EMERGENCY USE AUTHORIZATION (EUA)
SUMMARY

Pro-AmpRT SARS-CoV-2 Test

(Pro-Lab Diagnostics)

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

(The Pro-Lab Diagnostics Pro-AmpRT SARS-CoV-2 Test will be performed at Pro-Lab Diagnostics, located at 21 Cypress Blvd. Suite 1155, Round Rock, TX 78665, that 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. The Laboratory Standard Operating Procedure was reviewed by the FDA under this EUA.)

INTENDED USE

The Pro-AmpRT SARS-CoV-2 Test is a reverse transcription isothermal amplification test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in upper respiratory specimens (anterior nasal, mid-turbinate, nasopharyngeal or oropharyngeal swabs, and nasopharyngeal wash/aspirate or nasal aspirate) and bronchoalveolar lavage from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to Pro-Lab Diagnostics, located at 21 Cypress Blvd, Suite 1155, Round Rock, Texas 78665, 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 samples 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 Pro-AmpRT SARS-CoV-2 Test is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of isothermal amplification and *in vitro* diagnostic procedures. The Pro-AmpRT SARS-CoV-2 Test is only for use under the Food and Drug Administration's Emergency Use Authorization.

DEVICE DESCRIPTION AND TEST PRINCIPLE

The Pro-AmpRT SARS-CoV-2 Test is a rapid, high throughput, reverse transcription isothermal amplification test. The SARS-CoV-2 primer set is designed to detect RNA from the SARS-CoV-2 in respiratory specimens from patients recommended for testing by public health authority guidelines. The primer set comprises 6 primers and amplifies a 206 base pair region of the ORF1ab gene of SARS-CoV-2 (Yu L, et al. Infectious Diseases; accessed from: http://medrxiv.org/lookup/doi/10.1101/2020.02.20.20025874, now published in Clinical Chemistry: https://academic.oup.com/clinchem/article/66/7/975/5823294). This test does not employ internal controls but uses several external controls as listed in the section *CONTROLS TO BE USED WITH THE Pro-AmpRT SARS-CoV-2 Test* (below).

Upper respiratory swabs are collected in 1mL viral transport medium. RNA is extracted manually using the Pro-Mag RNA Extraction Kit and eluted in 40µl elution buffer. 5µl of RNA eluate is reverse transcribed to cDNA and subsequently amplified using the Genie HT instrument with Software version v4.00rc2 using loop-mediated isothermal amplification process. Fluorescence intensity is monitored at each PCR cycle by the Genie HT instrument and the time to cross the threshold of 10,000 RLU (Relative Fluorescence Units) is measured as result output.

In addition, after the amplification, a melting curve analysis of the amplification products are performed. The result output from the melting curve analysis is an annealing temperature appropriate for the correct amplification product (i.e., 84±1ºC).

The Genie HT instrument (OptiGene) can process 12 strip-tubes of 8 samples each (i.e., 82 samples plus 14 controls) at once; each run takes 30 minutes to complete (excluding extraction).

INSTRUMENTS USED WITH TEST

Pro-AmpRT SARS-CoV-2 Test is to be used with Genie HT instrument manufactured by OptiGene (United Kingdom) for reverse transcription and isothermal amplification using software version v4.00rc2.

EQUIPMENT, REAGENTS AND MATERIALS

The following equipment/reagents/materials are required to run this test in addition to the consumables for the extraction and PCR process:

Table 1: Reagents, Material and Equipment Required

Item	Vendor	Catalog number
Specimen collection vial	Pro-Lab Diagnostics	PL.185
SARS-CoV-2 transport media	Pro-Lab Diagnostics based on https://www.cdc.gov/coronavirus/2019-ncov/downloads/Viral-Transport-Medium.pdf	(1mL)
Sterile flocked nylon swab	Cleanmo International	CM-FS-913
Pro-Mag RNA Extraction Kit	Pro-Lab Diagnostics	PLM-2000
Extraction control (γ-irradiated SARS-CoV-2)	BEI	NR-52287
Isothermal Master Mix	OptiGene	ISO-004
SARS-CoV-2 Primers	IDT (or equivalent)	
AMV Reverse Transcriptase	Promega	M9004
Positive control (synthetic DNA)	IDT, GBlocks (or equivalent)	
Genie Testing Strips	OptiGene	OP-0008-50
Genie HT instrument	OptiGene	GENHT

