LIGHTHOUSE Lab Services

Making Quality Lab Testing More Accessible

CovidNow SARS-CoV-2 Assay Kit
rRT-PCR Diagnostic Panel

Instructions for Use

2000 reactions
Catalog #9731816

For *In-vitro* Diagnostic (IVD) Use

This test has not been FDA cleared or approved; this test is for use by laboratories certified under CLIA, 42 U.S.C. § 263a, to perform high complexity tests. Review under the EUA program is pending. Distributed in accordance with the guidance on Policy for Coronavirus Disease-2019 Tests During the Public Health Emergency, Section IV.C.2.

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Intended Use

The CovidNow SARS-CoV-2 Assay Kit is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) in human upper respiratory specimens (i.e. nasopharyngeal swab, anterior nasal swab, and mid-turbinate nasal swab specimens) from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to laboratories designated by Lighthouse Lab Services and certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a that meet requirements to perform high complexity tests.

Results are for the detection and identification of SARS-CoV-2 RNA. SARS-CoV-2 RNA is generally detectable in respiratory specimens during 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 positive 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 Kit is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time RT-PCR and in vitro diagnostic procedures.

Summary and Explanation of the Test

The CovidNow SARS-CoV-2 Assay Kit 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)

Table 1. Reagents provided in the CovidNow SARS-CoV-2 Assay Kit

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)

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

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

Consumables (Not Provided)

Table 3. Consumables required, but not provided, for the CovidNow SARS-CoV-2 Assay Kit

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

Equipment (Not Provided)

Table 4. Equipment required, but not provided, for the CovidNow SARS-CoV-2 Assay Kit

Component Name
ThermoFisher Applied Biosystems QuantStudio 5 Real-Time PCR System
ANDiS 350 Automated Nucleic Acid Extraction System
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

Warnings, Precautions, and Best Practices

- The CovidNow SARS-CoV-2 rRT-PCR Assay is for *in vitro* diagnostics use (IVD).
- Laboratories should include a statement that this test has been validated, but FDA's independent review of this validation is pending in test reports to healthcare providers.
- This test has not been FDA cleared or approved; this test is for use by laboratories certified under CLIA, 42 U.S.C. § 263a, to perform high complexity tests. Review under the EUA program is pending. Distributed in accordance with the guidance on *Policy for Coronavirus Disease-2019 Tests During the Public Health Emergency*, Section IV.C.2.
- This test has is only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
- 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 sooner terminated or revoked.
- 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.
- Performance characteristics have been determined with human upper respiratory specimens and lower respiratory tract specimens from human patients with signs and symptoms of respiratory infection.
- 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.
- 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).

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.
- 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.

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

RNA Extraction

1. RNA extraction is performed with ANDiS Viral RNA Auto Extraction and Purification Kit (Cat No. 3103010026) on ANDiS 350 Automated Nucleic Acid Extraction System (Cat No. 3105020003).
2. Please follow the product instructions to conduct viral RNA extraction and purification.
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 in Table 5, below:

Table 5. '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.
    c. Seal with a transparent plastic qPCR seal. Centrifuge briefly 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 qPCR 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: Cy5.5
        iii. Quencher Dye: (none)
4. Run the assay as per the thermocycling conditions given in Table 6, below:

Table 6. 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 'Baseline 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

1. 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 should target Ct value for N1 established by lot but should be less than 35. The control's Ct value should fall within the established range. 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: All clinical samples should exhibit fluorescence growth curves in the RNase P reaction that cross the threshold line within 38.00 cycles (< 38.00 Ct), thus indicating the presence of the human RNase P (RP) gene and proper extraction. Failure to detect RP in any clinical specimens is considered invalid and the specimen should be rerun.
    - Negative extraction control: This control should target Ct value for RNAse P established by lot but should be less than 35. The control's Ct value should fall within

the established range. 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.

Table 7. 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" RNase P: "Not Detected"
Positive Control	Synthetic SARSCoV-2 RNA control	To monitor for properly functioning reagents	Every batch of samples	N1: "detected" RNase P: "Not Detected"
Internal Process Control	Primer/Probe set detecting RNaseP	To assess specimen quality and appropriate extraction	Each Sample	RNaseP: CT<38
Negative Extraction Control	Synthetic RNAse P control	To confirm effective RNA extraction	With each extraction batch	RNaseP: CT<38

Interpretation of Clinical Specimens

1. 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 <38.0 in the N1 and with an acceptable RNAse P, are reported as "Detected" for SARS-CoV-2 RNA.
    - 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.
    - Inconclusive Results: Specimens with unacceptable RNAse P Ct value (>38) and in which N1 was detected <38 will be re-extracted and repeated. If results are again the same the specimen will be reported out as inconclusive.
    - Invalid Results: Specimens with an unacceptable RNAse P and N1 Ct value will be re-extracted and repeated. If results are again the same, the specimen will be reported out as invalid.

