Simoa® SARS-CoV-2 N Protein Antigen Test

INSTRUCTIONS FOR USE

IFU 0002

Quanterix Corporation

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Customer Support

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For use under an Emergency Use Authorization (EUA) Only

Prescription Use Only

For In Vitro Diagnostic Use Only

Read this package insert prior to use. Package insert instructions must be carefully followed. Reliability of assay results cannot be assured if there are deviations from the instructions in this package insert.

NAME

Simoa® SARS-COV-2 N Protein Antigen Test

INTENDED USE

The Simoa SARS-CoV-2 N Protein Antigen Test is an automated paramagnetic microbead-based immunoassay intended for the qualitative detection of the nucleocapsid protein (N protein) antigen from SARS-CoV-2 in nasopharyngeal swab and anterior nasal swab specimens collected and frozen in Huachenyang iClean Viral Transport Medium (VTM), CDC's formulation of VTM, normal saline, or phosphate buffered saline (PBS) from individuals who are suspected of COVID-19 by their healthcare provider within 14 days for nasopharyngeal swabs and within five (5) days of symptom onset for anterior nasal swab specimens. For anterior nasal swab specimens collected and frozen in transport media only, this test is also authorized for individuals without symptoms or other epidemiological reasons to suspect COVID-19, when tested twice over two (or three) days with at least 24 hours (and no more than 36 hours) between tests. This test is also intended for qualitative detection of the nucleocapsid protein (N protein) antigen from SARS-CoV-2 in saliva specimens that have been collected and frozen from individuals who are suspected of COVID-19 by their healthcare provider within seven (7) days of symptom onset. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet the requirements to perform moderate or high complexity tests.

The Simoa SARS-CoV-2 N Protein Antigen Test does not differentiate between SARS-CoV and SARS-CoV-2.

Results are for the detection of SARS-CoV-2 nucleocapsid protein antigen. Antigen is generally detectable in nasopharyngeal and anterior nasal swab specimens and saliva specimens during the acute phase of infection. Positive results indicate the presence of viral antigens, but clinical correlation with patient history and other diagnostic information is necessary to determine infection status. Positive results do not rule out co-infection with other viruses. The antigen detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities.

Negative results should be treated as presumptive and confirmation with a molecular assay, if necessary, for patient management, may be performed. Negative results do not rule out SARS-CoV-2 infection and should not be used as the sole basis for treatment or patient management decisions, including infection control decisions. Negative results should be considered in the context of a patient's recent exposures, history and the presence of clinical signs and symptoms consistent with COVID-19.

For serial testing programs, additional confirmatory testing with a molecular test for negative results may be necessary, if there is a high likelihood of SARS-CoV-2 infection, such as an individual with a close contact with COVID-19 or with suspected exposure to COVID-19 or in communities with high prevalence of infection. Additional confirmatory testing with a molecular test for positive results may also be necessary, if there is a low likelihood of SARS-CoV-2 infection, such as in individuals without known exposures to COVID-19 or residing in communities with low prevalence of infection.

The Simoa SARS-CoV-2 N Protein Antigen Test is intended for use by trained clinical laboratory personnel specifically instructed and trained in in vitro diagnostic procedures. The Simoa SARS-CoV-2 N Protein Antigen Test is intended for use on the Simoa HD-X Analyzer. The Simoa SARS-CoV-2 N Protein Antigen Test is only for use under the Food and Drug Administration's Emergency Use Authorization.

SUMMARY AND EXPLANATION OF TEST

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is a recently identified coronavirus strain responsible for the Coronavirus Disease 2019 (COVID-19) and pandemic. SARS-CoV-2 emerged in China in December 2019 and is transmitted mainly through droplets and surface contact routes. The virus infects human cells through interaction between angiotensin converting enzyme 2 (ACE2) on respiratory cells and spike or S-protein on the outer envelope of the virion particle. COVID-19 affects people in different ways. Symptoms can include signs and symptoms of acute respiratory illness, such as fever, cough, shortness of breath, but the infection can also be asymptomatic. Symptomatic, pre-symptomatic and asymptomatic infected individuals can all be sources for viral transmission. The current gold standard for diagnosis of SARS-CoV-2 infection is real-time reverse transcription polymerase chain reaction (rRT-PCR), which detects the presence of SARS-CoV-2 nucleic acid material in upper respiratory specimens, such as nasopharyngeal swab and oropharyngeal swab. In contrast, SARS-CoV-2 protein antigen detection directly detects the presence or absence of proteins from the SARS-CoV-2 virus. One of these proteins, nucleocapsid protein (or N protein) is elevated in respiratory fluids during the initial acute phase of the infection. This N protein elevation can be used to detect a SARS-CoV-2 infection in its earliest stages, prior to the development of a significant anti-SARS-CoV-2 antibody response.[1]

PRINCIPLES OF THE PROCEDURE

The Simoa SARS-COV-2 N Protein Antigen Test is a 2-step microbead-based sandwich ELISA that uses single molecule array (Simoa) technology.[2] In the first step, anti-N protein antibody coated paramagnetic capture beads, sample, and biotinylated anti-N protein detector antibody are combined. Nucleocapsid protein molecules present in the sample are captured by the anti-N protein capture beads and labeled with biotinylated detector. After washing, a conjugate of streptavidin-ß-galactosidase (SBG) is mixed with the capture beads. SBG binds to the biotinylated detector antibodies, resulting in enzyme labeling of captured nucleocapsid protein. Following a second wash, the capture beads are resuspended in a resorufin ß-D-galactopyranoside (RGP) substrate solution for signal generation. Digital processing occurs when beads are transferred to the Simoa array disc which is composed of microarrays of femtoliter reaction wells. Individual capture beads are then sealed within microwells in the array through the addition of oil, which forms a liquid seal trapping the labeled immunocomplexes and RGP within the wells. If nucleocapsid protein from the sample has been captured and labeled, the ß-galactosidase hydrolyzes the RGP substrate into a fluorescent product that provides the signal for digital counting. The fraction of bead-containing microwells counted with an enzyme is converted into 'average enzymes/bead' (AEB). AEB values are converted into N protein concentration in unknown samples by interpolation from a calibration curve obtained by 4-parameter logistical regression fitting. Total time to first result on a single sample is 80 minutes. Time to perform 96 tests is approximately two hours and 30 minutes.

For additional information on system and assay technology, refer to the *Simoa HD-X Analyzer User Guide (EUA Edition) (USER-0105)*.

