CovidNow
SARS-CoV-2 Assay

Instructions for Use

For Emergency Use Authorization (EUA) Only

For In-Vitro Diagnostic Use

Rx only

Lighthouse Lab Services,
1337 Hundred Oaks Drive - Suite A
Charlotte, NC 28217
General Inquiries: contact@lighthouselabservices.com
Technical Support: support@lighthouselabservices.com

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Intended Use

The CovidNow SARS-CoV-2 Assay is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in upper respiratory specimens (i.e., nasopharyngeal, oropharyngeal, anterior nasal, and mid-turbinate nasal swab specimens, nasopharyngeal washes/aspirates or nasal aspirates) from any individual including individuals without symptoms or other reasons to suspect COVID-19. Testing is limited to laboratories designated by Lighthouse Lab Services that are also certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a and meet requirements to perform high complexity tests.

This test is also authorized for use with anterior nasal swab specimens that are self-collected at home (unobserved) (which includes in a community-based setting) from individuals 18 years of age or older when suspected of COVID-19 and determined to be appropriate by a healthcare provider, using the CovidNow Collection Kit.

Results are for the detection and identification of SARS-CoV-2 RNA. SARS-CoV-2 RNA is generally detectable in upper respiratory specimens during 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 CovidNow SARS-CoV-2 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 CovidNow SARS-CoV-2 Assay is only for use under the Food and Drug Administration's Emergency Use Authorization.

Summary and Explanation of the Test

The CovidNow SARS-CoV-2 Assay is a molecular *in vitro* diagnostic kit intended for the qualitative detection of SARS-CoV-2 in upper respiratory specimens (such as nasopharyngeal swabs (NPS), oropharyngeal swabs (OPS), or nasal swabs. The assay is based on widely used real-time reverse transcription polymerase chain reaction (rRT-PCR) technology, which employs oligonucleotide primers and probes labeled with fluorescent reporter dyes and quenchers. The CovidNow SARS-CoV-2 Assay detects a conserved region of SARS-CoV-2 nucleocapsid (N) gene as well as sequences to target the human RNase P (RP) for detection of human nucleic acids.

The qualified laboratories in which all users, analysts, and any person reporting results from use of this device should be trained to perform and interpret the results from this procedure by a Lighthouse Lab Services-designated instructor prior to use.

Test Principles

The oligonucleotide primers and probes for detection of 2019-nCoV were selected from a region of the virus nucleocapsid (N) gene. The panel is designed for specific detection of the 2019-nCoV. An additional primer/probe set to detect the human RNase P gene (RP) in control samples and clinical specimens is also included in the panel.

Viral RNA is first extracted from patient samples and then in the one-step rRT-PCR process, RNA is converted to cDNA. Next, the probes anneal to specific target sequences on the cDNA located between the forward and reverse primers. During the extension phase of the PCR cycle, the 5' nuclease activity of the DNA polymerase degrades the probes, 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 the QuantStudio 5 Real-Time PCR instrument (Applied Biosystems). The PCR cycle at which the fluorescence intensity surpasses a defined threshold value is used to determine results.

Materials Required

Materials Required (Provided)

Reagents provided in the CovidNow SARS-CoV-2 Assay

Component Name	Contents	Storage
CovidNow SARS-CoV-2 Primer/Probe Mix	1600 µL x 5	Store at -20°C
CovidNow SARS-CoV-2 Positive Control	200 µL x 1	Store at -80°C
CovidNow SARS-CoV-2 Neg Extraction Control	15mL x 1	Store at -20°C

Reagents Required (Not Provided)

General Reagents required, but not provided, for the CovidNow SARS-CoV-2 Assay

Component Name	Contents	Storage
ThermoFisher Applied BioSystem TaqPath™ 1-Step Multiplex Master Mix Cat No. A28522	10mL	Store at -20°C
ANDis Auto Extraction and Purification Reagent Kit Cat No. 3103010026	128 tests	Store at -20°C
(Alternative to above ANDis) MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit (Cat. No. A42352)	2,000 rxn	Store at 4°C
Fisher BioReagent Ethanol, Absolute, Molecular Biology Grade, or equivalent (0.9 ml per rxn) 4L	10,000	Store at 4°C

Consumables (Not Provided)

Consumables required, but not provided, for the CovidNow SARS-CoV-2 Assay

Component Name
Reagent Reservoirs
20 µL barrier DNA/RNAse free pipette tips
200 µL barrier DNA/RNAse free pipette tips
1000 µL barrier DNA/RNAse free pipette tips
MicroAmp™ Fast Optical 384-Well Reaction Plate
MicroAmp™ Optical Adhesive Film
1.5mL DNA/RNAse free micro centrifuge tubes
Molecular grade water, nuclease-free
Cold block(s) or ice
Appropriate PPE supplies
KingFisher 96 Deep-well Plates (if using KingFisher)
KingFisher Microwell Plates (if using KingFisher)
KingFisher 96 tip comb for deep-well magnets (if using KingFisher)

