SalivaNow
SARS-CoV-2 Assay

Instructions for Use

For Use Under Emergency Use Authorization (EUA) Only

For *In-Vitro* Diagnostic Use

Rx only

Table of Contents

Intended Use

The SalivaNow SARS-CoV-2 Assay is a real-time RT-PCR *in vitro* diagnostic test intended for the qualitative detection of nucleic acid from severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) in human saliva specimens from individuals suspected of COVID-19 by their healthcare provider. Testing is limited 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.

Results are for the identification of SARS-CoV-2 RNA. SARS-CoV-2 RNA is generally detectable in saliva specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definitive cause of disease.

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. Negative results for SARS-CoV-2 RNA from saliva should be confirmed by testing of an alternative specimen type if clinically indicated.

The SalivaNow SARS-CoV-2 Assay is intended for use by qualified laboratory personnel specifically instructed and trained in the techniques of real-time RT-PCR and *in vitro* diagnostic procedures. The SalivaNow 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 SalivaNow SARS-CoV-2 Assay is a molecular *in vitro* diagnostic kit intended for the qualitative detection of SARS-CoV-2 in human saliva specimens. 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.

All laboratory personnel using this test 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 (N1) 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 saliva specimens is also included in the panel.

Patient saliva specimens are first lysed 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

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 (Provided by Lighthouse Separately)

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

Component Name	Contents	Storage
SalivaNow SARS-CoV-2 1-Step Multiplex Master Mix	10 mL	Store at -20°C
SalivaNow SARS-CoV-2 Proteinase K	10 mL	Store at Room Temperature (15-25°C)

Consumables (Not Provided)

Table 3. Consumables required, but not provided, for the SalivaNow 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 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

Component Name
ThermoFisher Applied Biosystems QuantStudio 7 Real-Time PCR System equipped with QuantStudio Design and Analysis software v2.3.3.
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

Qualifying Your Applied Biosystems QuantStudio 7 Real-Time PCR Instrument

To qualify your Applied Biosystems QuantStudio 7 Real-Time PCR Instrument to run the SalivaNow SARS-CoV-2 Assay, ensure that the instrument has been properly maintained and calibrated. Below is the instrument maintenance schedule and a link to the RUO instrument Maintenance Guide (Table 5). All instruments should be properly calibrated and maintained prior to use.

Table 5. Instrument for rRT-PCR

Instrument Model	Maintenance Guide Link	Maintenance Schedule
Applied Biosystems QuantStudio 7 Real-Time PCR Systems	QuantStudio RT-PCR Maintenance Guide	Every 12 months

To further qualify the performance of the instrument prior to testing, run 3 SalivaNow SARS-CoV-2 Positive Controls according to Instructions for Use. The instrument will be qualified if the SalivaNow SARS-CoV-2 Positive Controls meet the acceptance criteria, i.e. Threshold Cycle (Ct) for N1 falls in the range of 29-35 and RNase P (RP) is undetermined or Ct>38.

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 below "Emergency Use Only" labeling. The instrument should retain this labeling throughout the EUA use of the SalivaNow SARS-CoV-2 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

- For *in vitro* diagnostics use only. Rx only. For use under Emergency Use Authorization only.
- This product has not been FDA cleared or approved, but has been authorized for emergency use by 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 diagnostic tests for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug and Cosmetic Act, 21 U.S.C. § 360bbb- 3(b)(1), unless the declaration is terminated or authorization is revoked sooner.
- 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.
- 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 Coronavirus Disease 2019 (COVID-19)* https://www.cdc.gov/coronavirus/2019-nCoV/lab/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 SARS-CoV-2 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 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.
- Do not use any reagents past the expiration date.
- Dispose of unused kit reagents and human specimens according to local, state, and federal regulations.
- Accurate results depend on proper specimen collection, storage, and handling procedures.

Specimen Collection, Handling, and Storage

Inadequate or inappropriate saliva 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. Limit the number of freeze/thaw cycles to minimize specimen degradation.