REAGENTS

Reagent Kit – Materials Provided

Simoa SARS-COV-2 N Protein Antigen Test kit

Bead Reagent	1 bottle (4.4 mL)	Anti-N protein (mouse monoclonal) antibody coated capture beads in Tris buffer with a protein stabilizer (bovine) and a surfactant. Preservative: ProClin 300.
Detector Reagent	1 bottle (4.0 mL)	Biotinylated anti-N protein (mouse monoclonal) antibody in phosphate buffer with a protein stabilizer (bovine) and a surfactant. Preservative: ProClin 300.
SBG Reagent	1 bottle (12.3 mL)	Conjugate of streptavidin-ß-galactosidase (SBG) in phosphate buffer with a protein stabilizer (bovine). Preservative: ProClin 300.
Sample Diluent	2 bottles (14.5 mL ea)	Phosphate buffer with a protein stabilizer (bovine), a heterophilic blocker, and a surfactant. Preservative: ProClin 300.
RGP Reagent	2 bottles (3.4 mL)	Resorufin ß-D-galactopyranoside (RGP) in phosphate buffer with a surfactant.
Calibrators A-H (0 plus 7 levels)	8 vials (1 mL each)	Recombinant N protein in phosphate buffer with a protein stabilizer (bovine), a surfactant. Preservative: ProClin 300.
Positive Control 1	1 vial (0.5 mL each)	Recombinant N protein in Sample Diluent.
Positive Control 2	1 vial (0.5 mL each)	Recombinant N protein in Sample Diluent.
Negative Control 3	1 vial (0.5 mL each)	Phosphate buffered saline with sodium azide as a preservative.

Materials Required but Not Provided

- Simoa HD-X Analyzer, Simoa software v3.1 (Item # 103385)
- Simoa HD-X System Buffer 1 (Item # 100486)
- Simoa HD-X System Buffer 2 (Item # 100487)
- Simoa HD-X Sealing Oil (Item # 100206)
- Simoa HD-X cuvettes (Item # 103346, 3000 ct box)
- Simoa disposable pipettor tips (Item # 101726)
- Simoa Discs (Item # 100001)
- Simoa microplate shaker (Item # 102899)
- X-Pierce™ XP-100 plate seals (K-1080, Sigma Aldrich item # Z722502)
- Simoa 96 well assay plates (Item # 101457)
- Standard nasopharyngeal or nasal swab collection kit — Users should obtain samples in accordance with the IFU procedures on sample collection
- 50 mL sterile conical screw top tube without preservatives (for saliva specimen collection)
- Sterile 1.7 mL microcentrifuge tubes
- P1000 pipet
- Sterile 1000 or 1250 µL pipet tips

WARNINGS AND PRECAUTIONS

For in vitro diagnostic and laboratory professional use. For emergency authorization use only.

- This product has not been FDA cleared or approved; but has been authorized by FDA under an Emergency Use Authorization (EUA) for use by authorized laboratories; laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet the requirements to perform moderate or high complexity tests.
- This product has been authorized only for the detection of proteins 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 Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated, or authorization is revoked sooner.
- Laboratories within the United States and its territories are required to report all results to the appropriate public health laboratories.

Safety Precautions

- **CAUTION:** This product requires the handling of human specimens. It is recommended that all human-sourced materials be considered potentially infectious and be handled in accordance with the OSHA Standard on Bloodborne Pathogens.[3] Biosafety Level 2[4] or other appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.
- **CHEMICAL AND SAFETY INFORMATION:**

  The reagents, calibrators and controls in the Simoa SARS-CoV-2 N protein Antigen Test contain the following hazardous ingredients:

Reagents	Hazards	Link to MSDS
ProClin 300	• Harmful if swallowed or inhaled. • Causes severe skin burns and eye damage. • May cause an allergic skin reaction. • Very toxic to aquatic life with long lasting effects.	https://www.sigmaaldrich.com/US/en/sds/sial/48914-u
Triton X-100	• Harmful if swallowed. • Causes skin irritation. • Causes serious eye damage. • Very toxic to aquatic life with long lasting effects.	https://www.sigmaaldrich.com/US/en/sds/sial/x100

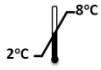
  The RGP reagent, bead reagent, detector reagent, SBG reagent, sample diluent reagent, calibrators and controls in the Simoa® SARS-CoV-2 N protein Antigen Test contain ProClin 300. Additionally, the sample diluent reagent, calibrators, and controls contain Triton X-100. If the reagent, calibrator, or controls solution contacts the skin or eye, immediately wash with plenty of running water. If irritation persists, please seek medical advice at: https://www.poison.org/contact-us or 1-800-222-1222.
- For a detailed discussion of safety precautions during instrument operation, refer to the *Simoa HD-X Analyzer User Guide (EUA Edition) (USER-0105)*.

Handling Precautions

- Proper specimen collection, storage, and transport are critical to the performance of this test. Inadequate or inappropriate specimen collection, storage, and transport may yield false negative test results.
- The reagents are one-time use; any remaining material should be discarded after the completion of assay run.
- Calibrators and controls are one-time use; any remaining material should be discarded after completion of assay run.
- Fresh RGP reagent (prepared as described in the Assay Procedure) should be prepared prior to the assay runs.
- Do not use reagent kits beyond the expiration date. When stored and handled as directed, reagents and calibrator are stable until the expiration date.
- Do not pool reagents within a kit or between reagent kits.
- Do not attempt to reuse tips, cuvettes, or Simoa Discs, as this will cause significant data quality deterioration and may result in incorrect results.
- Dispose of test components and clinical specimens in accordance with Federal, State and Local regulatory requirements. The Safety Data Sheets (SDS) for kit components are available upon request. Contact Quanterix Customer Service (1-877-786-8749).

Shipping and Storage Instructions

Simoa SARS-COV-2 N Protein Antigen Test reagents are shipped on cold packs. If the reagents arrive at room temperature or frozen, reagent integrity may be compromised. Contact Quanterix Customer Service (1-877-786-8749).

- 

Simoa SARS-COV-2 N Protein Antigen Test reagents must be stored at 2–8°C in an upright position.
- Simoa SARS-COV-2 N Protein Antigen Test Calibrators and Controls are shipped on dry ice. If these components arrive in a partially frozen or unfrozen state, their integrity may be compromised. Contact Quanterix Customer Service.
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Simoa SARS-COV-2 N Protein Antigen Test Calibrators and Controls must be stored at –80°C and should be kept upright.
- When stored and handled as directed, reagents and calibrators are stable until the expiration date.

Indications of Reagent Deterioration

If a control sample returns a concentration value out of the expected range, this may indicate deterioration of reagents or errors in technique. Associated test results may be invalid and may require retesting. Assay recalibration may be necessary. Refer to the Calibration and Quality Control Procedures section of this document.

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

Acceptable specimen types for the SARS-COV-2 N Protein Antigen Test are nasopharyngeal swab, anterior nasal swab, and saliva. It is critical that correct specimen collection and preparation methods be followed to ensure accurate results. **Note**: Only one specimen type can be tested within a single run.

