EMERGENCY USE AUTHORIZATION (EUA) SUMMARY
SARS-CoV-2 DUCoM-PDL Modified Tetracore Assay
(Drexel University College of Medicine)

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

The SARS-CoV-2 DUCoM-PDL Modified Tetracore Assay will be performed at Drexel University Drexel Medicine Diagnostics, located at 245 N. 15th Street, Room 5401, Philadelphia, PA 19102, 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 Laboratory Standard Operating Procedures that were reviewed by the FDA under this EUA.

INTENDED USE

The SARS-CoV-2 DUCoM-PDL Modified Tetracore Assay is an in vitro real-time reverse transcription polymerase chain reaction (rRT-PCR) test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in mid-turbinate nasal swab specimens from individuals suspected of COVID-19 by their healthcare provider.

Testing is limited to Drexel University Drexel Medicine Diagnostics, located at 245 N. 15th Street, Room 5401, Philadelphia, PA 19102, 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 mid-turbinate nasal swab 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 definitive cause of disease. Laboratories within the United States and its territories are required to report all test 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/or epidemiological information.

The SARS-CoV-2 DUCoM-PDL Modified Tetracore Assay is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time RT-PCR and in vitro diagnostic procedures. The SARS-CoV-2 DUCoM-PDL Modified Tetracore Assay is only for use under the Food and Drug Administration's Emergency Use Authorization.

DEVICE DESCRIPTION AND TEST PRINCIPLE

Device Description

The SARS-CoV-2 DUCoM-PDL Modified Tetracore Assay is a RT-PCR Test Kit designed to detect RNA from SARS-CoV-2 in nasal mid-turbinate swab specimens collected in Edge Biological saline 0.85% by a healthcare provider.

The SARS-CoV-2 DUCoM-PDL Modified Tetracore Assay utilizes two primer and probe sets to detect a conserved region in the SARS-CoV-2 nucleocapsid (N1 and N2) gene utilizing a single reporter dye (FAM) for detection of all N gene targets. It also utilizes one primer and probe set to detect a human S9 ribosomal gene in a clinical sample. RNA from swab specimens is reverse transcribed to cDNA and subsequently amplified using either a QuantStudio 6 Pro or QuantStudio 7 Flex instrument. During the RT-PCR reaction the probe anneals to a specific target sequence between the forward and reverse primers, the 5' exonuclease activity of Taq polymerase degrades the bound probe during the extension phase of the PCR cycle, which causes the 5' labeled reporter dye separate from the quencher generating a fluorescent signal. During the PCR amplification, fluorescence generated by degradation of the target-specific probe is monitored by the QuantStudio instrument(s).

Description of Test Steps:

1. Specimen Transport and Storage

Mid-turbinate nasal swab specimens are collected on nylon flocked nasopharyngeal swabs, placed in Edge Biological saline 0.85% and transported, stored, and processed according to CLSI MM13-A. Specimens are stored at 4°C up to 6 days.

2. Specimen Testing

Prior to performing RT-PCR, crude samples are diluted. All sample steps including dilution of crude sample and preparation of the RT-PCR plate are automated by the INTEGRA ASSIST PLUS liquid handler. RT-PCR amplification is performed in a 96-well or 384-well format using either the Applied Biosystems QuantStudio 6 Pro with software version 1.5 and Instrument Firmware 1.5.0 or Applied Biosystems QuantStudio7 Flex Real-Time PCR System with software version 1.3 and Instrument Firmware 1.0.4. Data interpretation and analysis are performed by QuantStudio Design and Analysis Software v2 version 2.6.0.

3. Result Reporting

All test results are reported to the requesting healthcare provider via the authorized distributor's Electronic Health Record (EHR) system and public health authorities in accordance with local, state, and federal requirements.

INSTRUMENTS USED WITH THE TEST

Table 1: Instruments and Software

Instruments	Manufacturer	Software	Firmware
QuantStudio 7 Flex	ThermoFisher	Version 1.3	1.0.4

Instruments	Manufacturer	Software	Firmware
QuantStudio 6 Pro	ThermoFisher	Version 1.5	1.5.0
QuantStudio Design	ThermoFisher	Version 2	2.6.0

REAGENTS AND MATERIALS

Reagents should not be used past their expiration date.

