Simoa® SARS-CoV-2 N Protein Antigen Test

INSTRUCTIONS FOR USE

IFU-0002

Version 11.0

Quanterix Corporation

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Billerica, MA 01821

Customer Support

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Techsupport@quanterix.com

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 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 of symptom onset for nasopharyngeal swabs and within five days of symptom onset for anterior nasal swab specimens. 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.

***Note**: This test has been validated for use with anterior nares specimens, but FDA's independent review of this validation is pending, and the test is being distributed in accordance with the Policy for Coronavirus Disease-2019 Tests During the Public Health Emergency, Section IV.C.

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 swab 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.

The Simoa SARS-CoV-2 N Protein Antigen Test is intended for use by trained clinical laboratory personnel specifically instructed and trained 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

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 laboratories certified under the CLIA 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.
- This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostic tests for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated, or authorization is revoked sooner.

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.
- Simoa reagents contain methylisothiazolones, which are components of ProClin and are classified per applicable European Community (EC) Directives as: Irritant (Xi). The following are the appropriate Risk (R) and Safety (S) phrases.

R43 May cause sensitization by skin contact.

S24 Avoid contact with skin.

S35 This material and its container must be disposed of in a safe way.

S37 Wear suitable gloves.

S46 If swallowed, seek medical advice immediately and show this container or label.

For a detailed discussion of safety precautions during instrument operation, refer to the S*imoa 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 used with every run.
- 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 suspect. Contact Quanterix Customer Service (1-877-786-8749).

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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 suspect. 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.
- Total time for RGP reagent on board the instrument should be limited to no more than eight hours.

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 and anterior nasal swab. 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, store the specimen at 2-8°C until ready for testing.
- Stability of nasopharyngeal specimens in saline has been established for up to 4 hours at 30°C, 3 days at 2-8°C, and 14 days at -80°C. 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, store the specimen at 2-8°C until ready for testing.
- Stability of anterior nasal swab specimens in saline has been established for up to four hours at 30°C, three days at 2-8°C, and 14 days at -80°C. Anterior nasal specimens in saline are stable to up to seven freeze/thaw cycles.

General Instructions for Both 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 insulated cold shipping containers with frozen gel packs sufficient to ensure cold conditions through the duration of shipment. The receiving laboratory must confirm the integrity of the samples upon receipt by confirming the frozen state of the gel packs. Upon receipt, transfer the samples to 2-8°C 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.
- If samples are frozen, thaw 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 thawed specimens prior to assay. Centrifugation conditions should be sufficient to remove small particulate matter and to clarify the sample, for example five minutes at 3,000 g.
- For optimal results, inspect all samples for bubbles immediately before placing on the instrument. Remove bubbles with an applicator stick 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.
- Specimens with obvious microbial contamination should not be used.

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
- 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 multiple smaller runs up to 48 tests, but total time on board the instrument should be limited to no more than eight hours.
  - In the run set up screen, specify **"neat"** protocol for calibrators and "**dilution"** protocol for controls and samples. The "dilution" protocol performed 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 of plate seals is recommended 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

Nasopharyngeal and anterior nasal swab specimens

- The assay clinical cutoff for the presence of N protein in nasopharyngeal and anterior nasal swab specimens is 3.0 (arbitrary units). The following table provides guidance in interpreting the information displayed in the Clinical Results Report following completion of the Simoa SARS-COV-2 N Protein Antigen Test. (Note: 'AEB' refers to 'average enzymes/bead,' the digital signal of the assay).

#	Result Displayed	Reason for Result	Interpretation
1	Numerical result <3.0	Undetectable SARS-CoV-2 N protein antigen. AEB within calibration range.	**Negative** for SARS-CoV-2 N protein antigen.
2	Numerical result ≥3.0	SARS-CoV-2 N protein antigen detected. AEB within calibration range.	**Positive** for SARS-CoV-2 N protein antigen.
3	"Undetectable"	AEB below calibration range.	**Negative** for SARS-CoV-2 N protein antigen.
4	"Positive, out of range"	AEB above calibration range.	**Positive** for SARS-CoV-2 N protein antigen.
5	"Positive, too much fluor"	Fluorescence exceeds optical range.	**Positive** for SARS-CoV-2 N protein antigen.
6	"NaN" (Not a Number)	Invalid.	Retest

Non-numerical result scenarios #3-5 are valid for determining the presence or absence of viral antigens in the sample. "NaN" results indicate one or more system errors (such as an aspiration error) 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 non-numerical results recur after repeat testing with recommended sample dilutions, contact Quanterix Customer Support.

Note: For both sample types, only the qualitative results from the Interpretation table should be reported. Semi-quantitative numerical results have not been clinically or analytically validated and may not correlate with patient disease status, duration of illness or severity of illness. Semi-quantitative results have not been demonstrated to correlate with the success or failure of any therapeutic interventions and should not be used to guide clinical management. **Note:** Results of this assay should always be interpreted in conjunction with the patient's medical history, clinical presentation, and other findings.

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.
- Only qualitative results should be reported. Semi-quantitative numerical results have not been clinically or analytically validated and may not correlate with patient disease status, duration of illness or severity of illness. Semi-quantitative results have not been demonstrated to correlate with the success or failure of any therapeutic interventions and should not be used to guide clinical management.
- 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.

- 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 nasopharyngeal specimens. Other specimen types have not been evaluated and should not be used with this assay.
- Bacterial contamination of specimens may affect the test results.
- Therapeutic doses of biotin can interfere with assays that utilize biotinylated reagents.

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/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 (at customerservice@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.

Cross Reactivity and Microbial Interference

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 an assay signal equivalent to 57.1 units.

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

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* thus homology-based cross-reactivity can 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 table above.

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

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%

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

- The clinical performance characteristics of the Simoa SARS-CoV-2 N Protein Antigen Test was 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 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%

Anterior nasal swab specimens

The clinical performance characteristics of the Simoa SARS-CoV-2 N Protein Antigen Test with 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 166 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 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 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*	131	135
Total	35	131	166
Positive Agreement: 31/35 **88.6%** (95% CI: 73.26% - 96.80%)			
Negative Agreement: 131/131 **100%** (95% CI: 97.22% - 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 166 samples used in the analysis. The table below shows the positive results by age.

Age	Simoa SARS-Cov-2 N Protein Antigen (n=166)		
	Total #	Positive	Prevalence
≤ 5 years	1	0	0.0%
6 to 21 years	11	1	9.1%
22 to 59 years	134	31	23.1%
≥ 60 years	20	3	15.0%

- 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	95% CI
0	0	0	N/A	N/A	N/A
1	10	8	80.0%	80.0%	44.4% - 97.5%
2	20	18	100%	90.0%	68.3% - 98.8%
3	29	25	77.8%	86.2%	68.3% - 96.1%
4	33	29	100%	87.9%	71.8% - 96.6%
5	35	31	100%	88.6%	73.3% - 96.8%
6	38	33	66.7%	86.8%	71.9% - 95.6%
7	43	37	80.0%	86.0%	72.1% - 94.7%

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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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