Nasopharyngeal Swab Specimens

- Use sterile rayon, foam, polyester or flocked flexible-shaft NP swabs to collect a nasopharyngeal sample.
- Follow universal collection precautions and guidelines according to your organization. For specimen collection of nasopharyngeal swabs, follow the Centers for Disease Control and Prevention (CDC) Swab Collection Guidelines and swab manufacturers' recommendations.
- To ensure proper collection for nasopharyngeal specimens, the swab should be passed a distance that is halfway from the nose to the tip of the ear. This is about half the length of the swab. The swab should travel smoothly with minimal resistance; if resistance is encountered, withdraw the swab a little without taking it out of the nostril. Then elevate the back of the swab and move it forward into the nasopharynx.
- Immediately place the swab in 3 mL of transport medium and cap the specimen tube. Huachenyang iClean Viral Transport Medium, Centers for Disease Control Viral Transport Medium, normal saline, and phosphate buffered saline have been validated as transport media for the Simoa SARS-COV-2 N Protein Antigen Test.
- After collection, the specimen must be stored at -80°C until ready for testing. The stability of frozen nasopharyngeal specimens in saline has been established for up to 14 days at -80°C.
- Alternatively, specimens may be stored for up to three days at 2-8°C or four hours at 30°C prior to freezing. **Specimens must first be frozen prior to testing.**
- If necessary, samples can be frozen prior to testing by transferring a 1 mL aliquot to a sterile 1.7 mL microfuge tube appropriate for vortexing and centrifugation. Freeze at -80°C after aliquoting for a minimum of 30 minutes.
- Prior to testing, thaw specimens at room temperature.
- Thawed specimens must always be mixed THOROUGHLY by low speed vortexing or inverting 10 times. Visually inspect the mixed specimens to confirm homogeneity.
- Centrifuge all specimens prior to assay. Centrifugation conditions should be sufficient to efficiently remove small particulate matter and to clarify the sample, for example ten minutes at 3,000 g.
- Nasopharyngeal specimens in saline are stable to up to seven freeze/thaw cycles.

Anterior Nasal Swab Specimens

- Use sterile rayon, foam, polyester or flocked flexible-shaft swabs to collect an anterior nasal swab sample.
- Follow universal collection precautions and guidelines according to your organization. For specimen collection of anterior nasal swabs, follow the Centers for Disease Control and Prevention (CDC) Swab Collection Guidelines and swab manufacturers' recommendations.
- To ensure proper collection, insert the entire absorbent tip of the swab (usually ½ to ¾ of an inch (1 to 1.5 cm) inside the nostril and firmly sample the nasal wall by rotating the swab in a circular path against the nasal wall at least 4 times.
- Immediately place the swab in 3 mL of transport medium and cap the specimen tube. Huachenyang iClean Viral Transport Medium, Centers for Disease Control Viral Transport Medium, normal saline, and phosphate buffered saline have been validated as transport media for the Simoa SARS-COV-2 N Protein Antigen Test.
- After collection, the specimen must be stored at -80°C until ready for testing. The stability of frozen anterior nasal swab specimens in saline has been established for up to 14 days at -80°C.
- Alternatively, samples may be stored for up to three days at 2-8°C or four hours at 30°C prior to freezing. **Specimens must first be frozen prior to testing.**
- Prior to testing, thaw specimens at room temperature.
- Thawed specimens must always be mixed THOROUGHLY by low speed vortexing or inverting 10 times. Visually inspect the mixed specimens to confirm homogeneity.
- Centrifuge all specimens prior to assay. Centrifugation conditions should be sufficient to efficiently remove small particulate matter and to clarify the sample, for example ten minutes at 3,000 g.
- Anterior nasal swab specimens in saline are stable to up to seven freeze/thaw cycles.

Saliva Specimens

- Collect saliva samples under supervision by a trained technician.
- Saliva donor should first rinse their mouth with water, followed by a 15-minute interval before collection.
- Collect saliva obtained from repeated ejecting saliva into a sterile, leak-proof screw cap container until approximately 2 ml of liquid is collected (excluding bubbles). No preservative is required.
- After collection, the specimen must be stored at -80°C until ready for testing. The stability of frozen saliva specimens has been established for up to 14 days at -80°C.
- Alternatively, specimens may be stored for up to three days at 2-8°C or four hours at 30°C prior to freezing. Saliva specimens are stable up to seven freeze/thaw cycles. **Specimens must first be frozen prior to testing.**

Preparing Saliva Specimens for Analysis

- If specimens were stored at -80°C, prior to testing thaw specimens at room temperature.
- If specimens were stored at 2-8°C, remove the 50 mL saliva collection containers from 2-8°C storage and allow to stand at room temperature for 30 minutes to allow for settling prior to freezing at -80°C.
- Thawed specimens must always be mixed THOROUGHLY by low speed vortexing or inverting 10 times. Visually inspect the mixed specimens to confirm homogeneity.
- Centrifuge all specimens prior to assay. Centrifugation conditions should be sufficient to efficiently remove small particulate matter and to clarify the sample, for example ten minutes at 3,000 g.
- Collect 1 mL of saliva from the middle of the specimen, avoiding any bubbles or sediment at the top or bottom of the specimen. **Note:** Use of a calibrated p1000 pipette and 1000uL or 1250uL sterile pipette tips, ideally extended

length versions, is recommended. Pipetting slowly and near the side of the container can help to prevent disruption of the bottom and top of the specimen.

- Transfer the collected saliva into a sterile 1.7 mL microfuge tube appropriate for vortexing and centrifugation. Freeze at -80°C after aliquoting for a minimum of 30 minutes.
- Thaw specimens at room temperature.
- Thawed specimens must always be mixed THOROUGHLY by low speed vortexing or inverting 10 times. Visually inspect the mixed specimens to confirm homogeneity.
  Centrifuge all specimens prior to assay. Centrifugation conditions should be sufficient to efficiently remove small particulate matter and to clarify the sample, for example ten minutes at 3,000 g.

General Instructions for all Sample Types

- If specimens are to be transported prior to testing, transportation must comply with all applicable regulations for the transport of etiologic agents. Samples to be transported outside the collection facility must be transported in appropriate insulated cold shipping containers with frozen gel packs or dry ice sufficient to ensure cold or frozen conditions through the duration of shipment. Specimen stability for all sample types has been established as three days at 2-8°C and 14 days at -80°C. The receiving laboratory must confirm the integrity of the samples upon receipt by confirming the frozen state of the gel packs or the presence of dry ice and the frozen state of the specimens. Upon receipt, transfer the samples to the appropriate storage. Samples to be transported within a facility may be maintained at room temperature (≤30°C) if transport time does not exceed four hours. If longer transport times are anticipated, follow the instructions above for shipment outside the facility.
- Specimens must first be frozen at -80℃ prior to testing. Frozen samples should be thawed completely at room temperature prior to processing. Thawed specimens must always be mixed THOROUGHLY by low speed vortexing or inverting 10 times. Visually inspect the mixed specimens to confirm homogeneity.
- Centrifuge all specimens prior to assay. Centrifugation conditions should be sufficient to efficiently remove small particulate matter and to clarify the sample, for example ten minutes at 3,000 g.
- For optimal results, inspect all samples for bubbles immediately before placing on the instrument. Remove bubbles with a sterile plastic pipette tip prior to analysis. Use a new applicator stick for each sample to prevent cross-contamination.
- Use caution when handling patient specimens to prevent cross-contamination. Use of disposable pipettes or pipette tips is recommended.