Table 2: Assay Reagents

Reagent	Manufacturer/Supplier	Catalog Number
Nylon Flocked Nasopharyngeal Swabs, 80 mm break point, sterile	Genesee Scientific	88-220C
Saline 0.85%	Edge Biological	T-0603
TetraCore EZ-SARS-CoV-2 Real-Time RT-PCR Test	TetraCore	TC-5048-192
Master Mix Blend	TetraCore	TC-5048-192
Enzyme Blend	TetraCore	TC-5048-192
Positive Control	TetraCore	TC-5048-192
Inhibition Control	TetraCore	TC-9111-100
NATtrol SARS-Related Coronavirus 2 (SARS-CoV-2) Inactivated Virus Stock	ZeptoMetrix	NATSARS(COV2)-ST
HyPure molecular Biology Grade Water, 500 mL, cytiva	HyClone	SH30538.02
Ultrapure Distilled Water, 500 mL	Invitrogen by life sciences	SH305538
Acid Blue 9	TCI America	TCI B0790
Gel Food Colors Blue No.1 Dye	Wilton Industries, Inc. Gel Food Colors | Blue	601-1006
Saline 0.85%	Edge Biological	T-0603
96 Well Plates, Sterile, Non-Pyrogenic	CELLTREAT	229597
384 Well PCR Plates, Olympus plastics	Genesee Scientific	24-305
Optical Adhesive PCR Film ThermalSeal RTS, EXCEL Scientific, Inc.	Genesee Scientific	12-537

CONTROLS

The SARS-CoV-2 DUCoM-PDL Modified Tetracore Assay utilizes an Endogenous Internal control (RNaseP), Inhibition control, Blank Control, Negative Control, and Positive Control (**Table 3**). The Endogenous and Inhibition controls are applied to each patient sample. The Blank Control, Negative Control, and Positive Control are used with each plate.

Table 3: Control Materials

Control Type	Description	Expected Results	Purpose
Endogenous Internal Control (RNaseP)	Primer and probe set to detect a human S9 ribosomal gene in clinical sample	Ct ≤ 37.5	Confirms that each sample being tested contains at least 10 pg. Monitors correct processing of patient material, reverse transcription of mRNA to cDNA, Taq DNA polymerase function, and bound probe is being hydrolyzed by 5'nuclease activity
Inhibition Control (IC)	IVT TetraCore proprietary control (8,000 copies/µL)	Ct ≤ 37.5	Inhibition of the RT-PCR process
Blank control	Ultrapure distilled water in blue dye	SAR-S CoV-2, RNaseP negative, IC positive	Monitors contamination of RT-PCR reagents
Negative control	A confirmed negative SARS-CoV-2 patient specimen	SARS-CoV-2 negative, IC and RNaseP positive	Control for specificity of the amplification reaction
Positive control	Positive control from TetraCore (100 copies/µL)	SARS-CoV-2 and IC positive	Control for amplification and N1/N2 primer-probe reagent integrity.

Table 4: Interpretation of Controls

Control Name	SARS-CoV-2	IC	RNaseP
Positive Control	28 ≤ Ct ≤ 33	≤ 37.5	-
Negative Control	-	≤ 37.5	≤ 37.5
Blank Control	-	≤ 37.5	-

INTERPRETATION OF RESULTS

Assessment of clinical specimen test results are performed after positive and negative controls have been examined and determined to be valid and acceptable (**Table 5**). If the controls are not valid, patient results cannot be interpreted. Any patient specimen that produces an indeterminate result is retested by processing the original sample and repeating the RT-PCR.

Table 5: Interpretation of Patient Results

SARS-CoV-2	Internal Control	RNaseP	Interpretation
+	+	+	SARS-COV-2 DETECTED
+	+	-	SARS-COV-2 DETECTED
+	-	+	SARS-COV-2 DETECTED

SARS-CoV-2	Internal Control	RNaseP	Interpretation
+	-	-	SARS-COV-2 DETECTED
-	+	+	SARS-COV-2 NOT DETECTED
-	+	-	SARS-COV-2 NOT DETECTED
-	-	+	INDETERMINATE
-	-	-	INDETERMINATE

PERFORMANCE EVALUATION

Limit of Detection (LoD) - Analytical Sensitivity:

The LoD of the SARS-CoV-2 DUCoM-PDL Modified Tetracore Assay was determined by using NATtrol SARS-Related Coronavirus 2 (SARS-CoV-2) Inactivated Virus Stock, ZeptoMetrix, (NATSARS(COV2)-ST) which has a starting concentration of 1 x 10^6 copies/µL. To estimate the LoD, three replicate samples were contrived at 6 different concentrations using negative, clinical matrix consisting of mid-turbinate nasal swab samples in saline. Samples were tested on the QuantStudio 6 Pro and QuantStudio 7 Flex instruments. The lowest concentration at which all three replicates produced positive results was defined as the preliminary LoD (**Table 6**). The preliminary LoD was then confirmed by testing an additional 20 replicates at the estimated LoD concentration (**Table 7**). The confirmed LoD of the SARS-CoV-2 DUCoM-PDL Modified Tetracore Assay was 30 copies/µL of starting sample.

Table 6: Preliminary LoD Determination (Saline)

Instrument	Concentration (cp/µL)					
	801	267	89	**30**	10	3
QuantStudio 6 Pro	3/3	3/3	3/3	**3/3**	3/3	2/3
QuantStudio 1 Flex 1	3/3	3/3	3/3	**3/3**	0/3	0/3
QuantStudio 7 Flex 2	3/3	3/3	3/3	**3/3**	0/3	0/3

There was 100% agreement on all analyzers except for one of the QuantStudio 7 Flex instruments which yielded 95% agreement (**Table 7**).

Table 7: Confirmation of LoD (Saline)

Instrument	Concentration (copies/µL)	Positive
QuantStudio 6 Pro	30	20/20
QuantStudio 7 Flex 1		20/20
QuantStudio 7 Flex 2		19/20

BD Universal Viral Transport (UVT) LoD

To determine equivalency between saline and BD Universal Viral Transport (UVT), for collection and transport of samples, the LoD for the SARS-CoV-2 DUCoM-PDL Modified Tetracore Assay with UVT was determined by creating serial dilutions of NATtrol SARS-Related Coronavirus 2 (SARS-CoV-2) Inactivated Virus Stock spiked at

known concentrations into mid-turbinate nasal swab clinical matrix from patient samples which were previously reported to be negative. Samples were tested at the same concentrations as the contrived samples in the original LoD with saline. Minimum concentration where all replicates gave positive signal were considered the preliminary LOD. The preliminary LoD for UVT was determined as 10 cp/µL (**Table 8**).

Table 8: Preliminary LoD Determination (UVT)

Instrument	Concentration (cp/µL)					
	801	267	89	30	**10**	3
QuantStudio 6 Pro	3/3	3/3	3/3	3/3	**3/3**	3/3
QuantStudio 7 Flex	3/3	3/3	3/3	3/3	**3/3**	2/3

Since the original preliminary LoD with saline yielded an LoD of 30 cp/µL and the preliminary LoD with UVT yielded an LoD of 10 cp/µL, the LoD was confirmed by testing 20 replicate samples at both LOD concentrations (30 & 10 cp/µL) on each instrument platform. NATtrol SARS-Related Coronavirus 2 (SARS-CoV-2) Inactivated Virus Stock was spiked to each LOD concentration in the BD Universal Viral Transport (UVT) negative mid-turbinate nasal clinical matrix. The confirmed LoD of the SARS-CoV-2 DUCoM-PDL Modified Tetracore Assay with both saline and UVT was determined to be 30 copies/µL (**Table 9**).

Table 9: Confirmation of LoD (UVT)

Instrument	Concentration (cp/µL)		Mean 30 cp/µL	SD 30 cp/µL	CV% 30 cp/µL
	30	10			
QuantStudio 6	**20/20**	20/20	32.374	0.353	1.09
QuantStudio 7	**20/20**	17/20	32.204	0.553	1.72

Inclusivity (analytical reactivity):

