FDA REVIEW MEMORANDUM FOR EMERGENCY USE AUTHORIZATION (EUA) OF THE PMLS SARS-CoV-2 Assay

PMLS SARS-CoV-2 Assay

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

The PMLS SARS-CoV-2 Assay will be performed at Premier Medical Laboratory Services which is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a and meets the 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

1) Intended Use

The PMLS SARS-CoV-2 Assay is an *in vitro* diagnostic real-time reverse transcription polymerase chain reaction (rRT-PCR) Test for the qualitative detection of nucleic acid from SARS-CoV-2 in anterior nasal swab specimens that are collected at home using the DoINeedaCOVID19Test.com Self-Collection Kit by any individual, 18 years or older (self-collected), 14 years and older (self-collected under adult supervision) or 2 years and older (collected with adult assistance), including individuals without symptoms or other reasons to suspect COVID-19, when determined to be appropriate by a healthcare provider.

This test is also for use with anterior nasal swab specimens that are collected using the RapidRona Self-Collection Kit when used consistent with its authorization.

Testing is limited to Premier Medical Laboratory Services, 6000A-1 Pelham Rd, Greenville, SC 29615, which is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263, and meets the 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 anterior 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 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 PMLS SARS-CoV-2 Assay is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures. The PMLS SARS-CoV-2 Assay and DoINeedaCOVID19Test.com Self-Collection Kit are only for use under the Food and Drug Administration's Emergency Use Authorization.

2) Special Conditions of Use Statements

For prescription use only
For in vitro diagnostic use
For Emergency Use only

This assay can be used with the RapidRona Self-Collection Kit. RapidRona, Inc. is owned by Diversified Medical Healthcare, which is the parent company of Premier Medical Laboratory Services, Inc. and therefore has right of reference to the data supporting use of this collection kit.

DEVICE DESCRIPTION AND TEST PRINCIPLE

Device Description
The PMLS SARS-CoV-2 Assay is a modification of the Centers for Disease Control and Prevention (CDC) 2019-novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel (EUA200001) and is designed to detect RNA from SARS-CoV-2 in anterior nasal swab specimens that are self-collected using either the DoINeedaCOVID19Test.com Self-Collection Kit or the RapidRona Self-Collection Kit (when used consistent with its authorization).

The CDC has granted a Right of Reference to the performance data contained in the CDC's EUA request for the 2019-novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel under EUA200001 to any entity seeking an FDA EUA for a COVID-19 diagnostic device.

The DoINeedaCOVID19Test.com Self-Collection Kit consists of a packaged sterile swab, sterile collection tube, transport medium (saline), shipping materials, barcode labels for specimen identification, and printed Instructions For Use (IFU) that state how to register, collect and return the sample to a drop-off location for shipment to the Testing laboratory. Each DoINeedaCOVID19Test.com Self-Collection Kit is intended to be dropped off on the day of specimen collection for return shipment overnight at ambient conditions for next day delivery.

The components of the DoINeedaCOVID19Test.com Self-Collection Kit and associated instructions for specimen collection are based on those for the previously authorized RapidRona Self-Collection Kit (EUA202347). RapidRona, Inc. has granted a Right of Reference to Premier Medical Laboratory Services to data contained in EUA202347 for the RapidRona Self-Collection Kit, including information regarding sample stability and usability.

DoINeedaCOVID19Test.com Self-Collection Kit Ordering and Processing
The DoINeedaCOVID19Test.com Self-Collection Kit will be available by prescription to individuals who request Testing for SARS-CoV-2 though eTrueNorth's on-line platform (ineedacovidTest.com). Individuals seeking a test register with eTrueNorth, complete an assessment questionnaire and chose a location at which to pick up their collection kit. This process triggers a physician order for the test. To pick up their collection kit, the individual presents a copy of the test order at their designated participating pharmacy. They then follow the Instructions For Use for kit registration, specimen collection, packaging in a biohazard bag and same day return of the kit to the drop-off location (either in person or to a drop-box located in the participating pharmacy). Collected specimens are packaged in bulk by appropriately trained staff for shipment overnight from the drop-off location to the testing laboratory.

