December 11, 2020

EMERGENCY USE AUTHORIZATION (EUA) SUMMARY FOR THE PACIFICDX Covid-19 Test

For *In vitro* Diagnostic Use

Rx Only

For use under Emergency Use Authorization (EUA) only

The PACIFICDX Covid-19 Test will be performed at Pacific Diagnostics located at 5 Mason Drive, Irvine, CA 92618, which is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, and meets requirements to perform high complexity tests, as described in the Standard Operating Procedure that was reviewed by the FDA under this EUA.

INTENDED USE:

The PacificDx Covid-19 Test is a Real-Time RT-PCR assay intended for the qualitative detection of nucleic acid from SARS-CoV-2 in upper respiratory specimens (nasopharyngeal, oropharyngeal, nasal, or mid-turbinate swabs collected by a healthcare provider (HCP), or nasal swabs self-collected in a healthcare setting) from individuals suspected of COVID-19 by a healthcare provider.

This test is also for use with nasal swab specimens that are self-collected at home without the supervision of an HCP, by individuals 18 years of age or older, using the RapidRona Self-Collection Kit when determined to be appropriate by an HCP based on results of a COVID-19 medical questionnaire.

Testing is limited to Pacific Diagnostics located at 5 Mason Drive, Irvine, CA 92618, which is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, and meets requirements to perform high complexity tests.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

The PacificDxCovid-19 Test is intended for use by qualified laboratory personnel specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures. The PacificDx Covid-19 Test is only for use under the Food and Drug Administration's Emergency Use Authorization.

December 11, 2020

SPECIAL CONDITIONS FOR USE

For Emergency Use Authorization (EUA) only
For prescription use only
For in vitro diagnostic use

This assay can be used with the RapidRona Self-Collection Kit which has received Emergency Use Authorization. RapidRona, Inc. has granted Pacific Diagnostics (PacificDx), Inc. a right of reference to the data supporting use of this collection kit.

INSTRUMENTS USED WITH TEST

Process	Instrument/Kit	Software Version
Sample Processing/ Liquid Handling	Hamilton Microlab STAR Liquid Handling System	4.1.1.5875.
PCR Platforms	Applied Biosystems QuantStudio Dx (Cat# 4480299)	Software version 1.0.3
	Applied Biosystems 7900HT (Cat# 4329001)	SDS 2.4

DEVICE DESCRIPTION AND TEST PRINCIPLE

The PacificDx Covid-19 Test is a modification of the CDC 2019-Novel Coronavirus (2019-CoV) Real-Time RT-PCR Diagnostic Panel that received Emergency Use Authorization (EUA) under EUA200001. The assay utilizes identical oligonucleotides, master mix (reverse transcriptase and DNA polymerase), thermocycler conditions, results interpretation and reporting guidelines as documented in EUA200001. The CDC has granted a right of reference to the performance data contained in EUA200001 to any entity seeking an FDA EUA for a COVID-19 diagnostic device.

PacificDx has also been granted a right of reference to the performance data generated in support of the RapidRona EUA and has validated the use of self-collected nasal swabs in accordance with the RapidRona instructions for use.

Following viral inactivation in lysis buffer containing a protease K and guanidine salt, RNA is isolated, converted to cDNA and amplified using Applied Biosystems QuantStudio Dx or 7900HT instrument. During the amplification process, the probe anneals to a specific target sequence located between the forward and reverse primers. During the extension phase of the PCR cycle, the 5’ nuclease activity of Taq polymerase degrades the bound probe, causing the reporter dye (FAM) to separate from the quencher dye (BHQ1), generating a fluorescent signal. Fluorescence intensity is monitored at each PCR cycle by the RT-qPCR instrument. Results interpretation and reporting is equivalent to the CDC 2019-Novel Coronavirus (2019-CoV) Real-Time RT-PCR Diagnostic Panel.

REAGENTS AND MATERIALS

Reagent	Storage Temperature
RNAdvance Viral Reagent Kit (Beckman Coulter CAT:C63510)	Reagent specific; Room temperature, 4°C, or -20°C
Proteinase K (Beckman Coulter CAT:C63510)	-20°C
PK Buffer (Beckman Coulter CAT:C63510)	Room temperature or -20°C when added to Proteinase K
Lysis LBF (Beckman Coulter CAT:C63510)	Room temperature
Bind BBD	4°C
Wash WBE	Room temperature
Isopropanol (Fisher Scientific CAT:A451-1)	Room temperature
Ethanol, Absolute (200 proof) (Fisher Scientific CAT:BP28184)	Room temperature
Nuclease Free Water (ThermoFisher Scientific CAT:AM9937)	Room temperature
Applied Biosystems TaqMan Fast Virus 1-Step Master Mix (Fisher Scientific CAT:44-444-34)	-20°C
2019-nCoV CDC EUA Kit (Integrated DNA Technologies CAT:10006606	-20°C
Negative Control (human total nucleic acids) (ResearchDx)	4°C
Positive control (Genomic RNA from 2019 Novel Coronavirus, strain: 2019-nCoV/USA-WA1/2020; ATCC VR-1986D, spiked into human total nucleic acids) (ResearchDx)	4°C

CONTROLS TO BE USED WITH THE TEST

1) **Positive Control:** The nCoVPC consists of Genomic RNA from 2019 Novel Coronavirus, Strain: 2019-nCoV/USA-WA1/2020; ATCC VR-1986D, spiked into a human genomic RNA background and is included on every PCR plate.