Equipment (Not Provided)

Equipment required, but not provided, for the CovidNow SARS-CoV-2 Assay

Component Name
ThermoFisher Applied Biosystems QuantStudio 5 Real-Time PCR System
ANDiS 350 Automated Nucleic Acid Extraction System Or Thermofisher King Fisher Flex
Pipettes (1-10 µL, 10-200 µL, and 100-1000 µL)
Vortex
Microfuge Centrifuge
Microplate Centrifuge
Class II or higher biological safety cabinet (laminar flow hood)
Freezer (manual defrost): -10 to -30°C
Freezer (manual defrost): -70 to -90°C
Refrigerator: 2 to 8°C

Preparing Your Instruments

To prepare your real-time PCR Instrument and automated extraction platform to run the CovidNow assay, ensure that the instruments have been properly maintained and calibrated. Below is the

instrument maintenance schedule for each of instruments and a link to the RUO instruments Maintenance Guide. All instruments should be properly calibrated and maintained prior to use.

Instrument Model	Maintenance Guide Link	Maintenance Schedule
Applied Biosystems QuantStudio 5 Real-Time PCR Systems	QuantStudio RT-PCR Maintenance Guide	Every 12 months
Applied Biosystems KingFisher Flex	KingFisher Flex User Manual (thermofisher.com)	Every 12 months
3D Med ANDiS 350 Automated Nucleic Acid Extraction System	ANDiS 350 User Manual (3D Med)	Every 12 months

Once an instrument has been qualified, please print and place the Emergency Use only label included in Appendix 1 on the front panel of the qualified instrument. If an instrument includes labeling indicating "For Research Use Only", please cover with the "Emergency Use Only" labeling. The instrument should retain this labeling throughout the EUA use of the CovidNow Assay. A label is also available to be mailed, please reach out to support@lighthouselabservices.com and one will be promptly shipped.

Warnings, Precautions, and Best Practices

- The CovidNow SARS-CoV-2 rRT-PCR Assay is for *in vitro* diagnostics use (IVD).
- Rx only
- This product has not been FDA cleared or approved but has been authorized for emergency use by the FDA under an EUA for use by authorized laboratories.
- 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 Act, 21 U.S.C. § 360bbb- 3(b)(1), unless the declaration is terminated or authorization revoked.
- 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 infection with SARS-CoV-2 virus and should not be used as the sole basis for treatment or other patient management decision.
- Positive results are indicative of the presence of SARS-CoV-2 RNA and Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.
- Follow standard precautions. All patient specimens and positive controls should be considered potentially infectious and handled accordingly.

- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in areas where reagents and human specimens are handled.
- Handle all specimens as if infectious using safe laboratory procedures. Refer to Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with 2019-nCoV https://www.cdc.gov/coronavirus/2019-nCoV/lab-biosafety-guidelines.html and Biosafety in Microbiological and Biomedical Laboratories (BMBL), a publication from the Centers for Disease Control and Prevention, and to your lab's safety protocol to limit biohazard risks. If biohazardous materials are used, properly label the equipment as a biohazard. For more information, see the BMBL guidelines online at https://www.cdc.gov/biosafety/publications/index.htm.
- Specimen processing should be performed in accordance with national biological safety regulations.
- If infection with 2019-nCoV is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions.
- Perform all manipulations of live virus samples within a Class II (or higher) biological safety cabinet (BSC).
- Use personal protective equipment such as (but not limited to) gloves, eye protection, and lab coats when handling kit reagents while performing this assay and handling materials including samples, reagents, pipettes, and other equipment and reagents.
- Amplification technologies such as PCR are sensitive to accidental introduction of PCR product from previous amplification reactions. Incorrect results could occur if either the clinical specimen or the real-time reagents used in the amplification step become contaminated by accidental introduction of amplification product (amplicon). Workflow in the laboratory should proceed in a unidirectional manner.
- Maintain separate areas for assay setup and handling of nucleic acids.
- Always check the expiration date prior to use. Do not use expired reagents. Do not substitute or mix reagents from different kit lots or from other manufacturers.
- Change aerosol barrier pipette tips between all manual liquid transfers.
- During preparation of samples, compliance with good laboratory techniques is essential to minimize the risk of cross-contamination between samples and the inadvertent introduction of nucleases into samples during and after the extraction procedure. Proper aseptic technique should always be used when working with nucleic acids.
- Maintain separate, dedicated equipment (e.g., pipettes, microcentrifuges) and supplies (e.g., microcentrifuge tubes, pipette tips) for assay setup and handling of extracted nucleic acids.
- Wear a clean lab coat and powder-free disposable gloves (not previously worn) when setting up assays.
- Change gloves between samples and whenever contamination is suspected.