PROCEDURE

Assay Procedure

- The Simoa SARS-COV-2 N Protein Antigen Test assay definition must be downloaded from the Quanterix customer portal website (portal.quanterix.com) and installed on the Simoa HD-X Analyzer prior to performing the assay. Do not open or attempt to edit the file or assay results could be compromised.
- A unique assay definition file is required for each specimen type. Select the appropriate assay definition according to the following list:
  - Nasopharyngeal swab specimens: assay definition file name CVDNAgNP
  - Anterior nasal swab specimens: assay definition file name CVDNAgAN
  - Saliva specimens: assay definition file name CVDNAgSal
- Calibrators, controls, and samples must be allowed to come to room temperature and mixed thoroughly before loading onto the Simoa HD-X Analyzer.
- Simoa reagents excluding RGP and Bead Reagent can be loaded onto the HD-X straight from refrigerated storage or after equilibration to room temperature.
- Solubilize RGP fully by heating at 30–37°C with constant vigorous shaking on the Simoa microplate shaker set to 800 rpm for a minimum of 30 minutes and a maximum of four hours. One RGP bottle is required for up to 48 tests. Two RGP bottles are required for 49-96 tests.
- Immediately before loading on the HD-X Analyzer, the Bead Reagent bottle must be mixed to resuspend the capture beads that may have settled. To resuspend the beads, vortex for a minimum of 30 seconds. Re-vortex if there is a delay of more than five minutes loading the Bead Reagent on the HD-X.
  Note: The bead diluent is formulated with an antifoam agent, but vortexing can still create foaming. If the foam does not dissipate within a few minutes, remove excess foam with a pipette prior to loading bead reagent onto the Simoa HD-X Analyzer.
- Set up the assay run on the instrument (see the *Simoa HD-X Analyzer User Guide, EUA edition) (USER-0105).*
  - Remove caps from reagent bottles and load the Simoa SARS-COV-2 N Protein Antigen Test reagents (Bead Reagent, Detector Reagent, SBG Reagent, Sample Diluent) into the reagent bay.
  - Load samples, calibrators, controls, and RGP into the sample bay. (Note: Two bottles of RGP are needed per 96-test run. One bottle of RGP may be used for a smaller run up to 48 tests.
  - In the run set up screen, specify **"neat"** protocol for calibrators and "**dilution"** protocol for controls and samples. The "dilution" protocol performs a 1:4 dilution of the controls and samples.
  - Replenish consumables and system resources as needed prior to initiating the run, as described in the *Simoa HD-X Analyzer User Guide (EUA Edition) (USER-0105).*
  - Initiate the run.
  - Upon completion of the run, generate and export the Clinical Results Report and place into the patient record as per the laboratory's standard operating procedures. Only qualitative results should be reported in the Clinical Results Report, with limitations as indicated in the Interpretation of Results Table included below.

Sample Inputs and Volumes

- The SARS-COV-2 N Protein Antigen Test was validated with Quanterix-supplied 96-well plates as the sample input. Each replicate test consumes 25 µL of sample. Multiple replicate tests of the same sample may be run from one well. The minimum sample volume in the well depends on the number of replicates to be run and the required dead volume of the well. The dead volume of a well is 30 µL, so the volume of sample to pipette for a single replicate is 55 µL. The maximum recommended sample volume for 96-well plates is 300 µL.
- If samples are left on board the instrument or pipetted samples are left on a lab bench for more than an hour, evaporation effects may influence the results depending on the volume of sample. Use plate seals to prevent evaporation. X-Pierce Sealing Films (cat # XP-100) are the only plate seals compatible with the Simoa HD-X Analyzer. When placing seals, care must be taken to center the black circular marks over all plate wells. Once seal is placed, do not tip the plate or sample well contents may wick up onto the seal and cause cross-contamination of wells.

Calibration

- To perform a SARS-COV-2 N Protein Antigen Test calibration, test Calibrators A through H in duplicate. All levels of SARS-COV-2 N Protein Antigen Test Controls should be tested in duplicate to evaluate the assay calibration. All assay control values should be within their expected concentration ranges for a valid calibration curve. Refer to the Certificate of Analysis for expected control values and ranges. If the results from one or more of the controls are outside their expected ranges, the assay calibration may not be valid, and a re-calibration is recommended.
- The HD-X Analyzer is capable of storing a calibration curve for analysis of samples on subsequent batch runs. Calibration curve storage has not been validated for the SARS-COV-2 N Protein Antigen Test.
- The SARS-COV-2 N Protein Antigen Test utilizes a 4 Parameter Logistic Curve fit data reduction method to generate a calibration curve. Specimen results are interpolated from the calibration curve.

Preparing Calibrators

- Calibrators should be brought to room temperature prior to pipetting. Do not heat the vial to accelerate thawing.

- When the solution is fully thawed, THOROUGHLY mix by multiple gentle inversions or vortexing. Frozen protein solutions can partition during freezing, so complete mixing of thawed material is critical for accurate calibrators.
- Each replicate test consumes 100 µL of calibrator. Multiple replicate tests of the same calibrator may be run from the same well. The minimum calibrator volume in the well depends on the number of replicates to be run and the required dead volume for the well. The dead volume of the well is 30 mL, so replicates of two require 230 µL of each calibrator level.

Preparing Controls

- Controls should be brought to room temperature prior to pipetting. Do not heat the vial to accelerate thawing.
- When the solution is fully thawed, THOROUGHLY mix by multiple gentle inversions or vortexing. Frozen protein solutions can partition during freezing, so complete mixing of thawed material is critical for accurate controls.

QUALITY CONTROL PROCEDURES

- Follow the specific quality control procedures in your laboratory.
- The SARS-COV-2 N Protein Antigen Test Negative Control and Positive Controls should be included in every run to assess run validity.
- Control values and ranges listed in the lot-specific Certificate of Analysis should be considered as guides. It is recommended that each laboratory establish statistically based control values and ranges with a sufficiently powered study. For guidance, it is recommended to consult with Clinical and Laboratory Standards, Institute (CLSI) Guideline C24, 4th ed., or other published guidelines for quality control recommendations.
- If quality control results do not meet acceptance criteria defined by your laboratory, sample results may be suspect. Follow the established quality control procedures for your laboratory. For troubleshooting information, refer to the *Simoa HD-X Analyzer User Guide (EUA Edition) (USER-0105)*.

Interpretation of Results

- The results are determined automatically by the HDX system by comparison to a cutoff verified in a clinical agreement study with RT-PCR. The following table provides guidance in interpreting the information displayed in the Clinical Results Report for all validated sample types following completion of the Simoa SARS-COV-2 N Protein Antigen Test.