2) **Negative Control (nCoVNC):** The nCoVNC consists a human genomic RNA background and is included on every PCR plate.

3) **No Template Control (NTC):** The NTC consists of molecular grade water in the RT-PCR reactions instead of RNA and is included one per PCR plate. If any of the NTC reactions yield a positive result, sample contamination may have occurred.

4) **Human Specimen Control (HSC) (Extraction Control):** The HSC is used as a nucleic acid extraction procedural control to demonstrate successful recovery of nucleic acid as well as extraction reagent integrity. One HSC is included per extraction batch. The HSC consists of ~10^5 noninfectious cultured human cells in a VTM background.

5) **Internal Control:** The assay includes primers and a probe for detection of endogenous RNase P (RP) nucleic acid that is extracted and amplified from every patient sample.

INTERPRETATION OF THE TEST

All test controls must be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted. Results interpretation and reporting is performed according to the CDC 2019-Novel Coronavirus (2019-CoV) Real-Time RT-PCR Diagnostic Panel (EUA200001). Results are reported to the ordering HCP or through the RapidRona medical oversight and result reporting process, as applicable.

Expected Performance of Controls

Control Type	External Control Name	Used to Monitor	2019 nCoV_ N1	2019 nCov_ N2	RP	Expected Ct Values
Positive	nCoVPC	Substantial reagent failure including primer and probe integrity	+	+	+	< 40.00 Ct
Negative	nCoVNC	Reagent and/or environmental contamination with SARS-CoV-2 reactive nucleic acids	-	-	+	< 40.00 Ct
Negative	NTC	Reagent and/or environmental contamination	-	-	-	None detected
Extraction	HSC	Failure in lysis and extraction procedure, potential contamination during extraction	-	-	+	< 40.00 Ct

Interpretation of Patient Specimens

N1	N2	RP	Result Interpretation	Actions
+ < 40.00 Ct	+ < 40.00 Ct	±	SARS-CoV-2 Detected	Report results
If only one of the two targets is positive		±	Inconclusive for SARS-CoV-2	Repeat testing of nucleic acid and/or re-extract and repeat RT-qPCR. If it is still "Inconclusive", report results.
-	-	+ < 40.00 Ct	SARS-CoV-2 Not Detected	Report results
-	-	-	Invalid Result	Repeat extraction and RT-qPCR. If the repeated result remains invalid, consider collecting a new specimen from the patient. If still "Invalid", report results.

PERFORMANCE EVALUATION OF THE PacificDx Covid-19 Test

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

The LoD studies assessed the lowest detectable concentration of SARS-CoV-2 at which greater or equal to 95% of all (true positive) replicates test positive. To determine the LoD, SARS-CoV-2 (BEI Resources, SARS-Related Coronavirus 2, Isolate USA-WA1/2020, Heat Inactivated, Catalog

No. NR-52286) was spiked into SARS-CoV-2-negative nasopharyngeal (NP) swab specimens in VTM media at 2-fold concentrations ranging from 100 to 3200 viral copies/mL. A total of three contrived specimens at each concentration were assayed. Preliminary LoD for the assay was determined to be 800 copies/mL.

Summary of Preliminary LoD determination (ABI 7900HT)

Target Level		**SARS CoV-2 (N1) Positive/Total**	**SARS CoV-2 (N2) Positive/Total**	**RNase P Positive/Total**
Copies / reaction	**Copies / mL**			
2	100	1/3	0/3	3/3
4	200	2/3	1/3	3/3
8	400	3/3	2/3	3/3
16	800	3/3	3/3	3/3
32	1600	3/3	3/3	3/3
64	3200	3/3	3/3	3/3

The LoD was confirmed by spiking SARS-CoV-2 at a concentration of 800 and 1600 copies/mL into 22 NP swab samples in VTM/UTM media previously confirmed to be negative for SARS-CoV-2. Nucleic acid was extracted from each of the contrived samples using the RNAdvance Viral Extraction Kit and the reverse transcription RT-PCR was performed using the QuantStudio Dx as well as the ABI 7900HT PCR system. The LoD of the PacificDx Covid-19 Test with ABI 7900HT and the QuantStudio Dx was confirmed to be 1600 copies/ml (22/22 positive).