- Keep reagent and reaction tubes capped or covered as much as possible.
- Primers, probes (including aliquots), and enzyme master mix must be thawed and maintained on a cold block at all times during preparation and use.
- Work surfaces, pipettes, and centrifuges should be cleaned and decontaminated with cleaning products such as 10% bleach, DNAZap™, or RNase AWAY™ to minimize risk of nucleic acid contamination. Residual bleach should be removed using 70% ethanol.
- RNA should be maintained on a cold block or on ice during preparation and use to ensure stability.
- Do not use any reagent past the expiration date.
- Dispose of unused kit reagents and human specimens according to local, state, and federal regulations.
- Modifications to assay reagents, assay protocol, or instrumentation are not permitted.
- Reliable results depend on proper specimen collection, storage, and handling procedures.

Specimen Collection, Handling, and Storage

Inadequate or inappropriate specimen collection, storage, and transport are likely to yield false test results. Training in specimen collection is highly recommended due to the importance of specimen quality. CLSI MM13-A may be referenced as an appropriate resource.

Collecting Specimens

- Refer to Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Patients Under Investigation (PUIs) for 2019 Novel Coronavirus (2019-nCoV) https://www.cdc.gov/coronavirus/2019-nCoV/guidelines-clinical-specimens.html
- Follow specimen collection device manufacturer instructions for proper collection methods.
- Swab specimens should be collected using only swabs with a synthetic tip, such as nylon or Dacron®, and an aluminum or plastic shaft. Calcium alginate swabs are unacceptable and cotton swabs with wooden shafts are not recommended. Place swabs immediately into sterile tubes containing 1-3 ml of appropriate transport media, such as viral transport media (VTM).
- Specimens may be self-collected at home using the [insert collection kit name].

Transporting Specimens

- Specimens must be packaged, shipped, and transported according to the current edition of the International Air Transport Association (IATA) Dangerous Goods Regulation. Follow shipping regulations for UN 3373 Biological Substance, Category B when sending potential 2019-nCoV specimens.
- Samples collected, packed and shipped consistent with the self-collection instructions for the CovidNow Assay are compliant with UN3373

- After collection, store specimens at 2-8°C and ship overnight on ice pack. If a specimen is frozen at -70°C or lower, ship overnight on dry ice.

Accessioning of Self-Collected Specimens

Laboratories testing anterior nasal swab specimens that are self-collected using the CovidNow Assay must follow the standard operating procedure "Specimen Receipt and Handling for the CovidNow Self-Collection Kit" when accepting specimens for testing, or must incorporate in their Laboratory SOP the following accessioning criteria:

- Sample collection tube must be intact and not visibly damaged.
- The tube barcode label must be present and readable by a barcode scanner.
- The tube cap must be properly secured onto the tube.
- The swab should be oriented correctly; bud at the bottom, shaft at the top.
- The expiration date on the Tube label is not exceeded.
- Accession date is within 56 hours of the collection date/time.

Storing Specimens

- Specimens can be stored at 2-8°C for up to 72 hours after collection.
- If a delay in extraction is expected, store specimens at -70°C or lower.
- Extracted nucleic acid should be stored at -70°C or lower.

Reagent Storage, Handling, and Stability

- Store primers and probe mix at -20°C.
- Store positive SARS-CoV-2 control materials at ≤ -80°C.
- Always check the expiration date prior to use. Do not use expired reagents.
- Protect fluorogenic probes from light.
- Primers, probes (including aliquots), and enzyme master mix must be thawed and kept on a cold block at all times during preparation and use.
- Controls and aliquots of controls must be thawed and kept on ice at all times during preparation and use.

Instructions for Use

Quality Control

For each extraction plate include the following external controls:

- Negative Extraction Control (CovidNow SARS-CoV-2 Negative Extraction Control)

For each RT-PCR plate include the following external controls:

- Positive Control (CovidNow SARS-CoV-2 Positive Control)
- The Negative Extraction Control from each extraction plate/run
  Note: For example, if RNA samples from 4 extraction runs are combined on one 384-well RT-PCR reaction plate, then 4 Negative Control wells must be run on that 384-well reaction plate.
- One No Template Control (Nuclease-free water)

Important Guidelines for RT-PCR

- For each RT-PCR reaction plate, include the following controls:
  - One Positive Control
  - One Negative Extraction Control from each extraction run. For example, if RNA samples from 4 extraction runs are combined on one 384-well RT-PCR reaction plate, then 4 Negative Control wells must be run on that 384-well reaction plate.
- Prepare the RT-PCR reaction plate on ice and keep it on ice until it is loaded into the real-time PCR instrument.
- Run the plate immediately after preparation. Failure to do so could result in degraded RNA samples.
- To prevent contamination, prepare reagents in a PCR workstation or equivalent amplicon-free area. Do not use the same pipette for controls and RNA samples, and always use aerosol barrier pipette tips.
- Maintain an RNase-free environment.
- Protect assays from light.
- Keep RNA samples and components on ice or cold block during use.