Result Displayed	Interpretation
"Positive"	**Positive** for SARS-CoV-2 N protein antigen
"Negative"	**Negative** for SARS-CoV-2 N protein antigen
"Invalid"	Retest

An "Invalid" result indicates one or more system errors (such as an aspiration error) may have occurred during processing. Inspect the Flags field of the Clinical Results Report for additional information and address the cause of any flags where required before repeating a sample. If an "Invalid" recurs after repeat testing with recommended sample dilutions, contact Quanterix Customer Support.

Testing anterior nasal swabs from asymptomatic subjects

A negative result should be confirmed with a second test two or three days after the initial negative result with at least 24 hours (and no more than 36 hours) between tests.

Note: Negative results should be treated as presumptive and confirmation with a molecular assay, if necessary, for patient management, may be performed. For serial testing programs, additional confirmatory testing with a molecular test for negative results may be necessary after a second negative result for asymptomatic patients, if there is a high likelihood of SARS-CoV-2 infection, such in an individual with as a close contract with COVID-19 or with suspected exposure to COVID-19 or in communities with high prevalence of infection. Additional confirmatory testing with a molecular test for positive results may also be necessary, if there is a low likelihood of SARS-CoV-2 infection, such as in individuals without known exposures to SARS-CoV-2 or residing in communities with low prevalence of infection. **Note**: For a description of the messages that may appear in the Flags field, refer to the *Simoa HD-X Analyzer User Guide (EUA Edition) (USER-0105)*.

LIMITATIONS OF THE PROCEDURE

- For in vitro diagnostic use on the HD-X under Emergency Use Authorization Only. Other Quanterix instrument platforms are not authorized for diagnostic testing.
- This test will indicate the presence of SARS-CoV-2 nucleocapsid protein antigen in the specimen from both viable and non-viable SARS-CoV-2 virus. Test performance depends on the amount of virus (antigen) in the sample and may or may not correlate with viral culture results performed on the same sample.
- Failure to follow the instructions for use may adversely affect test performance and/or invalidate the test result.
- Test results should be considered in the context of all available clinical and diagnostic information, including patient history and other test results.
- Positive test results do not differentiate between SARS-CoV and SARS-CoV-2.
- Positive test results do not rule out co-infection with other pathogens.
- Negative test results are not intended to rule in other non-SARS viral or bacterial infections.
- Negative results should be treated as presumptive and confirmation with a molecular assay, if necessary, for patient management, may be performed.
- A negative test result may occur if the level of antigen in a sample is below the detection limit of the test or if the sample was collected or transported improperly.
- The amount of antigen in a sample may decrease as the duration of illness increases. Specimens collected after six days are more likely to be negative compared to RT-PCR.
- If the differentiation of specific SARS viruses and strains is needed, additional testing, in consultation with state or local public health departments, is required.
- Performance has only been established with frozen nasopharyngeal, anterior nasal and saliva specimens. Other specimen types and fresh specimens have not been evaluated and should not be used with this assay.
- For specimen transport/shipping prior to testing, stability has been established for all sample types as four hours at 30°C, three days 2-8°C, and two weeks -80°C.
- Bacterial contamination of specimens may affect the test results.
- Therapeutic doses of biotin can interfere with assays that utilize biotinylated reagents.
- This test is only indicated for screening asymptomatic individuals in the context of serial testing when testing anterior nasal swab specimens.
- The performance of this test does not support screening for use with saliva. Saliva specimens are only indicated for symptomatic individuals.
- The performance of fresh nasopharyngeal, anterior nasal and saliva specimens has not been established. Only specimens that have been frozen and thawed should be tested.
- If a negative result is obtained with a saliva specimen and COVID-19 is still suspected based on exposure history together with other clinical findings, testing an alternative specimen type should be considered by healthcare providers in consultation with public health authorities.
- The performance of this test was established based on the evaluation of a limited number of clinical specimens collected between 07/10/2020 and 01/29/2021. The clinical performance has not been established in all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.

Conditions of Authorization for the Laboratory

The Simoa SARS-COV-2 N Protein Antigen Test 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/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/in-vitro-diagnostics-euas.

Authorized laboratories* using the Simoa SARS-COV-2 N Protein Antigen Test, must adhere to the Conditions of Authorization indicated in the Letter of Authorization as listed below:

- Authorized laboratories using the Simoa SARS-COV-2 N Protein Antigen Test 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 Simoa SARS-COV-2 N Protein Antigen Test must use the Simoa SARS-COV-2 N Protein Antigen Test as outlined in the Instructions for Use. 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 the Simoa SARS-COV-2 N Protein Antigen Test are not permitted.
- Authorized laboratories that receive the Simoa SARS-COV-2 N Protein Antigen Test must notify the relevant public health authorities of their intent to run the Simoa SARS-COV-2 N Protein Antigen Test prior to initiating testing.
- Authorized laboratories using the SARS-COV-2 N Protein Antigen Test 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 Simoa SARS-COV-2 N Protein Antigen Test and report to DMD/OHT7-OIR/OPEQ/ CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and Quanterix Corporation (atcustomerservice@quanterix.com) any suspected occurrence of false reactive or false non-reactive results and significant deviations from the established performance characteristics of the Simoa SARS-COV-2 N Protein Antigen Test of which they become aware.
- All laboratory personnel using the Simoa SARS-COV-2 N Protein Antigen Test must be appropriately trained in in performing and interpreting the results laboratory and personal protective equipment when handling this kit and use the product in accordance with the authorized labeling.
- Quanterix Corporation, authorized distributors, and authorized laboratories using the Simoa SARS-COV-2 N Protein Antigen Test must ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

* 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 moderate and high complexity tests" as "authorized laboratories."

PERFORMANCE CHARACTERISTICS

Limit of Detection (Analytical Sensitivity)

Nasopharyngeal and anterior nasal swab specimens

The Limit of Detection (LoD) was determined by evaluating different dilutions of gamma-inactivated SARS-CoV-2 virus eluted from swab tips into viral transport medium. The LoD range was first estimated by assaying a 2-fold series of seven dilutions of virus. In a second range finding step, a smaller series of six dilutions near the LoD estimated from the previous range finding was tested. The LoD was then confirmed with five additional dilutions tested in replicates of 20. The LoD is defined as the lowest concentration where at least 95% of the replicates (19/20) from this dilution series read positive relative to the clinical cutoff. The results of this final dilution series are summarized in the table below.

	Dilution 1	Dilution 2	Dilution 3	Dilution 4	Dilution 5
Number positive replicates/total	20/20	20/20	20/20	20/20	17/20
$TCID_{50}$/ml	0.44	0.37	0.33	0.29	0.26

The LoD in nasopharyngeal and anterior nasal swab specimens was confirmed as 0.29 $TCID_{50}$/mL.