CONTROLS TO BE USED WITH THE Pro-AmpRT SARS-CoV-2 Test

Positive control: synthetic DNA homologous to the assay's target: a region of the ORF1ab in SARS-CoV-2. This control is run at 2x the LoD, once in each strip-tube, to ensure that amplification reagents are working within each amplification batch (PCR), and also to ensure each module in the instrument is working.

Negative control (NTC): nuclease-free water. The negative control is a "no template control" and it is included once with each extraction or amplification batch (PCR)

Positive extraction control: γ-irradiated SARS-CoV-2 (BEI #NR-52287) or heat-inactivated SARS-CoV-2 (ATCC #VR-1986HK). The positive extraction control is included once with each RNA extraction batch for the 1mL procedure.

Procedural control: The annealing temperatures of the amplicons is determined in a melting curve analysis that is performed through an automated step at the end of the amplification process on the Genie HT. The annealing temperature of the amplified products must be 84°C±1°C. This step is an internal processing control that ensures amplicons of the correct size are generated and detected in the reaction. A decrease in the annealing temperature may be indicative of an inhibitory substance in the patient's sample restricting optimal amplification of any pre-existing viral nucleic acid.

INTERPRETATION OF 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. A detailed decision flow chart for interpretation of assay controls is included in the attached SOP. All reactions are run for 24 minutes. Reactive and non-reactive patient sample determinations are based on a 24-minute cutoff for amplification.

a. Interpretation of assay controls

- The positive control amplification curve must reach a minimum fluorescence of 10,000 RLU and the annealing temperature must be 84°C±1°C for the specimen results in that amplification batch to be valid.
- Neither amplification nor annealing curves should be generated from the negative control. If amplification or annealing curves appear for the negative control, it is likely that the isothermal amplification reagents are contaminated. Specimen from such an amplification batch will need to be retested.
- The extraction control amplification curve must reach a minimum fluorescence of 10,000 RLU and the annealing temperature must be 84°C±1°C for the specimen results in that extraction batch to be valid.
- The annealing temperature serves as the internal control in this assay. The annealing temperature is based on the GC content of an amplicon. For the amplicon generated in this assay, the annealing temperature is 84°C±1°C. An annealing temperature out of this range indicates that the incorrect DNA sequence was amplified. If the annealing temperature of the positive control or extraction control is out of range, the entire amplification and/or RNA extraction batch must be retested. If the annealing temperature of a patient specimen is out of range, but an amplification curve appears, this specimen should be retested.

b. Interpretation of patient samples

- A reactive (positive/detected) patient sample must produce an amplification curve with a minimum fluorescence of 10,000 RLU before 24 minutes *and* have an annealing temperature of 84°C±1°C.
- A non-reactive (negative/non-detected) patient sample must have both the absence of an amplification curve *and* the absence of an annealing curve within 24 minutes.
- An invalid (no result reported) patient sample is one in which the results cannot be determined because the assay controls are not valid. Trouble shooting must be performed according to the SOP and these samples must be processed again. If amplification occurs but no annealing curve appears, this sample must be repeated.

  Note: If the annealing temperature of an amplicon is out of range it indicates an incorrect amplification product, such as a contaminant. In this case the product would have an annealing temperature outside of the specified 84°C±1°C, in which

case that sample would be reported as invalid because an incorrect amplicon has been produced.

PERFORMANCE EVALUATION

All samples in the validation studies are interpreted based on the acceptance criteria (cutoff) described above, i.e., reaching a minimum of 10,000 RLU within 24 minutes and an annealing temperature of the amplification product of 84°C±1°C.