RNA Extraction

1. RNA extraction is performed automated with either the ANDiS Viral RNA Auto Extraction and Purification Kit (Cat No. 3103010026) on ANDiS 350 Automated Nucleic Acid Extraction System (Cat No. 3105020003) or the KingFisher Flex (Cat No 5400630) with the MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit (Cat No. A42352).

2. Please follow the product instructions to conduct viral RNA extraction and purification.

Extraction Kit	Extraction User Guides	Input/Elution Summary
MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit	MagMAX Extraction IFU	Input:0.2mL Elution: 0.05mL
3D Med ANDiS Viral RNA Auto Extraction & Purification Kit	ANDiS Extraction IFU	Input:0.2mL Elution: 0.1mL

3. Please note one negative extraction control should be extracted with each batch of patient specimens.

CovidNow SARS-CoV-2 Assay rRT-PCR Batch Set Up

1. Equilibrate all reagents and controls in cooler or on ice.
2. On ice, prepare a master mix containing the following (account for 10% extra lost during pipetting). Briefly vortex and centrifuge reagents before use.
3. Mix all the reagents and control by low vortex for 5 seconds, centrifuge briefly as needed to collect the contents to the bottom of the tube.
4. Prepare an 'Assay Reaction Mix' according to the formula described below:

'Assay Reaction Mix' for rRT-PCR

	Component	Volume per Reaction	Volume per N Reaction
1	Master Mix	5 µL	5 µL x (N + 1)
2	CovidNow SARS-CoV-2 Primer Mix	4 µL	4 µL x (N + 1)
3	Nuclease-free water	6 µL	6 µL x (N + 1)

5. Place the 384-well plate on the PCR plate cooler and add 15 µL of 'Assay Reaction Mix' to each designated well for all patient and quality control samples.
6. Bring the extracted samples and the PCR 'Assay Reaction Mix' to a biosafety cabinet.
    a. Add 5 µL of extracted samples to each designated well of the master mix plate. Mix by pipetting, taking care to avoid introducing bubbles. Change gloves often and when necessary to avoid contamination.
    b. Add 5 µL of CovidNow SARS-CoV-2 Positive Control to the designated PCR control well. Mix by pipetting, taking care to avoid introducing bubbles. It is recommended that 2 positive controls be run on each plate.
7. Seal the plate thoroughly with MicroAmp Optical Adhesive Film. It's important to ensure pressure is applied across the entire plate and there is a tight seal on each well to avoid potential contamination.
8. Vortex the plate at the highest setting speed for approximately 15 seconds with medium pressure. Move the plate around to ensure equal contact on the vortex platform.
9. Centrifuge the plate for approximately 1 minute to remove bubbles if present. Store in the dark at 2-8°C or on a cooling block until ready (not to exceed one hour from the time the reaction mix is prepared).

rRT-PCR

1. Load the plate into the PCR machine and run the following thermocycler conditions:
2. Set up the assay as follows:
    a. Block type: 384-well Block
    b. Experiment type: Standard curve
    c. Reagent: Taqman
    d. Instrument properties: Standard
    e. Passive reference: None

f. Sample volume: 20 µL

3. Assign the targets as shown below:
   a. Create the N1 Detector. Include the following:
      i. Name: N1
      ii. Reporter Dye: FAM
      iii. Quencher Dye: (none)
   b. Create RNase P detector. Include the following:
      i. Name: RNase P
      ii. Reporter Dye: Cy5
      iii. Quencher Dye: (none)
4. Run the assay as per the thermocycling conditions given below:

ThermoCycling Conditions for rRT-PCR

Step	Temperature	Time	Number of cycles
1	52˚C	10 min	1
2	95˚C	2 min	1
3	95˚C	10 sec	45
4	55˚C	30 sec	

Data Analysis

1. Analyze the data by opening the appropriate .eds file in Design and Analysis software v2.3.3.
2. Select 'Actions' → 'Primary Analysis Setting' → change the Algorithm Setting to 'Relative Threshold' → save.
3. The data should automatically reanalyze, but if it does not, select 'analyze again'.
4. Export results by selecting 'Actions' → 'Export'.
5. Assess the test results of the clinical specimens after positive, negative, and internal controls have been evaluated and determined to be acceptable.
6. Interpret the positive and negative results by comparing the Ct values from each fluorescent channel to its respective expected Ct value.

