Simoa® SARS-CoV-2 N Protein Antigen Test
Quick Reference Instructions

For use under an Emergency Use Authorization (EUA) Only.

Prescription Use Only. For In Vitro Diagnostic Use Only.

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 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.

Study the HD-X Analyzer User Guide and Simoa SARS-CoV-2 N Protein Antigen Test Instructions for Use thoroughly before using these Quick Reference Instructions or performing a test. This is not a complete product insert. Refer to the Simoa SARS-CoV-2 N Protein Antigen Test Instructions for Use for Warnings, Precautions and Limitations.

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.

EMERGENCY USE AUTHORIZATION – WARNING AND PRECAUTIONS

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. In the USA, - this test 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 the declaration is terminated or authorization is revoked sooner.

Please see the Simoa SARS-CoV-2 N Protein Antigen Test Instructions for Use for the full instructions for use, including Limitations, Warnings and Precautions

(https://www.quanterix.com/wp-content/uploads/2021/01/Simoa_SARS-Cov2_N_Protein_Antigen_Test_Instructions_for_Use.pdf).

1. Prepare Instrument

Note: The system must be shut down once every 24 hours.

1. **Turn on the HD-X Analyzer:** Turn on the PC > Start the HD-X Analyzer (switch on the right side of instrument) > Open the Simoa® software. When instrument **initialization** is complete the system will say **Ready**.
2. **Pre-Run Maintenance**: **Maintenance** Tab **>** Check the **Start of Day** Task > click **Run Task** (about 20 minutes).

Note: If Start of Day Task was performed, but system has been idle for over 4 hours, run **Idle System Prime.**

3. **Adjust the Lot-Specific Calibrator Concentrations:** For each new lot of kits to be used, in the **Custom Assay** Tab > select the SARS-CoV-2 N Protein Antigen Assay > deselect **Read Only** > select **Plexes** > select the **SARS-CoV-2 N Protein Antigen Plex** > Modify the concentration value for each calibrator level according to the lot-specific CoA.

2. Set Up Assay

1. **Solubilize RGP:** Place RGP vial(s) on a temperature-controlled shaker set at 30°C for ≥30 minutes at a shaking speed of 800 rpm. One vial is sufficient for up to 48 tests. Do not shake for > 4 hours prior to use. Do not re-use RGP vials.
2. **Prepare Samples:** Equilibrate Calibrators, Controls, and Specimens to room temperature, then mix thoroughly by low speed vortexing or inverting 10 times. Visually inspect the specimens to confirm homogeneity. Centrifuge samples at 10,000g for 5 minutes to clear any debris.
3. **Determine volume required per well:** Each well of the assay plate requires 30 uL dead volume + the volume consumed during testing. Calibrators are run neat, and 100 uL is consumed per test. Controls and Specimens are run with an on-board 4X dilution, and 25 uL is consumed per test.
4. **Prepare Assay Plate:** Pipette required volumes of calibrators, controls and specimens into a Simoa 96-well microplate. Cover with an X-Pierce XP-100 sealing film.

Table 1: *Sample volumes required. A minimum of two replicates should be run for calibrators and controls.*

Replicates	Calibrators No Dilution Required	Controls and Specimens 4X Dilution on Instrument
Tests/well	Minimum volume per well (uL)	Minimum volume per well (uL)
1	not recommended	55
2	230	80
3	prep in separate wells	105

3. Load Instrument and Run Assay

Note: Only the RGP reagent needs to be warmed before use. Other assay reagents can go immediately from 2 – 8 ˚C storage on to the instrument.

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1. **Vortex Bead Reagent:** Vortex Bead Reagent for about 30 seconds immediately before loading. If beads are not loaded within 5 minutes, vortex again.
2. **Load Reagents:**

   a. Load Bead, Sample Diluent, Detector and SBG reagents into Rack: Remove all caps. Make sure beads are in one of the three shaking positions on the rack. Other reagents should be in non-shaking positions. Position bottles so barcodes are readable on the right side of the rack.

