LIGHTHOUSE Lab Services

Making Quality Lab Testing More Accessible

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

Instructions for Use

2000 reactions
Catalog #9731816S

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 SalivaNow 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 saliva 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 saliva 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 SalivaNow 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.

For prescription use only.

Summary and Explanation of the Test

The SalivaNow SARS-CoV-2 Assay Kit is a molecular *in vitro* diagnostic kit intended for the qualitative detection of SARS-CoV-2 in saliva samples. 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 SalivaNow 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.

Patient samples are first treated with proteinase K 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 7 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 SalivaNow SARS-CoV-2 Assay Kit

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

Reagents Required (Not Provided)

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

Component Name	Contents	Storage
ThermoFisher Applied BioSystems TaqPath™ 1-Step Multiplex Master Mix Cat No. A28522	10mL	Store at -20°C
ThermoFisher Applied Biosystems MagMAX Viral/Pathogen Proteinase K Cat No. A42363	10ml	Ambient Temperature

Consumables (Not Provided)

Table 3. Consumables required, but not provided, for the SalivaNow 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 96-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 SalivaNow SARS-CoV-2 Assay Kit

Component Name
ThermoFisher Applied Biosystems QuantStudio 7 Real-Time PCR 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 SalivaNow SARS-CoV-2 rRT-PCR Assay is for *in vitro* diagnostics use (IVD) under Emergency Use Authorization only.
- This test has not been FDA cleared or approved; this test has been authorized by FDA under an EUA for use by laboratories certified under CLIA, 42 U.S.C. § 263a, to perform high complexity tests.
- This test has been authorized 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 saliva 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 proteinase K treatment 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 treated samples.
- 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 and are in violation of the product Emergency Use Authorization.
- 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.
- Saliva specimens should be collected using a sterile RNase free tube. A minimum of 1mL is required for testing. Patients should avoid eating or drinking 10 minutes prior to collection.

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 packs. 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 specimen processing is expected, store specimens 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

Important Guidelines for RT-PCR

- For each RT-PCR reaction plate, include the following controls:
  - One Positive Control
  - One Negative Control
  - One No Template Control
- 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.

SalivaNow SARS-CoV-2 Sample Treatment

1. In the Class II BSC, add 2.5 µL of ThermoFisher Scientific MagMAX™ Viral/Pathogen Proteinase K to designated PCR plate (200 µL capacity).
2. Briefly vortex each saliva sample until homogeneous or pipette up and down, and immediately transfer 50 µL saliva to each PCR plate containing proteinase K. If saliva sample is too viscous or lumpy, then vortex the sample on high speed until homogenized.
3. Close PCR plate lids tightly.
4. Vortex the PCR plate for 1 minute at 3,000-5,000 RPM.
5. Briefly spin down the rack using a plate spinner.
6. Inactivate the proteinase K by heating samples for 5 minutes at 95˚C on a PCR instrument or equivalent thermocycler.

7. Allow the plates to cool down and then centrifuge plate 1 min in plate spinner.
8. Store samples at -80˚C or proceed immediately to rRT-PCR testing.

SalivaNow 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	SalivaNow 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 a 96-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 treated samples and the PCR 'Assay Reaction Mix' to a biosafety cabinet.
    a. Add 5 µL of treated sample 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 SalivaNow 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 is 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 qPCR machine and run the following thermocycler conditions:
2. Set up the assay as follows:
    a. Block type: 96-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 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 '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

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 target Ct value for N1 is established by lot but should be less than 38. 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 proteinase K treatment.
    - Negative control: This target Ct value for RNAse P is established by lot but should be less than 38. 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 proteinase K treatment	Each Sample	RNaseP: CT<38
Negative Control	Synthetic RNAse P control	To confirm effective proteinase K treatment	With each batch	RNaseP: CT<38

Note: Expected Ct ranges for positive control are established for all lot numbers. Targeted Ct between 28-33.