Interpretation of Results

Interpretation of Quality Controls

All test controls should be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted.

- Negative Template Control (NTC): This control must NOT have a detectable Ct in the N1 or RNase P reactions. If this control has a detectable Ct in any of the reaction wells, this indicates contamination of the PCR run and it is considered invalid and must be repeated.

- Positive control: This control targets Ct value for N1 should fall within 29-35. If there is amplification of N1 but it falls outside of established ranges, a supervisor should be contacted to investigate. This control must NOT have a detectable Ct in the RNase P reaction.
- Internal control: An internal control targeting the human RNaseP (RP) gene is used to monitor proper extraction and sample collection. Detection of RP is required to report a negative SARS-CoV-2 results (RP: Ct ≤38). Failure to detect RP (>38) and N1(>40) in a clinical specimen is considered invalid and the specimen should be rerun. A high viral load of pathogen nucleic acid may create competitive inhibition of RP, therefor a positive N1 in the presence of RP >38 is considered to be a positive result per the table below.
- Extraction control: This control should target Ct value for RNAse P and should be <35. If there is amplification of RNAse P but it falls outside of established ranges, a supervisor should be contacted to investigate. This control must NOT have a detectable Ct in the N1 reaction.

Assay Control Reporting

Control	Description	Purpose	Frequency	Results
Negative Template Control (NTC)	Nuclease-free water	To monitor for contamination of rRT-PCR reagents	Every batch of samples	N1: not detected or >40 RNase P: not detected or >38
Positive Control	Synthetic SARSCoV-2 RNA control	To monitor for properly functioning reagents	Every batch of samples	N1: 29-35 RNase P: not detected or >38
Internal Process Control	Primer/Probe set detecting RNaseP	To assess specimen quality and appropriate extraction	Each Sample	RNaseP: Ct≤38 if N1 >40 RNaseP: Ct=any if N1<40
Negative Extraction Control	Synthetic RNAse P control	To confirm effective RNA extraction	With each extraction batch	RNaseP: Ct≤35

Interpretation of Clinical Specimens

Assessment of clinical specimen test results should be performed after the positive and negative controls have been examined and determined to be valid and acceptable. If the controls are not valid, the patient results cannot be interpreted.

- Detected/Positive Specimens: Specimens with Ct values of ≤40.0 in the N1 are reported as "Detected" for SARS-CoV-2 RNA independent of the RNase P signal.
- Not Detected/Negative Specimens: Specimens with undetectable Ct values for N1 and with an acceptable RNAse P (Ct≤38) are reported as "Not Detected" for SARS-CoV-2 RNA.
- Invalid Results: Specimens with an RNAseP Ct value > 38 and N1 Ct value >40 will be re-extracted and repeated. If results are again the same, the specimen will be reported out as invalid.

Interpretation Criteria for Patient Results

Interpretation	RP CT	nCov_N1 CT
Not Detected/Negative	≤38	>40 or Undetermined
Invalid	>38 or Undetermined	>40 or Undetermined

Detected/Positive	Any	≤40

Limitations of the Procedure

- Performance of the CovidNow SARS-CoV-2 Real-Time RT-PCR Diagnostic Panel has only been established in nasopharyngeal swab specimens, however, oropharyngeal swabs, and nasal swab specimens collected per this IFU are also acceptable specimen types.
- All users, analysts, and any person reporting diagnostic results should be trained to perform this procedure by a competent instructor. They should demonstrate their ability to perform the test and interpret the results prior to performing the assay independently.
- 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.
- Negative results do not preclude 2019-nCoV infection and should not be used as the sole basis for treatment or other patient management decisions. Optimum specimen types and timing for peak viral levels during infections caused by 2019-nCoV have not been determined. Collection of multiple specimens (types and time points) from the same patient may be necessary to detect the virus.
- 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.
- Positive and negative predictive values are highly dependent on prevalence. False-negative test results are more likely when prevalence of disease is high. False-positive test results are more likely when prevalence is moderate to low.
- If the virus mutates in the rRT-PCR target region, 2019-nCoV may not be detected or may be detected less predictably. Inhibitors or other types of interference may produce a false-negative result. An interference study evaluating the effect of common cold medications was not performed.
- Test performance can be affected because the epidemiology and clinical spectrum of infection caused by 2019-nCoV is not fully known. For example, clinicians and laboratories may not know the optimum types of specimens to collect, and during the course of infection when these specimens are most likely to contain levels of viral RNA that can be readily detected.
- Detection of viral RNA may not indicate the presence of infectious virus or that 2019-nCoV is the causative agent for clinical symptoms.
- The performance of this test has not been established for monitoring treatment of 2019-nCoV infection.