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

a) *Tentative LoD study*

To determine the LoD of samples, sterile swabs were spiked with virus suspension derived from two-fold serial dilutions of γ-irradiated SARS-CoV-2 (BEI, NR-52287). The swabs were then eluted in 1 mL transport medium and processed with the extraction step per laboratory SOP. The tentative limit of detection was determined as the lowest dilution detected in 24 minutes, with an annealing temperature of 84°C±1°C.

b) Confirmatory LoD

The LoD was confirmed by testing 20 replicates using 1 µl of viral suspension spiked onto nasopharyngeal swabs confirmed negative for SARS-CoV-2. For spiking γ-irradiated SARS-CoV-2 was used. Swabs were then inserted into 1 mL transport medium and viral RNA was extracted and tested per laboratory SOP. All 20 replicates achieved at least 10,000 RLU within the 24 minutes run time and had annealing temperatures of the resulting amplification product within the required of 84°C±1°C. The LoD of the Pro-AmpRT SARS-CoV-2 Test was confirmed with 20/20 replicates at 125 genome equivalents per swab.

***Table 2. LoD confirmations* at 125 copies/swab**

Sample Processing Method	Mean Amplification Time (min)	Mean Annealing Temperature*	Positivity Rate
1 mL VTM **with** extraction	15.25	83.62	100% (20/20)

***** All 20 replicates achieved at least 10,000 RLU within the 24 minutes run time and had annealing temperatures of the resulting amplification product within the required of 84°C±1°C.

2) ***Inclusivity***

In silico analysis was conducted with all six primer sequences used in the Pro-AmpRT SARS-CoV-2 Test. Each sequence was BLASTed against the 15,000 Betacoronavirus sequences using the NCBI and GenBank Betacoronavirus database (as of August 12, 2020). Two of the primer sequences showed 100% identity for all sequences. There were only 8 SARS-CoV-2 sequences in the NCBI database that contained one bp mismatch with one of the primers other sequences. None of the sequences showed mismatches with multiple primer/probe sequences. In all mismatched sequences the location is in the middle of the primers and is not expected to impact binding and amplification. Therefore, there is no risk of false negatives results due to the failure to detect certain SARS-CoV-2 sequences.

3) ***Analytical Inclusivity/Specificity:***

a. Cross-reactivity

In silico analysis for possible cross-reactions with all organisms listed in Table 9 was conducted by mapping the three primer sets of the Pro-AmpRT SARS-CoV-2 Test target nucleic acid sequence to the sequences of the organisms available from the NCBI GenBank databases using Primer BLAST. Results are included in the table below. "Mismatch" means that Primer BLAST could not use the primers input to generate an amplicon product. "100% match" means that an amplicon product would be generated by Primer BLAST given the primer pair input. None of the SARS-CoV-2 primer sets showed significant combined homologies with the organisms listed, except FIP/BIP with SARS-coronavirus. However, any amplicon generated by these two primers would be a different length (158 base pairs rather than 206 base pairs) and of a different annealing temperature, so it would not change patient sample results interpretation.

Table 3. Potential cross-reactivity of the Pro-AmpRT SARS-CoV-2 Test primer sets with other respiratory pathogens

Organism	F3/B3	FIP/BIP	LF/LB
Human coronavirus 229E	Mismatch*	Mismatch	Mismatch
Human coronavirus OC43	Mismatch	Mismatch	Mismatch
Human coronavirus HKU1	Mismatch	Mismatch	Mismatch
Human coronavirus NL63	Mismatch	Mismatch	Mismatch
SARS-coronavirus	Mismatch	100% match**	Mismatch
MERS-coronavirus	Mismatch	Mismatch	Mismatch
Adenovirus (e.g., C1 Ad. 71)	Mismatch	Mismatch	Mismatch
Human Metapneumovirus (hMPV)	Mismatch	Mismatch	Mismatch
Parainfluenza virus 1	Mismatch	Mismatch	Mismatch
Parainfluenza virus 2	Mismatch	Mismatch	Mismatch
Parainfluenza virus 3	Mismatch	Mismatch	Mismatch