Saliva specimens

The LoD was determined by evaluating different dilutions of gamma-inactivated SARS-CoV-2 virus spiked into pooled negative saliva. The LoD range was first estimated by assaying a 2-fold series of seven dilutions of virus. In a second range finding step, a smaller series of five dilutions near the LoD estimated from the previous range finding was tested. The LoD was then confirmed with seven additional dilutions tested in replicates of 20. The LoD is defined as the lowest concentration where at least 95% of the replicates (19/20) from this dilution series read positive relative to the clinical cutoff. The results of this final dilution series are summarized in the table below.

	Dilution 1	Dilution 2	Dilution 3	Dilution 4	Dilution 5	Dilution 6	Dilution 7
Number positive replicates/total	20/20	20/20	19/20	15/20	14/20	6/20	2/20
$TCID_{50}$/ml	0.22	0.19	0.16	0.14	0.12	0.11	0.10

The LoD in saliva specimens was confirmed as 0.16 $TCID_{50}$/mL.

Cross Reactivity and Microbial Interference

Nasopharyngeal and anterior nasal swab specimens

Cross reactivity and potential interference were evaluated by testing 30 commensal and pathogenic microorganisms (16 viruses, 12 bacteria, two fungi and pooled human nasal fluid) that may be present in the nasal cavity. Each of the organisms, viruses, and fungi were tested in triplicate in the absence or presence of gamma-inactivated SARS-CoV-2 virus in a pool of eluates from PCR-confirmed negative NP swabs. No cross-reactivity or interference was seen with the microorganisms presented in the table below with the exception of SARS-Coronavirus. When tested at a concentration of 1.0 E+05 CFU/mL in negative matrix, SARS-Coronavirus gave a positive result.

Potential Cross-Reactant		Test Concentration
Virus	Enterovirus Type 68 (2007 Isolate)	3.78E+05 $TCID_{50}$/mL
	Adenovirus Type 07 (Species B)	3.53E+04 $TCID_{50}$/mL
	Human Metapneumovirus 16 Type A1	9.50E+05 $TCID_{50}$/mL
	Influenza A H1N1 (New Cal/20/99)	2.88E+06 $TCID_{50}$/mL
	Influenza B (Florida/02/06)	3.53E+04 $TCID_{50}$/mL
	Parainfluenza Virus Type 1	2.28E+08 $TCID_{50}$/mL
	Parainfluenza Virus Type 2	2.88E+06 $TCID_{50}$/mL
	Parainfluenza Virus Type 3	1.65E+06 $TCID_{50}$/mL
	Parainfluenza Virus Type 4A	7.05E+06 $TCID_{50}$/mL
	Respiratory Syncytial Virus Type A	9.50E+05 $TCID_{50}$/mL
	Rhinovirus Type 1A	8.88E+04 $TCID_{50}$/mL
	Coronavirus (Strain: 229E)	1.04E+05 $TCID_{50}$/mL
	Coronavirus (Strain: OC43)	2.63E+05 $TCID_{50}$/mL
	Coronavirus (Strain: NL63)	4.25E+04 $TCID_{50}$/mL
	MERS-Coronavirus (Strain: FL/USA-2 Saudi 2014)	1.0 E+05 $TCID_{50}$/mL
	SARS-Coronavirus	1.0 E+05 PFU/mL
Bacteria	Bordetella pertussis A639	1.13E+09 CFU/mL
	Legionella pneumophila Philadelphia	1.88E+09 CFU/mL
	Mycobacterium tuberculosis H37Ra-1	6.86E+06 CFU/mL
	Mycoplasma pneumoniae M129	3.16E+07 CFU/mL
	Pseudomonas aeruginosa	8.44E+08 CFU/mL
	Streptococcus pyogenes Z018	1.64E+08 CFU/mL
	Streptococcus salivarius Z127	4.19E+07 CFU/mL
	Chlamydia pneumoniae (TWAR Strain)	1.49E-01 mg/mL
	Haemophilus influenzae type b	5.43E+06 CFU/mL
	Streptococcus pneumoniae 19F	2.26E+07 CFU/mL
	Staphylococcus aureus	1.48E+06 CFU/mL
	Staphylococcus epidermidis MRSE	1.21E+09 CFU/mL
	Pooled Human Nasal Fluid	15% v/v
Fungus	Candida albicans Z006	6.27E+07 CFU/mL
	Pneumocystis jiroveci	3.45E+07 CFU/mL

Saliva specimens

Cross reactivity and potential interference were evaluated by testing 12 commensal and pathogenic microorganisms that may be present in the oral cavity. Each of the organisms was tested in triplicate in the absence or presence of gamma-inactivated SARS-CoV-2 virus in pools of human saliva. No cross-reactivity or interference was seen with the microorganisms presented in the table below with the exception of SARS-Coronavirus. When tested at a concentration of 1.0 E+05 PFU/mL in negative matrix, SARS-Coronavirus gave a positive result.

Potential Cross-Reactant	Test Concentration
Irradiated SARS-CoV	1.00E+05 PFU/mL
Herpes Simplex Virus - 1 (HSV-1)	1.41E+04 TCID50/mL
Epstein-Barr Virus (EBV)	1.03E+06 CP/mL
Moraxella catarrhalis	1.00E+06 CFU/mL
Porphyromonas gingivalis	10% v/v
Prevotella oralis	10% v/v
Streptococcus oralis	1.00E+06 CFU/mL
Nocardia asteroides	1.00E+06 CFU/mL
Streptococcus mutans	1.00E+06 CFU/mL
Streptococcus mitis	1.00E+06 CFU/mL
Eikenella corrodens	10% v/v
Neisseria mucosa	1.00E+06 CFU/mL

To estimate the likelihood of cross-reactivity with SARS-CoV-2 of organisms that were not available for wet testing, in silico analysis using the Basic Local Alignment Search Tool (BLAST) managed by the NCBI was used to assess the degree of protein sequence homology.

- No protein sequence homology was found between *M. tuberculosis* and *P. jirovecii*, however, homology-based cross-reactivity cannot be ruled out.
- Homology for Coronavirus-HKU1 and MERS-Coronavirus was 39.1% and 50.3% across 76% and 88% of the sequence respectively. Cross-reactivity of the assay with Coronavirus-HKU1 and MERS-Coronavirus cannot be ruled out. For reference, sequence homology with SARS-Coronavirus was 90.5% across 100% of the sequence, leading to the cross reactivity observed in the wet testing described in the tables above.