Reagent Storage, Handling, and Stability

- Store SalivaNow SARS-CoV-2 Primer/Probe Mix at -20°C.
- Store SalivaNow SARS-CoV-2 Positive Control material at ≤ -80°C.
- Store SalivaNow SARS-CoV-2 Negative Control material at -20°C.
- Store SalivaNow SARS-CoV-2 1-Step Multiplex Master Mix at -20°C.
- Store SalivaNow SARS-CoV-2 Proteinase K at room temperature (15-25°C).
- Do not perform more than 4 freeze/thaw cycles of any assay reagents
- 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 rRT-PCR plate include the following external controls:

- Negative Control (SalivaNow SARS-CoV-2 Negative Control)

- Positive Control (SalivaNow SARS-CoV-2 Positive Control)
- No Template Control (Nuclease-free water)

Important Guidelines for RT-PCR

- For each rRT-PCR reaction plate, include the following external controls:
  - One SalivaNow SARS-CoV-2 Positive Control
  - One SalivaNow SARS-CoV-2 Negative Control
  - One No Template Control (Nuclease-free water)

Prepare the RT-PCR reaction plate on ice and keep it on ice until it is loaded into the QuantStudio 7 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 SalivaNow SARS-CoV-2 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, transfer enough SalivaNow SARS-CoV-2 1-Step Multiplex Master Mix to evaluate all control and patient samples. To account for volume lost during pipetting, include 10% extra volume of the SalivaNow SARS-CoV-2 1-Step Multiplex Master Mix. 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 6, below:

Table 6. 'Assay Reaction Mix' for rRT-PCR

	Component	Volume per Reaction	Volume per N Reaction
1	SalivaNow SARS-CoV-2 1-Step Multiplex Master Mix	5 µL	5 µL x (N + 1)
2	SalivaNow SARS-CoV-2 Primer/Probe 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.
   c. Add 5 µL of SalivaNow SARS-CoV-2 Negative Control to the designated PCR control well. Mix by pipetting, taking care to avoid introducing bubbles.
7. Add 5 µL of SalivaNow SARS-CoV-2 No Template Control (i.e. nuclease-free water) to the designated PCR control well. Mix by pipetting, taking care to avoid introducing bubbles.
8. 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.
9. 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.
10. 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 QuantStudio 7 Real-Time PCR Instrument 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 7, below:

Table 7. 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.

- No Template Control (NTC): This control must NOT have a detectable Ct in the N1 or RP reactions, or the Ct value should be greater than 38. 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.

- SalivaNow SARS-CoV-2 Positive Control: The Ct value for N1 should be between 29-35. If there is amplification of N1 but it falls outside of established ranges, a supervisor should be contacted to investigate and clinical samples reran. This control must NOT have a detectable Ct in the RP reaction, or it should be greater than 38.

- Internal Control: All clinical samples should exhibit fluorescence growth curves in the RP reaction that cross the threshold line less than or equal to ≤ 38 cycles, thus indicating the presence of the human RP gene and proper proteinase K treatment. Failure to detect RP in a saliva specimen is considered invalid and the specimen should be rerun, unless N1 has a Ct value ≤ 38 then it should be reported as positive.

- SalivaNow SARS-CoV-2 Negative Control: The Ct value for RP should be between 29-35. If there is amplification of RP but it falls outside of established ranges, a supervisor should be contacted to investigate and clinical samples reran. This control must NOT have a detectable Ct in the N1 reaction, or the Ct value should be greater than 38.

Table 8. Assay Control Reporting

Control	Description	Purpose	Frequency	Results
No Template Control (NTC)	Nuclease-free water	To monitor for contamination of rRT-PCR reagents	Every batch of samples	N1: Undetermined, or Ct>38 RP: Undetermined, or Ct>38
SalivaNow SARS-CoV-2 Positive Control	Synthetic SARS-CoV-2 RNA control	To monitor for properly functioning reagents	Every batch of samples	N1: Ct 29-35 RP: Undetermined, or Ct>38
Internal Process Control	Primer/Probe set detecting RNaseP	To assess specimen quality, appropriate proteinase K treatment, and reverse transcription/	Each Sample	RP: Ct≤38
SalivaNow SARS-CoV-2 Negative Control	Synthetic RNase P control	To confirm effective proteinase K treatment (i.e., cell lysis)	Every batch of samples	RP: Ct 29-35 N1: Undetermined, or Ct>38

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 ≤ 38 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 RP (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 > 38 will be re-processed according to the assay workflow and repeat tested. If results are again the same, the specimen will be reported out as invalid.