The oligonucleotide primers and probes used in this test are designed to detect regions of the virus nucleocapsid protein (N) gene of SARS-CoV-2 and consist of two Center for Disease Control (CDC) SARS-COV-2 specific sequences (i.e., 2019-nCoV_N1 forward and reverse, and 2019-nCoV_N2 forward and reverse) that are detected on the same reporter dye (FAM). Sequences were searched for each primer set on the Global Initiative on Sharing Avian Influenza Data (GISAID) website (www.gisaid.org) using their PrimerChecker (v3.04) application. Sequences were examined for at least one mutation in each primer sequence. Genomes which showed at least one mutation in at least one member of a PCR primer pair were examined for all possible primer combinations. Only one single genome was found in both the United States of America that contained a mutation in both the reverse primers of 2019-nCoV-N1 and 2019-nCoV-N2. According to the GISAID website in their figure "Common Primer Check for High Quality Genomes 2022-10-18", the number of mutations among all ~1.2 million high quality uploaded genome sequences was approximately 0.1% for the 2019-nCoV-N2 primer pair,

and 0.4% for the 2019-nCoV-N1 pair which has been determined as an acceptable level of risk.

To continue to monitor the levels of mutations among the possible primer pair combinations present in the US population of SARS-COV-2 sequences, inclusivity analysis is performed monthly. If primer pair sequence mutation levels ever increase past an acceptable degree (~5% sequence mismatch), new primer combinations will be considered to mitigate sensitivity loss.

Cross Reactivity (analytical specificity)

N/A - The primers and probes are the same as those used in the CDC assay and the sponsor is relying on the blanket Right of Reference for cross-reactivity.

Microbial Interference:

N/A - The primers and probes are the same as those used in the CDC assay and the sponsor is relying on the blanket Right of Reference for microbial interference.

Endogenous/Exogenous Interference Evaluation:

The impact of potential interfering substances on the SARS-CoV-2 DUCoM-PDL Modified Tetracore Assay was evaluated via spiking potential interfering substances into positive and negative specimens. Positive specimens were created via spiking inactivated virus to create samples at 2-3x LoD. Each interfering substance was tested in 3 replicates. No false negative or false positive results occurred during the study (**Table 10**).

Table 10: Interference Testing

Substance	Brand	Concentration	SARS-CoV-2 Concentration	Positive Sample Results (#Pos/Total)	Negative Sample Results (#Pos/Total)
Nasal Wash Mucus Solvent ad Cleaner	Alkalol	15% v/v	2-3 x LoD	3/3	0/3
Fluticasone Propionate Nasal Spray	CVS Health	15% v/v	2-3 x LoD	3/3	0/3
Afrin Nasal Spray	Afrin	15% v/v	2-3 x LoD	3/3	0/3
VapoCool Sore throat	Vicks	5% v/v	2-3 x LoD	3/3	0/3
Chloroseptic Sore Throat spray	Chloraseptic	5% v/v	2-3 x LoD	3/3	0/3
Antiseptic Mouth Wash	CVS Health	5% v/v	2-3 x LoD	3/3	0/3
Robitussin Cough Syrup	Robitussin	5% v/v	2-3 x LoD	3/3	0/3
Mucin: bovine submaxillary gland, type I-S	ThermoScientific	2.5 mg/ml	2-3 x LoD	3/3	0/3

Substance	Brand	Concentration	SARS-CoV-2 Concentration	Positive Sample Results (#Pos/Total)	Negative Sample Results (#Pos/Total)
Nicotine	Alta Aesar	0.03 mg/mL	2-3 x LoD	3/3	0/3
Optic White Advanced Toothpaste (Saliva)	Colgate	5% v/v	2-3 x LoD	3/3	0/3
Saliva	From Lab Tech	5% v/v	2-3 x LoD	3/3	0/3
Blood	From Lab Tech	5% v/v	2-3 x LoD	3/3	0/3

Sample Stability:

Specimen stability for 7 days at 4˚C was evaluated via testing of positive and negative mid-turbinate nasal swab samples in 0.85% saline. Cycle Threshold (Ct) values were recorded at the initial PCR run (Time 0), at 3 days, and 7 days after the initial test. No significant loss in Ct value was observed over the course of 7 days (**Table 11**). Based on the results, this study supports storage of specimens for a maximum of 6 days at 4°C.

