EMERGENCY USE AUTHORIZATION (EUA) SUMMARY
PSOMA COVID-19 RT TEST
(PSOMAGEN, INC.)

For *In vitro* Diagnostic Use
Rx Only
For use under Emergency Use Authorization (EUA) only

(The Psoma COVID-19 RT Test will be performed at Psomagen, Inc., 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 per the Instructions of Use that were reviewed by the FDA under this EUA.)

INTENDED USE

The Psoma COVID-19 RT Test is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in anterior nasal, mid-turbinate, nasopharyngeal, and oropharyngeal swab specimens as well as bronchoalveolar lavage specimens from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to Psomagen, Inc. 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 Psoma COVID-19 RT Test 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 Psoma COVID-19 RT Test is only for use under the Food and Drug Administration's Emergency Use Authorization.

DEVICE DESCRIPTION AND TEST PRINCIPLE

The Psoma COVID-19 RT Test is a real-time reverse transcription polymerase chain reaction (rRT-PCR) test. The primer and probe sets used in this test are designed to detect RNA of SARS-CoV-2 nucleocapsid (N) gene (Nl and N2 target) and RNase P gene from anterior nasal, mid-turbinate, nasopharyngeal, and oropharyngeal swab specimens as well as bronchoalveolar lavage (BAL) specimens from individuals suspected of COVID-19. RNA extracted from respiratory specimens are reverse transcribed to cDNA and amplified using Light cycler 480 (Roche Molecular Systems, Inc.)

Nucleic acids are isolated and purified from respiratory specimens using NucleoSpin RNA Virus (Takara Bio USA, Inc.). The purified nucleic acid is then reverse transcribed into cDNA and is subsequently amplified in Light Cycler 480 using TOPreal One-step RT qPCR kit. During this process, the probe anneals to a specific target sequence located between the forward and reverse primers of Psoma COVID-19 RT Test. During the extension phase of the PCR cycle, the 5' nuclease activity of Taq polymerase degrades the probe, causing the reporter dye to separate from the quencher dye, generating a fluorescent signal. With each cycle, additional reporter dye molecules are cleaved from their respective probes, increasing the fluorescence intensity. Fluorescence intensity is monitored at each PCR cycle by Light Cycler 480 (Roche Molecular Systems, Inc.)

INSTRUMENTS USED WITH TEST

The Psoma COVID-19 RT Test is to be performed using following equipment:

- Light cycler 480 instrument (Roche Molecular Systems, Inc.) and Light Cycler 480 software Version l.5.
- NucleoSpin RNA Virus kit (Takara Bio USA, Inc. cat # 740956.10)

REAGENTS AND MATERIALS

- Light Cycler 480 system (Roche Molecular Systems, Inc. Product # 05015278001)
- NucleoSpin RNA Virus kit (Takara Bio USA, Inc. cat # 740956.10)
- SARS-CoV-2 Research Use Only qPCR Primer & Probe Kit (IDT Technologies, cat# 10006713)
- HSC RPP30 positive control and 2019-nCoV-N positive control (IDT Technologies cat# 10006625 and 100066236)
- TOPreal One-step RT qPCR kit (Enzynomics Cat# RT430M)

CONTROLS TO BE USED WITH THE PSOMA COVID-19 RT TEST

Controls that will be provided with the test kit include:

- A "no template" (negative) control (NTC) is used on every plate and is needed to confirm that there is no contamination on the assay. DEPC-treated water is used as the negative control.
- A positive template control (COVID-19_N_Positive, IDT, # 10006625) targeting N gene (N1 and N2) is used on every plate and is needed to confirm that the assay is completed by the intended design.
- An internal control targeting the RNase P gene is used on every sample and is needed to confirm that there is nucleic acid in each sample. This also serves as a positive control for RNA extraction step and reverse transcription step.
- A negative extraction control (NEC) (pooled RNA from negative patients) is used on every plate and is needed to confirm that there is no contamination on RNA extraction step.

INTERPRETATION OF RESULTS

Assessment of clinical specimen test results should be performed after the controls have been examined and determined to be valid and acceptable. If the controls are not valid, the patient results cannot be interpreted. Please see table below for guidance on interpretation and reporting of the results. If the results do not follow these guidelines, the sample(s) is to undergo re-extraction and re-testing.