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 are reported as "Detected" for SARS-CoV-2 RNA independent of the RNase P signal.
- Not Detected/Negative Specimens: Specimens with Ct >40 or undetectable for N1 and with an acceptable RNAse P (Ct ≤38) are reported as "Not Detected" for SARS-CoV-2 RNA.
- Inconclusive Results: Specimens with acceptable RNAse P Ct value (≤38) and in which N1 was detected between >38 - ≤40 will be repeated. If results are again the same the specimen will be reported out as inconclusive.
- Invalid Results: Specimens with an unacceptable RNAse P **and** an unacceptable N1 Ct value (>38) will be repeated. If results are again the same, the specimen will be reported out as invalid.

Table 8. Interpretation Criteria for Patient Results

Output	Interpretation	RP CT	nCov_N1 CT
0	Negative	≤38	> 40 or Undetermined
1	Invalid	>38 or Undetermined	>38 or Undetermined
2	Positive	Any	≤38
3	Inconclusive	≤38	>38 - ≤40

Limitations of the Procedure

- Performance of the SalivaNow SARS-CoV-2 Real-Time RT-PCR Diagnostic Panel has only been established in saliva specimens.
- 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.

Conditions on Authorization for Laboratories

To assist clinical laboratories using SalivaNow SARS-CoV-2 Assay, the relevant Conditions of Authorization are listed below:

- Authorized laboratories using CovidNow SARS-CoV-2 Assay will 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 SalivaNow SARS-CoV-2 Assay must use the product as outlined in the authorized labeling.
- Deviations from the procedures, including the instruments, clinical specimen types, control materials, other ancillary reagents and materials specified in these instructions are not permitted.
- Authorized laboratories that receive the SalivaNow 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 SalivaNow 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 SalivaNow 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 SalivaNow 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 labeling.
- Authorized laboratories using SalivaNow 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 SalivaNow SARS-CoV-2 Assay Kit at the Limit of Detection (LoD) for specimens. In this study, the SalivaNow SARS-CoV-2 Assay was tested with quantified inactivated virus stocks (ZeptoMetrix) spiked into SARS-CoV-2 negative saliva specimens. Multiple RNase free collection tubes were utilized for this study. Tubes varied in vendor and size to ensure accuracy across a wide range of collection devices. For preliminary LoD testing, three replicates of each concentration in a dilution series were tested to estimate LoD. The LoD was confirmed by running 20 replicates at the preliminary LoD. LoD of the SalivaNow SARS-CoV-2 Assay was defined as the lowest concentration with ≥95% detection of 20 replicates (20 out of 20) and is 6.25 copies / µL.

Interfering Substances

Interfering substance testing was completed to determine the extent to which endogenous and exogenous substances interfered with the performance of the test. Potentially interfering substances tested were:

- Altoids-spearmint (1% v/v)
- Benzocaine/menthol Lozenges (3 mg/mL)
- Chloroseptic Sore Throat Spray (5% v/v)
- Cough Syrup-OTC (1% v/v)
- Halls Cough Drops (3mg/mL)
- Listerine Mouth Wash (5% v/v)
- Nasal Decongestant Spray (15% v/v)
- Saline-based nasal moisturizing gel spray (12.5% v/v)
- Toothpaste (0.5% v/v)
- Whole blood (2% v/v)
- Nicotine (0.03mg/mL)

With one exception (Colgate toothpaste), no endogenous or exogenous substances interfered with the test at the concentration of the substances that were added into the saliva for either the negative samples or the low positive samples spiked with SARS-CoV-2 for SalivaNow. The one exception was Colgate toothpaste at 0.5% v/v in which the low-level positive samples reported as negative or inconclusive for the 3 positive replicates. Certain toothpaste residues have been shown to cause interference problems, specifically those similar to Colgate tested here. It is

important to follow the collection guidelines outlined in the IFU to minimize the potential for false negatives.

Clinical Performance

For clinical correlation, 164 SARS-CoV-2 paired nasal or nasopharyngeal swabs and saliva samples were collected from patients. For the saliva specimens multiple RNase free collection tubes were utilized. Tubes varied in vendor and size to ensure accuracy across a wide range of collection devices. Nasal or nasopharyngeal swabs for SARS-CoV-2 were tested by a CLIA laboratory with TaqPath COVID combo kit (previously validated). 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 SalivaNow SARS-CoV-2 Assay kit.