Genetic Variants of Interest and Concern

Genetic variants of SARS-CoV-2 were evaluated for reactivity in the assay. The variants evaluated were B.1.1.7 (Alpha), B.1.2, B.1.351 (Beta), B.1.375, B.1.427 (Epsilon), B.1.429, B.1.525, B.1.526, P1 (Gamma), B.1.617.2 (Delta) and P2. Alpha, Beta, Gamma, and Delta are considered Variants of Concern (VOC), while the remaining variants are considered Variants of Interest (VOI). For Alpha, Beta, Gamma, and the Variants of Interest, the evaluation was performed using individual panels, each containing one of the variants. Panels were prepared according to the NIH RADx Variant Task Force protocol. Each VOC/VOI panel was prepared with 6-10 heat inactivated patient samples. Serial dilutions of each panel were made to simulate a range of viral loads as determined by a RT-PCR assay based on the CDC EUA primer/probe set (cycle thresholds from ~20 – 35). Gamma irradiated wild type virus (BEI NR-52287, Lot 70035888) served as the control antigen. Across the variants, comparable results to wild type virus were obtained for positive and negative panels. The data indicated that the Simoa SARS-COV-2 N Protein Antigen Test detected the variants used in the VOC/VOI panels at a similar sensitivity level as wild type virus. For Delta variant testing, a sequence-confirmed single clinical nasopharyngeal swab sample was diluted 1:10 and tested with the Simoa SARS-CoV-2 N Protein Antigen test, and by RT-PCR using the CDC EUA primer/probe set. This sample yielded a strongly positive result in the Simoa test, and a Ct value of 25.3 by RT-PCR. **Note**: due to the genetic diversity of the variants tested, it is not possible to conclude that every sub strain can be detected by the assay.

High Dose Hook Effect

High dose hook was evaluated with gamma-inactivated SARS-CoV-2 virus added to an eluate pool from negative NP swabs. No high dose hook effect was observed with increasing antigen concentration across the optical range of the instrument (to 137 $TCID_{50}$/mL). The highest concentration of SARS-CoV-2 virus tested to yield a positive qualitative result was 2.8x10^5 $TCID_{50}$/mL

Endogenous Interference

Nasopharyngeal and anterior nasal swab specimens

Potentially interfering substances, naturally present in respiratory specimens or that may be artificially introduced into the nasal cavity or nasopharynx, were evaluated in the absence or presence of gamma-inactivated SARS-CoV-2 virus in an eluate pool from negative NP swabs. The substances listed in the table below were found not to affect test performance.

Substance	Active Ingredient	Test Concentration
Afrin – nasal spray	Oxymetazoline	15%
Alkalol Allergy Relief	N/A	1:10 dilution
Blood (human)	Blood	5%
Chloraseptic	Benzocaine, Menthol	1.5 mg/mL
CVS Nasal Spray	Phenylephrine	15%
Flonase	Fluticasone	5%
Halls Relief Cherry Flavor	Menthol	0.8 g/mL
Homeopathic (Alkalol Nasal Wash)	N/A	1:10 dilution
Mupirocin	Mupirocin	10 mg/mL
Nasacort Allergy 24 hour	Triamcinolone	5%
NasalCrom Nasal Spray	Cromolyn sodium	15%
Naso GEL (NeilMed)	N/A	5%
Neo-Synephrine	Phenylephrine hydrochloride	5%
Oseltamivir	Oseltamivir	2.2 µg/mL
Purified mucin protein	Mucin protein	2.5 mg/mL
Rhinocort	Budesonide (Glucocorticoid)	5%
Saline nasal spray	Saline	15%
Sore Throat Phenol Spray	Phenol	15%
Tobramycin	Tobramycin	2.5 mg/mL
Zanamivir	Zanamivir	400 ng/mL
Zicam Cold Remedy	Galphimia glauca, Luffa operculata, Sabadilla	5%
Zicam Oral Mist	Zinc	5%

Saliva specimens

Potentially interfering substances, naturally present in saliva specimens or that may be artificially introduced into the oral cavity were evaluated in the absence or presence of gamma-inactivated SARS-CoV-2 virus in pools of human saliva. The substances listed in the table below were found not to affect test performance.

Description	Active Ingredient	Test Concentration
human Leukocytes	N/A	5E+06 cells/mL
Alcohol	Alcohol	5% v/v
Cepacol Lozenges	Benzocaine, Menthol	3mg/mL
Sore Throat & Cough Lozenges	Benzocaine, Dextromethorphan HBr	3mg/mL
Robitussin	Dextromethorphan HBr, Guaifenesin	5% v/v
Chloraseptic Sore Throat Spray	Phenol, Glycerin	5% v/v
Emergen-C	Zinc, Magnesium, Riboflavin, Vitamin C	5mg/mL
Zicam Oral Mist	Zinc	5% v/v
Listerine Mouth Wash	Eucalyptol, menthol, Methyl Salicylate, Thymol	5% v/v
Act dry mouth lozenges	Isomalt, xylitol, Glycerin	3mg/mL
Toothpaste (Colgate)	N/A	0.5% v/v
Nyquil	Acetaminophen, Doxylamine succinate, Dextromethorphan	5% v/v
Nicotine	Nicotine	0.03mg/mL
Mucin	Mucin	2.5mg/mL

Biotin Interference

Biotin was added to pools of VTM eluants from negative NP swabs at test concentrations ranging from 412.5 to 3500 ng/mL. Three positive pools were prepared by adding inactivated SARS-CoV-2 virus to levels 2-3X the clinical cutoff. The qualitative results from the negative and positive pools were unaffected by the added biotin. One of the 3 positive pools exhibited +18% bias in numerical assay results at biotin concentrations above 1750 ng/mL.

Clinical Performance

Nasopharyngeal swab specimens

- *Retrospective Study*. The clinical performance characteristics of the Simoa SARS-CoV-2 N Protein Antigen Test were evaluated in a single-site retrospective study conducted in the U.S. The study evaluated 126 nasopharyngeal swab specimens in viral transport medium from patients suspected of SARS-CoV-2 infection within 14 days of symptom onset. Specimens were transported, stored frozen, and tested following validated specimen stability information in the instructions for use. The table below exhibits the positive and negative percent agreement between the Simoa antigen test and matched results from a high sensitivity EUA RT-PCR.

Simoa SARS-CoV-2 N Protein Antigen Test	Comparator method (RT-PCR)		
	Positive	Negative	Total
Positive	86	0	87
Negative	2	38	39
Total	88	38	126
Positive Agreement: 86/88 **97.7%** (95% CI: 92.03% - 99.72%)			
Negative Agreement: 38/38 **100%** (95% CI: 90.75% - 100.0%)			

- Patient demographics (gender, age, time elapsed since onset of symptoms) are available for the 126 samples used in the analysis. The table below shows the positive results by age.

Age	Simoa SARS-Cov-2 N Protein Antigen (n=126)		
	Total #	Positive	Prevalence
≤ 5 years	0	0	0 %
6 to 21 years	4	3	75.0 %
22 to 59 years	69	55	79.7 %
≥ 60 years	53	30	54.7 %

- The table below shows positive results from days since symptom onset.