Test results are reported directly to the consumer, the ordering physician and relevant public health authorities in accordance with local, state and federal requirements using appropriate LOINC and SNOMED codes.

Specimen Transport and Storage
Anterior nasal swabs collected in Phosphate Buffered Saline (PBS) using the DoINeedaCOVID19Test.com Self-Collection Kit may be transported and stored at ambient temperature for up to 48 hours prior to testing.

Specimen Accessioning
Specimens received in the laboratory undergo accessioning prior to acceptance for testing. A summary of the criteria used for specimen accessioning is provided in **Table 1**. All acceptable specimens are processed by the laboratory. The Accessioning Supervisor is notified of any specimens that do not meet the accessioning acceptance criteria and procedures are implemented to gather missing information, as appropriate. If the measures to remediate specimen rejection cannot be rectified, the status is logged as "Test Not Performed" and the individual is notified that the specimen has been rejected with the option to re-collect a specimen.

Table 1. Accessioning criteria applied to specimens collected with the DoINeedaCOVID19Test.com Self-Collection Kit received for analysis with the PMLS SARS-CoV-2 Assay

Rejection Reason	Description
Missing requisition	Kits that are received but for which there is no Test requisition
Improper packaging/physical damage	Samples not received in a biohazard bag containing one vial with transport medium and one swab
Expired shipping time	Kits received ≥ 48 hours after specimen collection
Expired collection kit	Kits that have exceeded their assigned expiration date
Collection kit other than DoINeedaCOVID19Test.com Self-Collection Kit (or RapidRona Self-Collection Kit)	Use of sample collection and transport devices other than those authorized
Damaged, leaking or empty tubes	--
Tubes with missing or damaged identifiers	--

1) *Specimen Testing*
   The PMLS SARS-CoV-2 Assay is a modified version of the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel (EUA200001) and includes primers and probes for the detection of the N1 and N2 regions of the SARS-CoV-2 nucleocapsid gene as well as human RNase P nucleic acid as an endogenous control for specimen adequacy and process integrity. Amplified products are detected in separate reactions using TaqMan fluorescent probes that are labeled with a fluorophore and a quencher. The 5' exonuclease activity of the Taq polymerase hydrolyses the probes during the annealing/extension phase of PCR amplification, leading to generation of target-specific fluorescent signal.

Nucleic acid extraction is performed using the Omega Bio-tek Mag-Bind Viral DNA/RNA 96 Kit on a Hamilton Microlab Star robot liquid handler (software V1.0.3).

RT-PCR amplification is performed in 384-well format using an Applied Biosystems QuantStudio 12K flex instrument (software V4.1.1.5875).

2) *Result Reporting for DoINeedaCOVID19Test.com Self-Collection Kit Collected Specimens*
Individuals will be notified by email that their results are available on the eTrueNorth platform and must log-in to their account to retrieve their results and obtain guidance for appropriate next steps, including access to the Patient Fact Sheet. All test results are reported to the requesting healthcare provider and public health authorities in accordance with local, state, and federal requirements.

INSTRUMENTS USED WITH THE TEST

Table 2. Instruments and software for use with the PMLS SARS-CoV-2 Assay

Instrument	Manufacturer	Software Version
Microlab STAR Liquid Handling System	Hamilton	4.1.1.5875
QuantStudio 12K Flex Real-Time PCR System	Applied Biosystems (Thermo Fisher)	1.0.3

REAGENTS AND MATERIALS

Table 3. Reagents and materials used to perform nucleic acid extraction for the PMLS SARS-CoV-2 Assay