Table 1: Psoma COVID-19 RT Test Controls Interpretation

Control type	Control name	Expected Result and Ct Value					
		Nl		N2		hRP	
		Result	Expected Ct	Result	Expected Ct	Result	Expected Ct
Positive	Positive Template Control (PT C)	+	< 40	+	< 40	+	< 40
Negative	No template Control (NTC)	-	None detected	-	None detected	-	None Detected

Control type	Control name	Expected Result and Ct Value					
		Nl		N2		hRP	
		Result	Expected Ct	Result	Expected Ct	Result	Expected Ct
Extraction	Negative Extraction Control	-	None detected	-	None detected	+	< 40

- Negative control (no template control): negative for all targets detected (Ct is not detected)
- Positive control: positive for all targets detected (Ct< 40)
- Internal control: positive for RNase P target (Ct< 40)

Table 2: Interpretation of Patient Specimen Results:

Nl	N2	hRP	Status	Result	Action
POS	POS	POS	Valid	Positive SARS-CoV-2	Report result (Positive SARS-CoV-2)
POS	NEG	POS	Valid	SARS-CoV-2 Inconclusive	Repeat test*
NEG	POS	POS	Valid	SARS-CoV-2 Inconclusive	Repeat test*
NEG	NEG	POS	Valid	SARS-CoV-2 Not Detected	Report result (Negative SARS-CoV-2)
NEG	NEG	NEG	Invalid	NA	Repeat test

*In case of SARS-CoV-2 Inconclusive, the sample would be re-tested and repeated result will be also reported to physician and appropriate public health authorities.

- N gene target 1 (Nl): When Ct value is less than 40, Nl is positive
- N gene target 2 (N2): When Ct value is less than 40, N2 is positive
- RNase P (hRP): When Ct value is less than 40, RNase P gene is positive

LIMITATIONS

- The use of this assay as an *in vitro* diagnostic under the FDA Emergency Use Authorization (EUA) is limited to Psomagen, Inc. 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. Use of this assay is limited to personnel who are trained in the procedure. Failure to follow these instructions may result in erroneous results.

- The Psoma COVID-19 RT Test can be used only with the specimens listed in the Intended Use statement. Other specimen types have not been evaluated and should not be tested with this assay.
- Samples must be collected, transported, and stored using appropriate procedures and conditions. Improper collection, transport, or storage of specimens may hinder the ability of the assay to detect the target sequences. Extraction and amplification of nucleic acid from clinical samples must be performed according the specified methods listed in this procedure. Other extraction approaches and processing systems have not been evaluated.
- False-negative results may arise from:
    - Improper sample collection
    - Degradation of the viral RNA during shipping/storage
    - Using unauthorized extraction or assay reagents
    - The presence of RT-PCR inhibitors
    - Mutation in the SARS-CoV-2 virus
    - Failure to follow instructions for use
- False-positive results may arise from:
    - Cross contamination during specimen handling or preparation
    - Cross contamination between patient samples
    - Specimen mix-up
    - RNA contamination during product handling
- The effect of vaccines, antiviral therapeutics, antibiotics, chemotherapeutic or immunosuppressant drugs have not yet been evaluated.
- Please note, negative results do not preclude infection of SARS-CoV-2 virus and should not be the sole basis of a patient management decision. A positive result indicates detection of nucleic acid from the relevant virus. Nucleic acid may persist even after the virus is no longer viable. Laboratories are required to report all positive results to the appropriate public health authorities.
- 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.

PERFORMANCE EVALUATION

1) Analytical Sensitivity:

To determine the Limit of Detection (LoD) and analytical sensitivity of the Psoma

COVID-19 RT Test studies were performed using serial dilutions of analyte and the LoD was determined to be the lowest concentration of template that could reliably be detected with 95% of all tested positive.

LoD of each target assay in the Psoma COVID-19 RT Test was evaluated and verified using SARS-CoV-2 RNA (BEI Cat# 52285, 2019-nCoV/USA-WA1/2020) that was spiked in SARS-CoV-2-confirmed-negative nasopharyngeal swab specimens at concentrations of 0.25 to 100 copies(cp)/µL. Nucleic acid was extracted from the contrived samples using the NucleoSpin RNA Virus kit and the reverse transcription RT-PCR was performed using the 480 LightCycler Real-Time PCR system. The preliminary LoD was determined to be 1 cp/µL.