Table 11: Sample Stability Analysis

Sample	Ct within 24hr.		Ct at Day 3		Ct at Day 7		% ΔCt SARS	% ΔCt RNaseP
	SARS Ct	RNase P Ct	SARS Ct	RNaseP Ct	SARS Ct	RNaseP Ct		
1	21.788	29.743	20.669	30.952	20.882	30.346	-4.16%	2.03%
2	30.529	29.990	28.189	30.223	28.174	29.167	-7.72%	-2.74%
3	32.600	29.511	30.485	29.752	30.451	28.531	-6.59%	-3.32%
4	34.448	29.139	34.142	28.649	33.583	28.143	-2.51%	-3.42%
5	25.666	30.030	25.524	30.280	25.563	29.291	-0.40%	-2.46%
6	Undetermined	29.611	Undetermined	28.305	Undetermined	27.039	None	-8.69%
7	Undetermined	30.924	Undetermined	30.247	Undetermined	29.563	None	-4.40%
8	Undetermined	26.628	Undetermined	26.275	Undetermined	25.418	None	-4.54%
9	Undetermined	29.840	Undetermined	30.499	Undetermined	29.492	None	-1.17%
10	Undetermined	28.999	Undetermined	28.493	Undetermined	27.231	-4.16%	2.03%

Clinical Evaluation for Patients Suspected of COVID-19:

Clinical performance of the SARS-CoV-2 DUCoM-PDL Modified Tetracore Assay was evaluated by testing a total of 70 mid-turbinate nasal swab patient samples collected in UVT from patients suspected of COVID-19 by a healthcare provider and by a highly sensitive FDA-authorized Molecular SARS-CoV-2 RT-PCR Assay. Among these specimens, 40 were positive and 30 were negative as determined by the comparator method. The positive percent agreement was 97.5% (39/40) and the negative percent agreement was 100% (30/30). Based on the Ct values obtained with the comparator method, at least 20% of positive samples have Ct values within 3 cycles of the average Ct at the LoD of the comparator assay and were considered “weak positive”. The results of this study support the use of the SARS-CoV-2 DUCoM-PDL Modified Tetracore Assay

for SARS-CoV-2 testing for individuals suspected of COVID infection and are presented in **Table 12**.

Table 12: Clinical evaluation results for patients suspected of COVID-19

		EUA Authorized Comparator Test	
		Positive	**Negative**
SARS-CoV-2 DUCoM-PDL Modified Tetracore Assay	**Positive**	39	0
	Negative	1	30
Positive Agreement		97.5% (CI: 87.1%, 99.6%)	
Negative Agreement		100% (CI: 88.7%, 100%)	

WARNINGS

- For use under Emergency Use Authorization (EUA) only.
- For *in vitro* diagnostic use.
- For prescription use only.
- This product has not been FDA cleared or approved, but has been authorized for emergency use by FDA under an EUA for use by the authorized laboratory.
- 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 Cosmetics Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.

LIMITATIONS

- The SARS-CoV-2 DUCoM-PDL Modified Tetracore Assay is intended and validated for use only with mid-turbinate nasal swab specimens. Testing of other sample types may result in inaccurate results.
- Primers and probes for the SARS-CoV-2 DUCoM-PDL Modified Tetracore Assay target highly conserved regions within the genome of SARS-CoV-2. Mutations rarely occur in these highly conserved regions, but if a mutation did occur in these regions, SARS-CoV-2 RNA could become undetectable.
- Negative results in the SARS-CoV-2 DUCoM-PDL Modified Tetracore Assay do not preclude SARS-CoV-2 infection and should not be used as the sole basis for treatment or other patient management decisions.
- A false negative result may occur if a specimen is improperly collected, transported, or handled. False negative results may also occur if amplification inhibitors are present in the specimen or if inadequate numbers of organisms are present in the specimen.
- If the virus mutates in the regions targeted by the RT-PCR assay, SARS-CoV-2 may not be detected or may be detected less predictably.
- A false positive result may occur if there is cross-contamination by target organisms, their nucleic acids or amplified product.

- Detection of viral RNA may not indicate the presence of infectious virus or that SARS-CoV-2 is the causative agent for clinical symptoms.
- This test cannot rule out diseases caused by other bacterial or viral pathogens.
- The performance of this test was established based on the evaluation of a limited number of clinical specimens. Clinical performance has not been established with 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.
- The clinical performance of this test was established based on the evaluation of mid-turbinate nasal swab samples collected in UVT. Analytical performance of mid-turbinate nasal swab samples collected in 0.85% saline were determined to be equivalent. However, clinical performance has not been assessed or determined.