Reagent/Material	Manufacturer/ Supplier	Catalogue Number
Mag-Bind Viral DNA/RNA Kit - Mag-Bind Particles CNR - TNA Lysis Buffer - VHB Buffer - Carrier RNA - Proteinase K Solution (40 mg/mL) - SPR Wash Buffer - Nuclease-Free Water	Omega Bio-Tek	M6246-03
Heat-inactivated SARS-CoV-2	Zeptometrix	810229CFHI
200 proof ethanol	--	--
Nuclease-free water	Ambion	AM99337
Isopropanol	--	--
Sani-Cloth Wipes	--	--
RNase Away	--	--
70% ethanol	--	--
Lint-free wipes (Kim wipes)	--	--
15, 50 and 80 mL sterile conical tubes	--	--
1.5 mL microfuge tubes	--	--
Reagent trough 50 mL	--	--
Reagent trough 120 mL	--	--
CO-RE tips, high volume tips without filters	--	--
MicroWell 96-well microplate (2 mL deep well)	Nunc	--
Adhesive PCR Plate Foils	Thermo Fisher	AB-0626
Black, ultra-fine tipped marker	--	--
Alcohol wipes	--	--
Forceps	--	--
Laboratory mats	--	--
Pipettes	--	--
Pipette tips, filtered	--	--

Table 4. Reagents and materials used to perform the PMLS SARS-CoV-2 Assay

Reagent/Material	Manufacturer/ Supplier	Catalogue Number
Laboratory mat	--	--
RNase AWAY	--	--
70% ethanol	--	--
Lint-free wipes (Kim wipes)	--	--
384-well green reaction plate	--	--
2 mL tubes	--	--
RNase-free water	--	--
Black, ultra-fine tipped marker	--	--
Adhesive PCR Plate Foils	--	--
Pipettes	--	--
Pipette tips, filtered	--	--
Optical adhesive film	--	--
Biohazard waste bin	--	--
Tube rack	--	--
Magnetic plate holder	--	--
Master Mix (store at -20°C)	Applied Biosystems	A15300
2019-nCoV-N Positive Control (store at -20°C)	Integrated DNA Technologies	10006625
2019-nCoV Kit [1] (store at -20°C)	Integrated DNA Technologies	10006606
2019-nCoV PCR Primers/Probes [1] (store at -20°C)	Integrated DNA Technologies	--

[1] PCR primers/probes may either be purchased as pre-qualified kit or as individual components that are qualified prior to use under the PMLS quality system

Table 5. DoINeedaCOVID19Test.com Self Collection Kit

Component [1]	Description	Supplier	Part Number
Swab	Sterile polyester-tipped swab with polypropylene shaft	Steripak	60564
Saline-filled Transport Tube	Sterile polypropylene tube containing 3 mL 0.9 % saline	Global Scientific	6101G
Sample Bag	Biohazard bag with absorbent pad	Elkay	10790-168
Instructions	Printed pamphlet	Printplace.com	Not Applicable

[1] 1 of each component per kit

CONTROLS

Table 6. Assay controls used with the PMLS SARS-CoV-2 Assay

Control	Description	Manufacturer	Purpose	Frequency of Use
SARS-CoV-2 External Run Control	Inactivated SARS-CoV-2 (50,000 copies/mL)	Zeptometrix (Cat. #NATSARS (COV2)-ERC)	Monitors for reverse transcription and PCR amplification with SARS-CoV-2-specific primers and probes	1 per PCR plate
Positive Control – nCoVPC	Control plasmids contain the complete nucleocapsid gene from 2019-nCoV.	Integrated DNA Technologies (Cat. #10006625)	Monitors for substantial reagent failure including primer and probe integrity	1 per PCR plate
Negative Control – nCoVNC	PCR Negative: human genomic RNA background	Premier Medical Lab	Monitors for reagent and/or environmental contamination with SARS-CoV-2 reactive nucleic acids	1 per PCR plate
No Template Control (NTC)	Molecular grade water that is added to one well during the RT-PCR reaction set up	Premier Medical Lab	Monitors reagent and/or environmental contamination	1 per PCR plate
RNase P nucleic acid	Endogenous human RNase P nucleic acid	N/A	Monitors for specimen adequacy and process integrity	Per patient sample or control

INTERPRETATION OF RESULTS

Assay Controls

The criteria for interpretation of the results obtained with the assay controls are shown in **Table 7**. All controls must produce the expected results to enable interpretation of the results from testing of patient samples.