Days since symptom onset	Cumulative RT-PCR positives	Cumulative Simoa antigen positives	PPA
0	8	8	100%
1	23	22	93.3%
2	34	33	100%
3	44	43	100%
4	53	52	100%
5	57	56	100%
6	61	60	100%
7	69	68	100%
8 to 14	88	86	94.7%

- *Prospective Study*. The clinical performance characteristics of the Simoa SARS-CoV-2 N Protein Antigen Test were evaluated in a single-site post-market prospective study conducted in the U.S. The study evaluated 898 nasopharyngeal swab specimens in viral transport medium from patients suspected of SARS-CoV-2 infection within 14 days of symptom onset. Specimens were transported, stored frozen, and tested following validated specimen stability information in the instructions for use. The table below exhibits the positive and negative percent agreement between the Simoa antigen test and matched results from a high sensitivity EUA RT-PCR.

Simoa SARS-CoV-2 N Protein Antigen Test	Comparator method (RT-PCR)		
	Positive	Negative	Total
Positive	26	1	27
Negative	5*	866	871
Total	31	867	898
Positive Agreement: 26/31 **83.9%** (95% CI: 66.27%, 94.55%)			
Negative Agreement: 866/867 **99.9%** (95% CI: 99.36%, 100.0%)			

*RT-PCR results for two of these samples were negative for one of two gene targets.

- Patient demographics (gender & age) are available for the 898 samples used in the analysis. The table below shows the positive results by age.

Age	Simoa SARS-Cov-2 N Protein Antigen (n=898)		
	Total #	Positive	Prevalence
≤ 5 years	3	1	33%
6 to 21 years	33	1	3%
22 to 59 years	469	19	4%
≥ 60 years	393	10	3%

- The table below shows positive results from days since symptom onset.

Days since symptom onset	Cumulative RT-PCR positives	Cumulative Simoa antigen positives	PPA	Cumulative PPA
0	4	1	25.0%	25.0%
1	8	5	100.0%	62.5%
2	11	8	100.0%	72.7%
3	15	12	100.0%	80.0%
4	17	14	100.0%	82.4%
5	19	16	100.0%	84.2%
6	22	19	100.0%	86.4%
7	25	22	100.0%	88.0%
8-14 days	31	26	66.7%	83.9%

Anterior nasal swab specimens

The clinical performance characteristics of the Simoa SARS-CoV-2 N Protein Antigen Test with anterior nasal swabs was evaluated in a single-site prospective study conducted in the U.S. The comparator method was a high sensitivity EUA RT-PCR assaying matched nasopharyngeal swab specimens. The study evaluated 175 anterior nasal swab specimens in viral transport medium from patients suspected of SARS-CoV-2 infection within five days of symptom onset. Specimens were transported, stored frozen, and tested following specimen stability information in the instructions for use. The table below exhibits the positive and negative percent agreement between the Simoa antigen test results from the anterior nasal swab specimens and the RT-PCR results with matched nasopharyngeal swab specimens.

Simoa SARS-CoV-2 N Protein Antigen Test	Comparator method (RT-PCR)		
	Positive	Negative	Total
Positive	31	0	31
Negative	4*	140	144
Total	35	140	175
Positive Agreement: 31/35 **88.6%** (95% CI: 73.26%, 96.80%)			
Negative Agreement: 131/131 **100%** (95% CI: 97.40%, 100.0%)			

*RT-PCR results for two of these samples were negative for one of two gene targets.

- Patient demographics (gender, age, time elapsed since onset of symptoms) are available for the 175 samples used in the analysis. The table below shows the positive results by age.

Age	Simoa SARS-Cov-2 N Protein Antigen (n=175)		
	Total #	Positive	Prevalence
≤ 5 years	1	0	0.0%
6 to 21 years	11	1	9.1%
22 to 59 years	142	31	21.8%
≥ 60 years	21	3	14.3%

- The table below shows positive results from days since symptom onset.

Days since symptom onset	Cumulative RT-PCR positives	Cumulative Simoa antigen positives	PPA	Cumulative PPA
0	0	0	N/A	N/A
1	10	8	80.0%	80.0%
2	20	18	100.0%	90.0%
3	29	25	77.8%	86.2%
4	33	29	100.0%	87.9%
5	35	31	100.0%	88.6%
6	38	33	66.7%	86.8%
7	45	38	71.4%	84.4%

Saliva specimens

- The clinical performance characteristics of the Simoa SARS-CoV-2 N Protein Antigen Test with saliva specimens was evaluated in a single-site prospective study conducted in the U.S. The comparator method was a high sensitivity EUA RT-PCR assaying matched nasopharyngeal swab specimens. The study

evaluated 201 saliva specimens from patients suspected of SARS-CoV-2 infection within seven days of symptom onset. Specimens were transported, stored frozen, and tested following specimen stability information in the instructions for use. The table below exhibits the positive and negative percent agreement between the Simoa antigen test results from the saliva specimens and the RT-PCR results with matched nasopharyngeal swab specimens.

Simoa SARS-CoV-2 N Protein Antigen Test	Comparator method (RT-PCR)		
	Positive	Negative	Total
Positive	37	3	40
Negative	7*	154	161
Total	44	157	201
Positive Agreement: 37/44 **84.1%** (95% CI: 69.93%, 93.36%)			
Negative Agreement: 154/157 **98.1%** (95% CI: 94.80%, 99.60%)			

*RT-PCR results for two of these samples were negative for one of two gene targets.

- Patient demographics (gender, age, time elapsed since onset of symptoms) are available for the 201 samples used in the analysis. The table below shows the positive results by age.

Age	Simoa SARS-Cov-2 N Protein Antigen (n=201)		
	Total #	Positive	Prevalence
< 5 years	1	0	0.0%
6 to 21 years	11	1	9.1%
22 to 59 years	160	36	22.5%
> 60 years	29	7	24.1%

- The table below shows positive results from days since symptom onset.

Days since symptom onset	Cumulative RT-PCR positives	Cumulative Simoa antigen positives	PPA	Cumulative PPA
0	0	0	N/A	N/A
1	10	7	70.0%	70.0%
2	19	16	100.0%	84.2%
3	28	23	77.8%	82.1%
4	32	27	100.0%	84.4%
5	34	28	50.0%	82.4%
6	37	30	66.7%	81.1%
7	44	37	100.0%	84.1%

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Assistance:

For additional information on Simoa technology, instrument operation maintenance, and data analysis, refer to the *Simoa HD-X Analyzer User Guide (EUA Edition) (USER-0105).*

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In the USA, this product has not been FDA cleared or approved; but has been authorized by FDA under an EUA for use by authorized laboratories; use by laboratories certified under the CLIA, 42 U.S.C. §263a, that meet requirements to perform moderate or high complexity tests.

This product has been authorized only for the detection of proteins from SARS-CoV-2, not for any other viruses or pathogens; and in the USA, the emergency use of this product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of the virus that causes COVID-19 under Section 564(b)(1) of the Federal Food, Drug and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless declaration is terminated or the authorization is revoked sooner.