Organism	F3/B3	FIP/BIP	LF/LB
Parainfluenza virus 4	Mismatch	Mismatch	Mismatch
Enterovirus (e.g., EV68)	Mismatch	Mismatch	Mismatch
Respiratory syncytial virus	Mismatch	Mismatch	Mismatch
Rhinovirus	Mismatch	Mismatch	Mismatch
Chlamydia pneumoniae	Mismatch	Mismatch	Mismatch
Haemophilus influenzae	Mismatch	Mismatch	Mismatch
Legionella pneumophila	Mismatch	Mismatch	Mismatch
Mycobacterium tuberculosis	Mismatch	Mismatch	Mismatch
Streptococcus pneumoniae	Mismatch	Mismatch	Mismatch
Streptococcus pyogenes	Mismatch	Mismatch	Mismatch
Bordetella pertussis	Mismatch	Mismatch	Mismatch
Mycoplasma pneumoniae	Mismatch	Mismatch	Mismatch
Pneumocystis jirovecii (PJP)	Mismatch	Mismatch	Mismatch
Candida albicans	Mismatch	Mismatch	Mismatch
Pseudomonas aeruginosa	Mismatch	Mismatch	Mismatch
Staphylococcus epidermis	Mismatch	Mismatch	Mismatch
Streptococcus salivarius	Mismatch	Mismatch	Mismatch

*Mismatch means that no amplicon could be generated by the Primer BLAST,
**100% Match means an amplification product would be obtained

4) Clinical Evaluation:

A panel of 30 confirmed leftover positive nasopharyngeal swab specimens and 30 confirmed leftover negative nasopharyngeal swab specimens all obtained in 1mL of viral transport media. All leftover samples were de-identified following the policy outlined in the FDA Guidance on *Informed Consent for In Vitro Diagnostic Device Studies Using Leftover Human Specimens that are Not Individually Identifiable*. Samples were processed according to the laboratory SOP including RNA extraction with the Pro-Mag RNA Extraction Kit. The sample result with the Pro-AmpRT SARS-CoV-2 Test were compared to the prior result of the samples obtained with an EUA authorized comparator test. All 30 negative samples were correctly identified and 29/30 (96.6%) positive samples were correctly identified.

Table 4. Clinical Study – Comparison of result obtained with the Pro-AmpRT-SARS-CoV-2 Test with results of an EUA authorized comparator

			FDA EUA RT-PCR Test		
		Positive (#)	Inconclusive (#)	Negative (#)	**Total (#)**
Pro-AmpRT SARS-CoV-2	Positive (#)	29	0	0	**29**
	Inconclusive (#)	0	0	0	**0**
	Negative (#)	1	0	30	**31**
	Total (#)	**30**	**0**	**30**	**60**
Positive Percent Agreement (PPA)		**96.6% (29/30) (95% CI: 83.3% - 99.4%)**			
Negative Percent Agreement (NPA)		**100% (30/30) (95% CI: 88.7% - 100%)**			

WARNINGS:

- For *in vitro* diagnostic use
- For prescription use only
- For use under FDA emergency use authorization (EUA) only
- The Pro-AmpRT SARS-CoV-2 Test has not been FDA cleared or approved;
- The test has been authorized by FDA under an Emergency Use Authorization (EUA) for use by Pro-Lab Diagnostics, located at 21 Cypress Blvd. Suite 1155, Round Rock, TX 78665 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;
- The Pro-AmpRT SARS-CoV-2 Test has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
- The Pro-AmpRT SARS-CoV-2 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 Federal Food, Drug and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.
- The performance of the Pro-AmpRT SARS-CoV-2 Test was established using nasal swab specimens. Nasopharyngeal, oropharyngeal and mid-turbinate swabs, nasopharyngeal wash/aspirate or nasal aspirate and BAL are also considered acceptable specimen types for use with the Pro-AmpRT SARS-CoV-2 Test, but performance has not been established.
- 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.
- This test cannot rule out diseases caused by other bacterial or viral pathogens.

LMITATIONS:

- As with any molecular test, if the virus mutates in the target region, SARS-CoV-2 may not be detected or may be detected less predictably.
- A negative result for SARS-CoV-2 RNA does not exclude infection with SARS-CoV-2.
- The clinical performance has not been established in 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.