Table 7. Interpretation of results for assay controls

Assay Control Name	Ct Value		
	N1	N2	RNase P
SARS-CoV-2 External Run Control	< 40	< 40	> 40 or Undetermined
Positive Control - nCoVPC	< 40	< 40	> 40 or Undetermined
Negative Control – nCoVNC	> 40 or Undetermined	> 40 or Undetermined	< 40
No Template Control (NTC)	> 40 or Undetermined	> 40 or Undetermined	> 40 or Undetermined

Clinical Specimens

Table 8. Interpretation of results from clinical specimens

Ct Value			Interpretation	Action
N1	**N2**	**RNase P**		
< 40	< 40	Any	SARS-CoV-2 Detected	Report results to CDC/state and sender.
< 40	> 40 or Undetermined	Any	Inconclusive	Repeat testing of nucleic acid and/or re-extract and repeat RT-PCR. If result is still "inconclusive", report to sender.
> 40 or Undetermined	< 40	Any	Inconclusive	
> 40 or Undetermined	> 40 or Undetermined	< 40	SARS-CoV-2 Not Detected	Report result to CDC/state and sender.
> 40 or Undetermined	> 40 or Undetermined	> 40	Invalid	Repeat extraction and RT-PCR. If result is still "invalid", consider collecting a new specimen. If result remains "invalid" report to sender.

PERFORMANCE EVALUATION

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

LoD Determination

The LoD of the PMLS SARS-CoV-2 Assay was determined using dilutions of a quantified positive clinical anterior nasal swab that was collected in saline. To estimate the LoD, three contrived specimens at each of 8 different concentrations were tested. The lowest concentration at which all three replicates produced positive results was determined to be the preliminary LoD (**Table 9**). The preliminary LoD was then confirmed by testing an additional 25 replicates at the estimated LoD concentration in addition to one higher dilution (**Table 10**). The confirmed LoD of the PMLS SARS-CoV-2 Assay was 6.25 copies/µL (625 copies/mL) of starting sample.

Table 9. Preliminary LoD estimation

Copies/µL	Positive	Mean Ct (SD)		
		N1	**N2**	**RNase P**
100	3/3	31.6 (0.20)	31.4 (0.44)	32.7 (0.67)
50	3/3	33.3 (0.22)	32.6 (0.30)	33.1 (0.46)
25	3/3	34.5 (0.72)	33.0 (1.29)	33.2 (0.29)
12.5	3/3	35.1 (0.96)	34.2 (1.05)	33.4 (0.12)
6.25	**3/3**	**36.4 (1.02)**	**35.6 (0.73)**	**32.6 (0.87)**
3.13	1/3	37.1 (N/A)	37.0 (0.71)	33.5 (0.15)
1.56	0/3	N/A	36.7 (0.77)	33.2 (0.19)
0.78	1/3	37.4 (0.83)	37.6 (N/A)	33.6 (0.28)

N/A: Not applicable; SD: Standard Deviation

Only Ct values < 40 are included in the calculations
The estimated LoD is highlighted in yellow

Table 10. LoD confirmation

Copies/µL	Positive (%)	Mean Ct (SD)		
		N1	N2	RNase P
6.25	**24/25 (96.0)**	**37.8 (0.37)**	**38.5 (0.65)**	**33.8 (1.49)**
3.13	14/25 (56.0) [1]	38.7 (0.72)	39.2 (0.48)	32.9 (0.73)