The LOD was confirmed by spiking 20 replicates of 1 cp/µL of SARS-CoV-2 RNA (BEI Cat# 52285, 2019-nCoV/USA-WA1/2020) into nasopharyngeal swab matrix previously confirmed to be negative for SARS-CoV-2. Nucleic acid was extracted from the contrived samples using the NucleoSpin RNA Virus kit and the reverse transcription RT-PCR was performed using the 480 LightCycler Real-Time PCR system. Nl, N2, and hRP sequences were detected on all 20 spiked replicates confirming the LOD at 1 cp/µL.

Table 3: LoD Confirmation

Gene Copies/µL	N1		N2		hRP	
	Mean Ct	Detection Rate	Mean Ct	Detection Rate	Mean Ct	Detection Rate
1 cp/µL	31.23	100% (20/20)	33.60	100% (20/20)	25.68	100% (20/20)

2) ***Analytical Specificity:***

a) *Inclusivity*:

An alignment was performed with the oligonucleotide primer and probe sequences of the Psoma COVID19 RT Test with 6,648 publicly available SARS-CoV-2 sequences from NCBI and GISAID to demonstrate the predicted inclusivity of the assay. All the alignments show 100% identity of the assay to the SARS-CoV-2 sequences.

b) *Cross-reactivity*:

As reported under the CDC EUA, the *in silico* analysis for the N1 primer/probe set showed high sequence homology of the N1 probe with SARS coronavirus and Bat SARS-like coronavirus genome. Combining primers and probe, there is no significant homologies with human genome, other coronaviruses or human microflora that would predict potential false positive rRT-PCR results.

Table 4: Cross-Reactivity *In-silico* Analysis

***In silico* Analysis for % Identity to Targeted Gene**			
Number	**Strain**	**N1-Gene**	**N2-Gene**
1	Adenovirus type 6	Not found	Not found
2	*Bacillus anthracis*	Not found	Not found
3	*Bordetella parapertussis*	Not found	Not found
4	*Bordetella pertussis*	Not found	Not found
5	*Candida albicans*	Not found	Not found
6	*Chlamydia pneumoniae*	Not found	Not found
7	*Chlamydia psittaci*	Not found	Not found
8	Coronavirus 229E	Not found	Not found
9	Coronavirus HKU1	Not found	Not found
10	Coronavirus NL63	Not found	Not found
11	Coronavirus OC43	Not found	Not found
12	*Coxiella burnetii*	Not found	Not found
13	*Enterovirus*	Not found	Not found
14	*Haemophilus influenzae*	Not found	Not found
15	Influenza A virus H1N1	Not found	Not found
16	Influenza A virus H3N2	Not found	Not found
17	Influenza B virus	Not found	Not found
18	*Legionella pneumophila*	Not found	Not found
19	*MERS*	Not found	Not found
20	Metapneumovirus	Not found	Not found
21	*Moraxella catarrhalis*	Not found	Not found
22	*Mycobacterium bovis*	Not found	Not found
23	*Mycoplasma pneumoniae*	Not found	Not found
24	*Neisseria elongata*	Not found	Not found
25	*Neisseria meningitidis*	Not found	Not found
26	Parainfluenza virus 1	Not found	Not found
27	Parainfluenza virus 2	Not found	Not found
28	Parainfluenza virus 3	Not found	Not found
29	Parainfluenza virus 4a	Not found	Not found
30	Parechovirus	Not found	Not found
31	*Pneumocystis jirovecii*	Not found	Not found
32	*Pseudomonas aeruginosa*	Not found	Not found
33	Respiratory syncytial virus	Not found	Not found
34	Rhinovirus	Not found	Not found
35	SARS coronavirus	Not found	Not found
36	*Staphylococcus aureus*	Not found	Not found
37	*Staphylococcus epidermidis*	Not found	Not found
38	*Streptococcus pneumoniae*	Not found	Not found
39	*Streptococcus salivarius*	Not found	Not found

No cross reactivity was observed with common respiratory panel organisms

Wet Testing Analysis

To confirm the cross-reactivity of the Psoma COVID-19 RT Test in wet testing conditions, 14 samples containing non-target organisms were prepared by spiking each standard organism (concentration of 250,000 copies/ml) into negative nasopharyngeal swab matrix. Nucleic acid was extracted using the NucleoSpin RNA Virus kit and the reverse transcription RT-PCR was performed using the 480 LightCycler Real-Time PCR system. None of the 14 non-target organisms were detected.