Table 9. Interpretation Criteria for Patient Results

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

Limitations of the Procedure

- The SalivaNow SARS-CoV-2 Assay is used for qualitative detection of SARS-CoV-2 RNA from human saliva specimens. The Ct result cannot directly reflect the viral load in the patient specimen.
- SalivaNow SARS-CoV-2 Assay performance was established with human saliva specimens, only.
- Negative results for SARS-CoV-2 RNA from saliva should be confirmed by testing of an alternative specimen type if clinically indicated.
- All laboratory personnel using this product 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.
- The performance of this test was established based on the evaluation of a limited number of clinical specimens. Clinical performance has not been established with all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.
- Negative results from the SalivaNow SARS-CoV-2 Assay do not preclude SARS-CoV-2 infection and should not be used as the sole basis for treatment or other patient management decisions.

- 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.
- A false-positive result may occur if a specimen is improperly collected, transported, or handled. False-positive result may occur if there is cross-contamination by SARS-CoV-2, nucleic acid or amplified product that is introduced in a patient specimen.
- The SalivaNow SARS-CoV-2 Assay has not been evaluated for asymptomatic testing or specimen pooling.
- 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, SARS-CoV-2 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.
- Detection of viral RNA may not indicate the presence of infectious virus or that SARS-CoV-2 is the causative agent for clinical symptoms.
- The performance of this test has not been established for monitoring treatment of SARS-CoV-2 infection.
- The impact of vaccines, antiviral therapeutics, antibiotics, chemotherapeutics, or immunosuppressant drugs on test performance have not been evaluated.
- The performance of this test has not been established for screening of blood or blood products for the presence of SARS-CoV-2.
- This test cannot rule out diseases caused by other bacterial or viral pathogens.
- Amplification of nucleic acid from saliva specimens must be performed according to the specified methods listed in these instructions. Other processing systems have not been evaluated.
- Amplification and detection of SARS-CoV-2 with this test has only been validated with the instruments specified in these instructions. Use of other instrument systems may cause inaccurate results.

Conditions on Authorization for Laboratories

The SalivaNow 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:

https://www.fda.gov/medical-devices/covid-19-emergency-use-authorizations-medical-devices/in-vitro-diagnostics-euas

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

- Authorized laboratories[1] using the SalivaNow 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 the SalivaNow SARS-CoV-2 Assay must use the product as outlined in the authorized labeling.
- Deviations from the authorized procedures, including the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use your product are not permitted.
- Authorized laboratories that receive the SalivaNow SARS-CoV-2 Assay test must notify the relevant public health authorities of their intent to run the SalivaNow SARS-CoV-2 Assay prior to initiating testing.
- Authorized laboratories using the SalivaNow 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 the SalivaNow SARS-CoV-2 Assay and must report any significant deviations from the established performance characteristics of the SalivaNow SARS-CoV-2 Assay of which they become aware to DMD/OHT7/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and LMSI, LLC d/b/a Lighthouse Lab Services (via email: support@lighthouselabservices.com).
- All laboratory personnel using the SalivaNow SARS-CoV-2 Assay must be appropriately trained in real-time RT-PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit, and use the SalivaNow SARS-CoV-2 Assay in accordance with the authorized labeling.
- LMSI, LLC d/b/a Lighthouse Lab Services, authorized distributor(s) and authorized laboratories using the SalivaNow SARS-CoV-2 Assay must ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records must be made available to FDA for inspection upon request.

Performance Characteristics

Limit of Detection (LoD)

A study was performed to determine the Limit of Detection (LoD) SalivaNow SARS-CoV-2 Assay for saliva specimens. In this study, the SalivaNow SARS-CoV-2 Assay was tested with quantified SARS-CoV-2 inactivated virus stocks (ZeptoMetrix) spiked into negative saliva specimens. For preliminary LoD testing, three replicates of 6 (25 to 0.78 copies/µL) concentrations in a dilution series were tested to estimate LoD. The preliminary LoD was

[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."

defined as the lowest concentration where 100% (3/3) replicates were positive. The LoD was confirmed by running 20 replicates at the preliminary LoD concentration. The LoD of the SalivaNow SARS-CoV-2 Assay was defined as the lowest concentration with ≥95% detection 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. The following potentially interfering substances were tested at the denoted concentrations:

- Afrin Original nasal spray (15% v/v)
- Sore throat and cough lozenges such as Cepacol Lozenges(benzocaine/menthol) (3 mg/mL)
- Chloroseptic Sore Throat Spray (5% v/v)
- Mouth Wash (5% v/v)
- Cough Syrup-OTC (5 % v/v)
- Mucin: bovine submaxillary gland, type I-S (2.5 mg/mL)
- Tobacco (0.03 mg/mL)
- Blood (2.5% v/v)
- Toothpaste (0.5% v/v)

Potentially interfering substances were evaluated in both the presence of SARS-CoV-2 (at 3x LoD) and the absence of SARS-CoV-2. No interference was observed for any of the evaluated substances in either the presence of absence of SARS-CoV-2.

Clinical Performance

For the clinical performance study, 63 SARS-CoV-2 subject paired nasopharyngeal swab (NPS) samples and saliva samples were collected from patients suspected of COVID-19 by their healthcare provider. Paired saliva samples arrived at the lab randomized and blinded by a unique identifier and were tested with the SalivaNow SARS-CoV-2 Assay according to the Instructions for Use. NPS specimens were tested with one of three FDA-EUA high sensitivity molecular comparator assays. ~20% of the NPS specimens were low positives by the comparator assay. One of the saliva samples was invalid twice with the SalivaNow SARS-CoV-2 Assay and was removed from analysis, leaving 62 samples for performance calculations. t Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) were calculated between the comparator NPS data and the SalivaNow SARS-CoV-2 Assay saliva data. The calculated PPA and NPA were found to be 100% (95% two-sided CI: 89.0-100.0%) and 96.8% (95% two-sided CI: 83.8-99.4%) respectively (Table 10).

Table 10. Clinical Performance Summary

Subject Paired Saliva vs. NPS		**SARS-CoV-2 Comparator Test (NPS)**	
		Positive	**Negative**
SalivaNow SARS-CoV-2 Assay (Saliva)[1]	**Positive**	31	1
	Negative	0	30

Positive Percent Agreement	100.0% (31/31), 95% CI: (89.0-100.0%)
Negative Percent Agreement	96.8% (30/31), 95% CI: (83.8-99.4%)

[1] One saliva sample was invalid twice with the SalivaNow SARS-CoV-2 Assay and was removed from analysis.

Inclusivity

The SalivaNow SARS-CoV-2 Assay 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. 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 Panel EUA (006-00019 Rev5).

Cross-reactivity

The SalivaNow SARS-CoV-2 Assay uses the well-established primer and probe sequences published by the CDC (EUA CDC-006-00019 Rev5). Extensive *in-silico* testing has been previously reported with additional analysis described below.

In summary, *in-silico* of the probe sequence of the SalivaNow SARS-CoV-2 Assay N1 target 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 the 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).

Additionally, Lighthouse Laboratory Services performed more *in-silico* cross-reactivity testing using NCBI BLAST (National Center for Biotechnology Information Basic Local Alignment Search Tool) for likely present organisms or high priority pathogens from the same genetic family that may be found in a saliva specimen. None of the organisms exhibited greater than 80% homology and an e-value less than 0.01. It should be noted, the e-value is a parameter that describes the number of hits one can "expect" to see by chance when searching a database of a particular size. It is widely accepted an e-value greater than 0.01 will include hits that cannot be considered as significant. None of the organisms were expected to be detected and therefore were not wet-tested. These results demonstrate a low chance of a false positive rRT-PCR result due to cross-reactivity.

Ongoing variant analysis associated with the continuing evolution of the SARS-CoV-2 virus is ongoing. As of May 23, 2023, we have accessed the following variants (Omicron and BQ.1) and have determined there is no predicted adverse effect on detection of SAR-CoV-2 using the SalivaNow SARS-CoV-2 Assay.

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 on contents of the SalivaNow SARS-CoV-2 Assay:

REF	Catalog number		Temperature limitation
	Consult instructions for use	LOT	Batch code
IVD	In vitro diagnostic medical device		Use by
	Manufacturer		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 Use Only Label: Please print and place the Emergency Use only label on the front panel of the qualified Applied Biosystems QuantStudio 7 Real-Time PCR 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 SalivaNow SARS-CoV-2 Assay.

Emergency Use Only

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