[1] 10 additional samples yielded inconclusive results, all of which were positive for the N1 target but negative for N2
The confirmed LoD is highlighted in yellow

2) Inclusivity (Analytical Sensitivity):

The PMLS SARS-CoV-2 Assay uses primer and probe sequences for the N1 and N2 regions of the viral nucleocapsid gene and human RNase P genes that were originally authorized for use in the CDC nCoV-2019 Real-Time RT-PCR Panel (EUA200001). The CDC has granted a Right of Reference to the performance data contained in the EUA request for the CDC nCoV-2019 Real-Time RT-PCR Panel, including the *in silico* analysis of inclusivity, to any entity seeking an EUA for a COVID-19 diagnostic device.

Independent *in silico* inclusivity analysis performed in December, 2021 predicted no significant impact from known SARS-CoV-2 mutations and/or variants of concern on the inclusivity of the primers and probes used in the PMLS SARS-CoV-2 Assay.

In addition to the *in silico* analyses described above, selected samples that were identified as positive for SARS-CoV-2 using the PMLS SARS-CoV-2 Assay were sequenced using the Illumina COVIDSeq Assay Kit and IlluminaNovaSeq 600 F1 Flow Cell. The results showed that the PMLS SARS-CoV-2 Assay reported positive results from samples containing isolates of the Alpha (B.1.1.7, n = 7), Delta (B.1, n = 2), Iota (B.1.526, n = 5), Epsilon (B.1.427, n = 1) and Gamma (P.1, n = 6) SARS-CoV-2 variants.

In silico analysis of available high-quality sequences of the Omicron variant (B.1.1.529) performed by PMLS showed that as of December 6, 2021, 97.7% (560/573) exhibited a single nucleotide mismatch at position 3 of the N1 probe sequence of the PMLS SARS-CoV-2 Assay. Based on the location and melting temperature of the mismatched N1 probe, this mutation is not expected to impair detection of the Omicron variant.

3) Cross-reactivity (Analytical Specificity):

As noted above, the CDC has granted a Right of Reference to the performance data contained in the EUA request for the CDC nCoV-2019 Real-Time RT-PCR Diagnostic Panel (EUA200001) to any entity seeking an FDA EUA for a COVID-19 diagnostic device. *In silico* analysis and laboratory studies performed by CDC demonstrated no potential for cross-reaction with common respiratory pathogens and commensal species.

4) Specimen Shipping Stability:

The stability of anterior nasal swab specimens in 0.9% saline was evaluated under simulated

shipping conditions that included exposer to temperature extremes that may reasonably be anticipated during specimen transport. The results of the study support the stability of anterior nasal swab specimens collected with the DoINeedaCOVID19Test.com Self-Collection Kit for up to 48 hours at ambient temperature.

5) Usability:

A Usability Study was performed to evaluate the ease-of-use of the DoINeedaCOVID19Test.com Self-Collection Kit for collection of specimens from individuals aged 2 to 18 years. The study included 38 participants who either self-collected an anterior nasal swab specimen according to the Instructions For Use (ages 14 to 18, n = 14) or who had a sample collected by their parent or guardian (ages 2-13, n = 24). Sample collection was performed in a simulated home environment and the collected samples were shipped to the laboratory for testing for the presence of human RNase P nucleic acid. All 38 samples received in the laboratory were accepted for testing and all 38 produced a positive result for the RNase P target, indicating collection of an acceptable sample. Fourteen of the participants (or their parents or guardians) completed a usability survey. None of the respondents indicated difficulty in understanding the instructions or using the collection kit.

As a Condition of Authorization, Premier Medical Laboratory Services will collect additional usability data based on analysis of results and customer feedback obtained from Testing the first 1000 samples collected using the DoINeedaCOVID19Test.com Self-Collection Kit using the PMLS SARS-CoV-2 Assay.