- The performance of this test has not been established for screening of blood or blood products for the presence of 2019-nCoV.
- This test cannot rule out diseases caused by other bacterial or viral pathogens.

Conditions of Authorization for Laboratories

The CovidNow SARS-CoV-2 Assay Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients, and authorized labeling are available on the FDA website: fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/in-vitro-diagnostics-euas.

However, to assist clinical laboratories using CovidNow SARS-CoV-2 Assay, the relevant Conditions of Authorization are listed below:

- Authorized laboratories[1] using CovidNow SARS-CoV-2 Assay must include with test result reports, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.
- Authorized laboratories using CovidNow SARS-CoV-2 Assay must use the assay as outlined in the authorized labeling. Deviations from the authorized procedures, including the authorized instruments, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use this product are not permitted.
- Authorized laboratories that receive the CovidNow SARS-CoV-2 Assay must notify the relevant public health authorities of their intent to run this product prior to initiating testing.
- Authorized laboratories using CovidNow SARS-CoV-2 Assay must have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.
- Authorized laboratories must collect information on the performance of CovidNow SARS-CoV-2 Assay and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and Lighthouse Lab Services (via email: support@lighthouselabservices.com) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of your product of which they become aware.
- All laboratory personnel using CovidNow SARS-CoV-2 Assay must be appropriately trained in RT-PCR techniques and use appropriate laboratory and personal protective equipment when handling this Test and use this product in accordance with the authorized labeling.
- Authorized laboratories testing specimens self-collected using the CovidNow Collection Kit with your product must follow the "Accessioning SOP for Nasal Samples Collected by the CovidNow Collection Kit" standard operating procedure when accepting specimens for testing.
- Authorized laboratories using CovidNow SARS-CoV-2 Assay must ensure that any records associated with this assay are maintained until otherwise notified by the FDA. Such records will be made available to the FDA for inspection upon request.

[1] The letter of authorization refers to, "Laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests" as "authorized laboratories".

Performance Characteristics

Limit of Detection (LoD)

A study was performed to assess the performance of the CovidNow SARS-CoV-2 Assay at the Limit of Detection (LoD) for specimens. In this study, the CovidNow SARS-CoV-2 Assay was tested with quantified inactivated virus stock ZeptoMetrix NATSARS(COV2)-ERC: NATtrol SARS-Related Coronovirus- 2 (SARS-CoV-2) External Run Control) spiked into SARS-CoV-2 negative NPS matrix. For preliminary LoD testing, three replicates of each concentration of 2-fold dilution series were extracted on the ANDiS 350 Automated Nucleic Acid Extraction System to estimate LoD and the KingFisher Flex. The preliminary LoD was determined to be 0.8 copies/ µL . The LoD was confirmed by extracting 20 replicates at the preliminary LoD of 0.8 copies / µL. LoD of the CovidNow SARS-CoV-2 Assay for each extraction was defined as the lowest concentration with ≥95% detection of 20 replicates (19 out of 20) and is 0.8 copies / µL. Results are included in below.

Extraction	Replicates Tested	SARS-CoV-2 N1 Mean Ct	Internal Control Mean Ct	% Positive per Result Interpretation
MagMax	20	36.4	29.9	100% (20/20)
ANDiS 350	20	36.6	30.3	95% (19/20)

Clinical Performance

Clinical Evaluation-Suspected

To evaluate the clinical performance of the CovidNow Assay, 67 de-identified clinical remnant samples were obtained from CLIA certified laboratories. All samples were symptomatic or close contact patients in transport media and were collected by a healthcare worker. The comparator result from the paired NP swab was determined using a highly sensitivity EUA authorized PCR assay with a chemical extraction/purification step. The samples arrived at the laboratory blinded by a unique identifier and were subsequently run on the CovidNow assay per the IFU protocol. This study contained 20% 'low positive' samples (samples within 3 Ct of the comparator's mean Ct at the LoD).

Clinical Performance of the CovidNow Test with Suspected Individuals.

		Comparator Assay	
CovidNow SARS-CoV-2 Assay		**Positive**	**Negative**
	Positive	33	0

	Negative	0	34

Positive Percent Agreement =33/(33+0)X100 = 100% (95% CI: 98.6 – 100%)
Negative Percent Agreement =34/(34+0)X100 = 100% (95% CI: 98.9 – 100%)

The calculated Percent positive agreement (PPA) and percent negative agreement (NPA) were both calculated to be 100%.