Table 8. Interpretation Criteria for Patient Results

nCoV_N1	RNaseP	Interpretation
+	+	Detected
+	-	Inconclusive
-	-	Invalid
-	+	Not detected

Limitations of the Procedure

- Performance of the CovidNow SARS-CoV-2 Real-Time RT-PCR Diagnostic Panel has only been established in upper respiratory specimens (such as nasopharyngeal, oropharyngeal swabs, nasal, etc.).

- 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.
- 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.
- Do not use any reagent past the expiration date.
- 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.

Additional Instructions for Laboratories

To assist clinical laboratories using CovidNow SARS-CoV-2 Assay, additional instructions are listed below

- Laboratories using CovidNow SARS-CoV-2 Assay must use the product as outlined in the the provided 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.

- Laboratories should include a statement that this test has been validated, but FDA's independent review of this validation is pending, in test reports to healthcare providers.
- Authorized laboratories that receive the CovidNow SARS-CoV-2 Assay test will 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 will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.
- Authorized laboratories will 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 nucleic acid amplification 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 using CovidNow SARS-CoV-2 Assay will 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.

Performance Characteristics

Limit of Detection (LoD)

A study was performed to assess the performance of the CovidNow SARS-CoV-2 Assay Kit at the Limit of Detection (LoD) for specimens. In this study, the CovidNow SARS-CoV-2 Assay was tested with quantified inactivated virus stocks (Twist Biosciences) spiked into SARS-CoV-2 negative NPS. 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. The LoD was confirmed by extracting 20 replicates at the preliminary LoD. LoD of the CovidNow SARS-CoV-2 Assay for each extraction was defined as the lowest concentration with ≥95% detection of 20 replicates (20 out of 20) and is 0.4 copies / µL.

Clinical Performance

For clinical correlation, 30 negative and 30 positive SARS-CoV-2 nasal or blinded nasopharyngeal swabs were collected from patients. The clinical evaluation was then confirmed on 2 additional QuantStudio 5 rRT-PCR instruments. In addition, the clinical evaluation was run on all instruments each day for a period of 3 days. All results agreed with above data. Patient samples collected in nasal or nasopharyngeal swabs for SARS-CoV-2 which were previously tested by a CLIA laboratory with TaqPath COVID combo kit (previously validated) were used for this study. All samples were prepared together with positive, negative and internal controls per the instructions for use described herein.

Percent Positive agreement and Percent Negative agreement was calculated between TaqPath COVID Combo kit data and the CovidNow SARS-CoV-2 Assay kit.

Table 9. PPA and NPA Calculation

		SARS-CoV-2 Comparator Test	
CovidNow SARS CoV-2 Assay		**Positive**	**Negative**
	Positive	29	0
	Negative	1	30
Positive Agreement		29/(29+1)X100=96.7%	
Negative Agreement		30/(30+0)X100=100%	

Results: The calculated Percent positive agreement and NPA was found to be 96.7% and 100% respectively.

Inclusivity

The CovidNow SARS CoV-2 Assay Kit uses the well-established primer and probe sequences published by the CDC (EUA CDC-006-00019 Rev5). These previous inclusivity analyses performed by the CDC (evaluated against 31,623 sequences available in the Global Initiative on Sharing All Influenza Data) demonstrate the predicted inclusivity of the CovidNow SARS CoV-2 Assay Kit. 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 Rev5).

Cross-reactivity

The CovidNow SARS CoV-2 Assay Kit uses the well-established primer and probe sequences published by the CDC (EUA CDC-006-00019 Rev5). Extensive in silico and wet testing has been previously reported.

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 is 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 PanelEUA (006-00019 Rev5).

Specimen Stability

To increase the ability to detect infection with CovidNow SARS-CoV-2 assay kit, specimens should be collected as soon as possible once a PUI is identified regardless of symptom onset. Maintain proper infection control when collecting specimens. The specimens should be tested withing 72 hours after collection when stored at 2-8°C. For long term storage, specimens should be kept at -70°C. The specimens should be shipped to resting lab on ice packs overnight.

Disposal

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