Table 9. PPA and NPA Calculation

		SARS-CoV-2 Comparator Test	
SalivaNow SARS CoV-2 Assay		**Positive**	**Negative**
	Positive	49	0
	Negative	3	112
Positive Agreement		49/(49+3)*100=94.2%	
Negative Agreement		112/(112+0)*100=100%	

Results: The calculated Percent positive agreement and NPA was found to be 94.2% and 100% respectively.

Inclusivity

The SalivaNow 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 SalivaNow 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 SalivaNow 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 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 Rev5).

Updated *In Silico* Analysis

In light of the new CoV-2 variants emerging updated *in silico* analysis was performed. BLASTn analysis queries of the 2019-nCoV rRT-PCR assays primers and probes were performed against public domain nucleotide sequences. The database search parameters were as follows:

1) The nucleotide collection consists of GenBank+EMBL+DDBJ+PDB+RefSeq sequences, but excludes EST, STS, GSS, WGS, TSA, patent sequences as well as phase 0, 1, and 2 HTGS sequences and sequences longer than 100Mb;
2) The database is non-redundant. Identical sequences have been merged into one entry, while preserving the accession, GI, title and taxonomy information for each entry;
3) Database was updated on 01/11/2021;
4) The search parameters automatically adjust for short input sequences and the expect threshold is 1000;
5) The match and mismatch scores are 1 and -3, respectively;
6) The penalty to create and extend a gap in an alignment is 5 and 2 respectively.

Probe sequence of the 2019-nCoV rRT-PCR assay N1 showed high sequence homology with SARS coronavirus and bat SARS-like coronavirus genome. However, the forward and reverse primers showed no sequence homology with SARS coronavirus and bat SARS-like coronavirus genome. Combining primers and probe, there are no significant homologies with the human genome, other coronaviruses, or human microflora that would predict potential false positive rRT-PCR results. The primer and probe sequences do not overlap with mutations identified in the novel B.1.17 and B.1.351 variants minimizing the risk of false negative rRT-PCR results due to samples infected with one of these strains.

Continued Variant Analysis

To address the concerns associated with the continuing evolution of the SARS-CoV2 virus, the following mitigation steps will be employed to ensure our assay accuracy is not impacted:

1. A screen in which sequences from GISAID are queried over the past month will be run to identify any novel variants with mutations within the primer or probe binding regions. If mutations are identified in more than 5% of all screened sequences, further analysis will be performed to ensure the point mutation does not negatively impact the outcome of the test.
2. When novel mutations are identified within the primer or probe binding regions, a mismatch analysis will be performed to determine the impact of the mutation to primer or probe binding. The mismatch Tm should be no lower than 52.3°C or 95% of the annealing temperature recommend for this multiplex. Any constellation of mutations resulting in a mismatch Tm below 52.3°C will require additional laboratory testing by

Lighthouse Lab Services to determine the impact of the mutation(N1) on the assay performance.

3. Anytime a predicted mismatch Tm falls below 52.3°C, a wet lab test of that variant sequence will be performed. To carry out the analysis, synthetic RNA templates of the variant and consensus amplicon regions will be synthesized to perform a Limit of Detection (LoD) test. Any shift within the LoD less than 3-fold will not be considered significant and no further analysis is needed. If the LoD shift is greater than 3-fold, then a risk assessment will be performed to determine the impact of the mutation(s). The risk assessment will include regional location of the variant, last reported date of the variant and as the variant isolated to one region. If it is determined that the mutations will impact the performance of the assay and affect testing within the USA, then altering the oligos with a degenerate base(s) to account for the mutations.

As of April 1st, 2021 no identified variant mutations have been identified in more than 5% of screened sequences.

Specimen Stability

To increase the ability to detect infection with SalivaNow 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 testing lab on ice packs overnight.

Disposal

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