Clinical Evaluation-Asymptomatic

To evaluate the clinical performance of the CovidNow assay in individuals without symptoms or other reason to suspect COVID-19, a performance evaluation at three geographically diverse testing sites over a period of 48 hours was conducted. Collection sites that perform routine serial screening were selected. Included in this study were individuals who were being routinely tested in either a school, work, or nursing home environment. Questionnaires were filled out for all patients, and those who admitted to symptoms or close contact events were excluded. The study included individuals across all ages (5-85 years of age).

All samples were HCP-collected NP swabs collected per the instructions for use. Patients with symptoms or previous exposure (i.e, close contact admissions) were excluded. The comparator result from the paired NP swab was determined using a highly sensitive EUA authorized RT-PCR assay. The samples arrived at the laboratory blinded by a unique identifier and were subsequently run on the CovidNow assay per the IFU protocol.

The calculated Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) were both calculated to be 96% and 100% respectively.

		Comparator Assay	
CovidNow SARS-CoV-2 Assay		**Positive**	**Negative**
	Positive	24	0
	Negative	1*	155

**This CovidNow false negative sample was 'presumptive positive' for the comparator assay with no detection of Target 1 and low Ct in Target 2.*

Positive Percent Agreement = 24/(24+1)X100 = 96% (95% CI: 80.5 – 99.3%)
Negative Percent Agreement = 155/(155+0)X100 = 100% (95% CI: 97.6 – 100%)

Inclusivity (Analytical Sensitivity)

The CovidNow SARS CoV-2 Assay uses the well-established primer and probe sequences published by the CDC (EUA CDC-006-00019 Rev7). These previous inclusivity analyses performed by the CDC (evaluated against 831,910 sequences available in the Global Initiative on Sharing All Influenza Data) demonstrate the predicted inclusivity of the CovidNow SARS CoV-2 Assay. These previous findings show a low risk of mismatches resulting in a significant loss in reactivity causing a false negative result due to the design of the primers and probes, with melting temperatures >60°C and with annealing temperature at 55°C that can tolerate up to two mismatches. A full summary can be found in the CDC's 2019-Novel Coronavirus (2019-nCoV) Real Time RT-PCR Diagnostics PanelEUA (006-00019 Rev7).

A verification analysis was conducted on September 11th to assess the oligonucleotide primer and probe sequences used in CovidNow assay. They were evaluated via two different methods to demonstrate the predicted inclusivity of the CovidNow assay.

1. The first *in-silico* method used the primer checker tool publicly available via the Global Initiative on Sharing All Influenza Data (GISAID, https://www.gisaid.org) initiative, which analyzed 49,394 recent (August 3, 2021 to September 4, 2021) high-quality U.S. genome sequences against CovidNow primer and probe sequences. It should be noted, the dates correspond to the most recent update of the GISAID database. All samples containing N and Rs were removed from the data analysis.

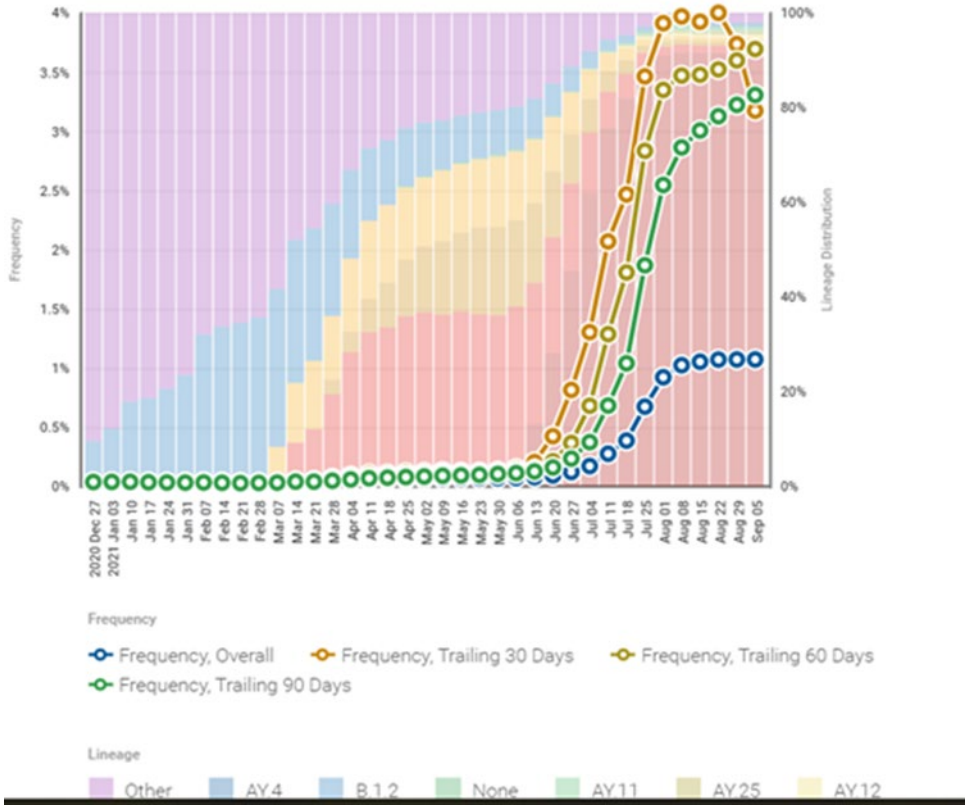
2. The second *in-silico* method used the Rosalind covid diagnostic monitoring system (DxM), a web-based platform, to monitor the latest viral variants and determine nucleotide mismatches against the CovidNow primer and probe sequences.

