following RT-qPCR instruments:

RT-qPCR instrument	Bio-Rad	CFX96 Touch Real-Time PCR Detection System
	ThermoFisher Scientific	Applied Biosystems 7500 Fast Real-Time PCR System
	ThermoFisher Scientific	Applied Biosystems 7500 Fast Dx Real-Time PCR System
	ThermoFisher Scientific	ABI QuantStudio 5 Real-Time PCR System (96 well format)
	ThermoFisher Scientific	ABI QuantStudio 6 Real-Time PCR System (96 well format)
	ThermoFisher Scientific	ABI QuantStudio 7 Real-Time PCR System (96 well format)
	Agilent	AriaMX Real-Time PCR System

Designated laboratories will receive an FDA accepted instrument qualification protocol included as part of the SalivaDirect IFU and will be directed to execute the protocol prior to testing clinical samples. Designated laboratories must follow the authorized IFU, which includes the instrument qualification protocol, as per the letter of authorization.

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 Kit	E3006S	2 mL	200 reactions
		E3006L	5 mL	500 reactions
		E3006X	10 mL	1,000 reactions
		E3006E	25 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
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	
New England Biolabs	Nuclease-free water	B1500S	25 mL	
		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:

Bio-Rad CFX96 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		
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 retreating the primary specimen with proteinase K. 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, including the proteinase K digestion and inactivation, 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.

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	Bio-Rad CFX96	6 copies/µL	100% (20/20)	36.7 (1.0)
NEB	NEB Luna	Bio-Rad CFX96	3 copies/µL	100% (20/20)	36.6 (1.0)
AmericanBio	NEB Luna	Bio-Rad CFX96	3 copies/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	6 copies/µL	100% (20/20)	36.4 (0.6)
Thermo	Thermo TaqPath	Bio-Rad CFX96	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)

A LoD study was conducted to validate an additional thermocycler, the Agilent AriaMX. 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 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	Agilent AriaMX	6 copies/µL	100% (20/20)	30.3 (0.4)

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. 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 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	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	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

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 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. 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)			

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 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 Provide d 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

WARNINGS:

- This test 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 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.