Summary of LoD confirmation (QuantStudio Dx)

Target Level		**SARS CoV-2 (N1)**	**SARS CoV-2 (N2)**	**RNase P**
Copies/reaction	Copies/mL	Positive/Total (Detection Rate)	Positive/Total (Detection Rate)	Positive/Total (Detection Rate)
16	800	13/22 (59%)	14/22 (64%)	22/22 (100%)
32	1600	22/22 (100%)	22/22 (100%)	22/22 (100%)

Summary of LoD Confirmation

Target	Thermocycler	Concentration (Copies/mL)	Ratio confirmed	Average Ct	SD
N1	QuantStudio DX	1,600	22/22	34.3	1.13
	ABI 7900	1,600	22/22	33.8	0.55
N2	QuantStudio DX	1,600	22/22	36.5	0.97
	ABI 7900	1,600	22/22	34.0	0.48
RP	QuantStudio DX	1,600	22/22	27.0	2.03
	ABI 7900	1,600	22/22	25.3	1.95

LoD Study in Support of Use of the RapidRona Self-Collection Kit:

An abbreviated study was performed to confirm LoD of the assay when used on nasal swab samples collected with the RapidRona Self-Collection Kit.

Forty-three samples were collected using the RapidRona Self-Collection Kit per the Instructions for Use and shipped to PacificDx. All samples were tested using the PacificDx Covid-19 Test upon receipt to assess their COVID-19 status. All specimens were determined to be SARS-CoV-2 negative, with neither of the two SARS-CoV-2 RNA targets detected in any of the samples, and RNase P detected in all (Mean Ct 27.3 ± 2.6).

The samples were then spiked with heat-inactivated SARS-CoV-2 viral particles (BEI Resources, Cat No. NR-52286) at 1900 viral particles / mL of saline medium and tested again using the PacificDx Covid-19 Test after a simulated shipping study. All 43 spiked samples returned a positive result, confirming detection of SARS-CoV-2 RNA in all and LoD with this sample type. The mean Ct values for N1, N2, and RP were 33.4, 33.8, and 26.1, respectively.

2) Inclusivity (analytical sensitivity)/ Cross-reactivity (analytical specificity):

The target sequences for the PacificDx Covid-19 Test are the N1 and N2 regions of the viral nucleocapsid gene and the endogenous RNase P internal control from the CDC 2019-Novel Coronavirus (2019-nCoV) Real-time RT-PCR Panel. The CDC has granted a Right of Reference to the performance data contained in the CDC's EUA request to any entity seeking an FDA EUA for a COVID-19 diagnostic device. Details are provided in the Instructions for Use for the CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel.

3) Clinical Evaluation:

The performance of the PacificDx Covid-19 Test was evaluated using a total of 163 clinical specimens, including specimens designated as nasopharyngeal/oropharyngeal, nasopharyngeal, and nasal swab specimens. RNA was extracted using the RNAdvance Viral Extraction Kit and the reverse transcription RT-PCR was performed using the ABI 7900HT PCR systems. The ABI 7900HT demonstrated 100% (89/89) positive percent agreement (PPA) and 97.0% (65/67) negative percent agreement (NPA) for all specimen types combined.

Clinical Performance of the PacificDx Covid-19 Test for All Specimen Types Combined

		Comparator Test			
		Positive	**Inconclusive***	**Negative**	**Total**
PacificDx Covid-19 Test	**Positive**	89	7	2	98
	Negative	0	0	65	65
	Total	89	7	67	163
PPA: 100% (89/89) (95% CI, 95.86~ 100%)					
NPA: 97.01% (65/67) (95% CI, 89.75~ 99.18%)					

*Not included in PPA or NPA calculations.

Clinical Performance by Specimen Type:

Nasopharyngeal/Oropharyngeal Swabs (NP/OP)		**Comparator test(s)**			
		Positive	**Inconclusive***	**Negative**	**Total**
PacificDx Covid-19 Test	Positive	15	N/A	0	15
	Negative	0	N/A	30	30
	Total	15	N/A	30	45
PPA: 100% (95% CI, 79.62~ 100%) NPA: 100% (95% CI, 88.65~100%)					

Nasopharyngeal Swabs (NPS)		**Comparator test**			
		Positive	**Inconclusive***	**Negative**	**Total**
PacificDx Covid-19 Test	Positive	30	0	0	30
	Negative	0	0	0	0
	Total	30	0	0	30
PPA: 100% (95% CI, 88.65~ 100%)					

Nasal Swabs (NS)		**Comparator test**			
		Positive	**Inconclusive***	**Negative**	**Total**
PacificDx Covid-19 Test	Positive	44	7	2	53
	Negative	0	0	34	34
	Total	44	7	36	87
PPA: 100% (95% CI, 91.97~ 100%) NPA: 94.44% (95% CI, 81.86~99.31%)					

*Not included in PPA or NPA calculations.

Summary of Ct values for Positive Clinical Specimens:

	N1		**N2**		**RP**	
	Median Ct	**Ct range**	**Median Ct**	**Ct range**	**Median Ct**	**Ct range**
NP/OP	26.83	11.08-36.7	27.22	11.56-36.06	24.14	22.26-28.91
NPS	17.13	12.11-30.71	17.04	12.1-21.24	23.96	21.06-29.57
NS	20.58	9.64-36.93	20.55	9.92-37.01	29.42	22.99-33.71

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 Pacific Diagnostics located at 5 Mason Drive, Irvine, CA 92618.
• This test has been authorized only for the detection of nucleic acid from SARSCoV-2, not for any other viruses or pathogens.
• The emergency use of this 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.