   Analysis using the first *in-silico* method demonstrated only one nucleotide mismatch with frequency greater than 1%, with all other nucleotide mismatches found to have a frequency lower than 0.2%. No nucleotide mismatches greater than 2 were found. The one nucleotide mismatch (G28326T) of the probe was found in variant B.1.617.2 sequences at a frequency of 4.99%. The second *in-silico* method also determined the same single nucleotide mismatch to be of significance; however, the frequency at 3.42% was lower due to the analysis of a higher number and more up to date database of U.S. sequences. Rosalind DxM also estimated a 2.81% drop in melting temperature from 60.8°C to 59.09°C, which is considered minimal.

   In summary, the assessment of homology between available sequences of SARS CoV-2 as of September 11, 2021 and the CovidNow assay primers and probes shows that the risk of significant loss in reactivity and false negative results is very low due to the absence of significant numbers of mismatches. No observations of 3 nucleotide mismatches occurred within the primers or probes. There were 19 individual instances of 2 nucleotide mismatches in the probe sequence, with 5 identified as a VOC and did not occur within the last 14 days of analysis. Our primers and probes were designed, with melting temperatures of >60°C and an annealing temperature of 55°C, to tolerate up to two mismatches depending on location without significant loss in assay sensitivity. It should be noted, the nucleotide mismatches did not occur at the end of our probe sequence. Ongoing in-silico analysis will be performed every three months based on recent FDA guidelines.

	Target Gene Fwd Primer	**Target Gene Rev Primer**	**Target Gene Probe**
Total Primer Length (nt)	20	24	24

Total Strains	49,394	49,394	49,394
100% Match	48,895	49,203	46,351
1 Mismatch	498	191	3,024
2 Mismatches	1	0	19
3 Mismatches	0	0	0
>3 Mismatches	0	0	0



Cross-reactivity (Analytical Specificity)

The CovidNow SARS CoV-2 Assay uses well-established primer and probe sequences published by the CDC (EUA CDC-006-00019 Rev7). Extensive in silico and wet testing has been previously reported for the CDC 2019-nCoV RT-PCR test and a general Right of Reference letter for commercial manufacturers and laboratories was provided by CDC to allow reference of that dataset.

In summary, in silico of the probe sequence of 2019-nCoV rRT-PCR assay N1 showed high sequence homology with SARS coronavirus and Bat SARS-like coronavirus genome. However, forward and reverse primers showed no sequence homology with SARS coronavirus and Bat SARS-like coronavirus genome. There are no significant homologies with human genome, other coronaviruses, or human microflora that would predict potential false positive

rRT-PCR results. A full summary can be found in the CDC's 2019-Novel Coronavirus (2019-nCoV) Real Time RT-PCR Diagnostics Panel EUA (006-00019 Rev7).

Disposal

Dispose of hazardous or biologically contaminated material according to the practice of your institution.

Glossary of Symbols Used on Packaging

The following symbols may appear contents of the CovidNow Assay:

REF	Catalog number		Temperature limitation
	Consult instructions for use	LOT	Batch code
IVD	In vitro diagnostic medical device		Use by
	Manufacturer	2	Do not reuse

Manufacturer Information

Manufactured by:
Lighthouse Lab Services
1337 Hundred Oaks Drive - Suite A
Charlotte, NC 28217
General inquires: contact@lighthouselabservices.com
Technical Support: support@lighthouselabservices.com
www.lighthouselabservices.com

Appendix 1

Emergency Only Label: Please print and place the Emergency Use only label on the front panel of the qualified instrument. If an instrument includes labeling indicating "For Research Use Only", please cover with the below "Emergency Use Only" labeling. The instrument should retain this labeling throughout the EUA use of the CovidNow Assay.

Emergency Use Only

This instrument is authorized for use with the CovidNow SARS-CoV-2 Assay.