Table 5: Cross-Reactivity Wet-Testing

Organism (250,000 cp/ml)	N1	N2
Parainfluenza I	Negative	Negative
Parainfluenza II	Negative	Negative
Parainfluenza III	Negative	Negative
Parainfluenza IV	Negative	Negative
Influenza A	Negative	Negative
Influenza B	Negative	Negative
Adenovirus	Negative	Negative
Respiratory syncytial virus A	Negative	Negative
Respiratory syncytial virus B	Negative	Negative
Rhino 8, A	Negative	Negative
Metapneumovirus	Negative	Negative
Beta Coronavirus OC43	Negative	Negative
Alpha Coronavirus 229E	Negative	Negative
Enterovirus	Negative	Negative
Human total RNA	Negative	Negative

3) ***Clinical Evaluation:***

Evaluation of Psoma COVID-19 RT Test Using Contrived Samples

Clinical evaluation of the Psoma COVID-19 RT Test was conducted with 60 contrived (30 negative and 30 positive) specimens. Nasopharyngeal swab samples that were confirmed to be negative for SARS-CoV-2 were contrived at 2x, 3x, and 5x LoD with SARS-CoV-2 RNA Isolate (BEI Resources, Cat # 52285). Nucleic acid was extracted from the contrived samples using the NucleoSpin RNA Virus kit and the reverse transcription RT-PCR was performed using the 480 LightCycler Real-Time PCR system. Data is summarized in the Table below:

Table 6: Summary of Contrived Clinical Sample Evaluation

Specimen Concentration	N	SARS-CoV-2			Mean Ct Values			Performance Agreement	95% CI
		N1 +	N2 +	RP +	N1 +	N2 +	RP +		
2X LoD	10	10/10	10/10	10/10	30.64	32.75	27.02	100%	72.2-100%
5X LoD	10	10/10	10/10	10/10	29.69	31.72	27.04	100%	72.2-100%
10X LoD	10	10/10	10/10	10/10	28.77	30.74	26.84	100%	72.2-100%
Negative	30	N/A	N/A	N/A	N/A	N/A	27.01	100%	88.7-100%

Clinical Evaluation of the Psoma COVID-19 RT Test

A clinical study was performed to evaluate the performance of the Psoma COVID-19 RT Test. Results obtained with a total of 110 clinical nasopharyngeal swab samples (55 negatives and 55 positives for SARS-CoV-2) tested with FDA EUA RT-PCR assays (TaqPath COVID-19 Method (EUA200010) and LabCorp COVID-19 RT_PCR Test (EUA200011) were compared to results obtained with the Psoma COVID-19 RT Test. Nucleic acid was extracted using the NucleoSpin RNA Virus kit and the reverse transcription RT-PCR was performed using the 480 LightCycler Real-Time PCR system. The results are summarized in the Table below:

Table 7: Clinical Evaluation of the Psoma COVID-19 RT Test

Psoma COVID-19 RT Test	FDA EUA RT-PCR Assay		Total	% Performance Agreement	95% CI
	Detected	Not Detected			
Detected	55	0	55	PPA 100%	93.5-100%
Not Detected	0	55	55	NPA 100%	93.5-100%
Total	55/55	55/55	110		

WARNINGS:

- This test has not been FDA cleared or approved;
- This test has been authorized by FDA under an EUA for use by the authorized laboratory;
- This test has been authorized only for the detection of nucleic acid from SARSCoV-2, not for any other viruses or pathogens; and
- This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostic tests for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.

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. The extraction method and instrument used were NucleoSpin RNA Virus (Takara Bio USA, Inc., Cat # 740956.10) and LightCycler 480 (Roche Molecular Systems, Inc. Product # 05015278001) respectively. The results are summarized in the following Table.

Table 8: Summary of LoD Confirmation Result using the FDA SARS-CoV-2 Reference Pane l

Reference Materials Provide d by FDA	Spe cimen Type	Product LoD	Cross-Reactivity
SARS-CoV-2	Nasopharyngeal Swab	$1.8x10^4$ NDU/mL	N/A
MERS-CoV		N/A	ND

NDU/mL = RNA NAAT detectable units/mL
N/A: Not applicable
ND: Not detected