6) Clinical Evaluation:

Symptomatic Patients

The clinical performance of the PMLS SARS-CoV-2 Assay was evaluated using anterior nasal swab specimens that were collected in 0.9% saline from individuals attending a drive-up clinic who reported having exhibited symptoms consistent with COVID-19 within the previous two weeks (i.e., either fever or chills, cough, shortness of breath, fatigue, muscle aches, headache, loss of taste or smell, sore throat, congestion, nausea, vomiting or diarrhea). The results obtained with the PMLS SARS-CoV-2 Assay were compared to those obtained with another FDA-authorized assay and showed 100% positive and negative agreement (PPA and NPA; **Table 11**).

Table 11. Performance of the PMLS SARS-CoV-2 Assay with anterior nasal swabs from symptomatic subjects

		FDA Authorized SARS-CoV-2 Assay		
		Positive	**Negative**	**Total**
PMLS SARS-CoV-2 Assay	**Positive**	30	0	**30**
	Negative	0	32	**32**
	Total	**30**	**32**	**62** [1]
Positive Agreement		**100 % (30/30); 88.7 - 100 %** [2]		
Negative Agreement		**100 % (32/32); 89.3 – 100%** [2]		

[1] 1 sample was reported as "negative" by the PMLS SARS-CoV-2 Assay and "inconclusive" by the comparator assay on both initial and repeat testing. Because no definitive comparator result was obtained, the sample was excluded from the performance calculations.

[2] Two-sided 95% score confidence interval

Asymptomatic Subjects

The clinical performance of the PMLS SARS-CoV-2 Assay in asymptomatic subjects was evaluated using anterior nasal swab specimens in 0.9% saline that were collected from individuals attending a drive-up clinic and who reported no exposure to a positive case of COVID-19 and no symptoms consistent with COVID-19 within the previous two weeks (i.e., no fever or chills, cough, shortness of breath, fatigue, muscle aches, headache, loss of taste or smell, sore throat, congestion, nausea, vomiting or diarrhea). All asymptomatic individuals who met the enrollment criteria were included in the study. The results obtained with the PMLS SARS-CoV-2 Assay were compared to those obtained with another FDA-authorized assay and showed 100% positive and negative agreement (PPA and NPA; **Table 12**).

Table 12. Performance of the PMLS SARS-CoV-2 Assay with anterior nasal swabs from asymptomatic subjects

		FDA Authorized SARS-CoV-2 Assay		
		Positive	**Negative**	**Total**
PMLS SARS-CoV-2 Assay	**Positive**	23	0	**23**
	Negative	0	100	**100**
	Total	**23**	**123**	**123**
Positive Agreement		**100 % (23/23); 85.7 - 100 %** [1]		
Negative Agreement		**100 % (100/100); 96.3 - 100%** [1]		

[1] Two-sided 95% score confidence interval

In addition to the consecutively collected samples from asymptomatic subjects described in **Table 12**, testing was also performed with 5 pre-selected samples from asymptomatic subjects that yielded high Ct values with the comparator assay and which were considered to contain low levels of SARS-CoV-2 RNA ("low positives"). All 5 samples (100%) yielded positive results with the PMLS SARS-CoV-2 Assay.

WARNINGS

- Caution: Federal Law restricts this device to sale by or on the order of a licensed practitioner.
- For *in vitro* diagnostic use.
- 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, Premier Medical Laboratory Services, 6000A-1 Pelham Rd, Greenville, SC 29615.
- This product has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
- 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 the authorization is revoked sooner.

LIMITATIONS

- 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.
- Asymptomatic individuals infected with COVID-19 may not shed enough virus to reach the limit of detection of the Test, giving a false negative result.
- Detection of RNase P indicates that human nucleic acid is present and implies that human biological material was collected and successfully extracted and amplified. It does not necessarily indicate that the specimen is of appropriate quality to enable detection of SARS-CoV-2.