   Select **Load Reagent** Tab > Select reagent lane > Enable the on-board barcode scanner > Insert reagent rack. Confirm that reagent barcodes have scanned correctly.

   b. Load RGP: Select an RGP lane > Make sure on-board barcode scanner is enabled > Place RGP bottle(s) in RGP rack (marked with an O) and remove cap > Insert RGP rack. Confirm RGP barcode has scanned correctly.

   c. Touch **Done Loading Reagents**.
3. **Create Plate Layout:**

   a. Setup Run Tab > Assign **Batch name** > Assign **Plate Barcode** > Click **Enter** on your keyboard.

   b. Assign Calibrators: Select **Assign Calibrators** Tab > Highlight the well containing Calibrator A > Select **CVDNAg** Assay Definition > Select Calibrator A from the **Select Calibrator** pop up > Click **Ascending/Descending** to populate the remaining calibrators in the plate column > Highlight all calibrator wells and set the **Replicates per well** to 2.

   c. Assign Samples: Select **Assign Sample** Tab > Highlight all wells that contain controls/samples > Select **CVDNAg** in the **Assay** section> Set the desired **Replicates per well**.

   d. If desired, select individual wells and enter sample IDs with the onscreen keyboard, USB keyboard, or handheld barcode scanner.

   e. When setup is complete, click on **List View** to confirm selections. Ensure dilutions are set properly for calibrators (neat), and controls/specimens (dilution). Ensure calibrator concentrations match those on the lot-specific CoA.

   f. Slide the prepared sample plate onto the plate rack with well A1 in the indicated position and the plate skirt under the catch screw. Clip plate into place. Insert plate rack, then touch **Done with Setup**.
4. **Check Liquid and Solid Consumables and Empty Waste**

   a. Load Liquid Consumables: Check the System Resources tab. If indicated by yellow or red color coding, fill the DI Water and Wash Buffer 1 secondary containers and the Wash Buffer 2 container.

   b. Load Cuvettes, Tips, and Discs, if Required:

   Load Cuvettes: Cuvettes are added by placing a full stack of 50 in the chute. Do not allow stacks to twist while loading.

   Note: After loading a stack, wait for the system to indicate **Ready** prior to loading the next stack.

   Load Tips: **System Resource** Tab > Select **Solid Resources** > Click **Unlock Drawers** > Place tip racks carefully into place and ensure they sit level > In the software, tap twice on each position where a new tip rack was loaded. The tip positions in the rack diagram turn light blue. Only load full racks of tips. Insert Drawer.

   Load Discs: **System Resource** Tab > Select **Solid Resources** > Click **Unlock Drawers** > Use the handheld scanner to scan the barcode on the wrapper > Remove blue base plate (from the old stack) from the disc pole in the drawer > Load the new stack on an empty disc pole > remove the wrapper and the top disc with the Quanterix logo. Insert Drawer.

   c. Empty Solid and Liquid Waste: If necessary, empty solid and liquid waste containers located in the system bay.

   Note: Only remove one liquid waste container at a time. Ensure the sensor has been re-inserted in the first waste container prior to removing the sensor from the second waste container.
5. **Start Run**: **System Resource** Tab **>** Select **All Resources** > Click **Start Run**. If button is not active, check for flags in **Resource Details**.
6. **Current Run Tab:** Use this screen to monitor progress of run. Run is finished when this tab reads 00:00 and status line at bottom left corner says **READY.**

4. Post-Run: Data Export, Maintenance, and Shut Down

1. **Remove Sample Plate, Reagents and RGP and dispose appropriately.** All kit reagents are single use only.
2. **Export the Clinical Results Report:** **History & Reports** Tab > Select **Reports** > Select **Clinical Results Report** > select appropriate batch name from pull down list > **Preview Report** > **Export**. Reports may be exported as .pdf files and saved to the instrument S:\ drive, a network location, or a USB. Reports should be exported promptly at the completion of each run.
3. **End of Day Maintenance:** After completing the final run of the day, navigate to **Maintenance** Tab > Check the **End of Day** task > Click **Run Task** (about 20 minutes).
4. **Shut down:** Shut down software > Turn off instrument > Shut down PC.

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5. Interpretation of Results

Prior to accepting assay results ensure that the control values are within the acceptance criteria guided by the lot-specific CoA and established by your laboratory. Results for controls and samples will be automatically corrected for the dilution applied during the run.

The assay clinical cutoff for the presence of N protein is 3.0 (arbitrary units). The following table provides guidance in interpreting the Clinical Results Report.

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

Note: Results of this assay should always be interpreted in conjunction with the patient's medical history, clinical presentation, and other findings.

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.

Negative results from patients with should be treated as presumptive and confirmation with a molecular assay, if necessary, for patient management, may be performed.

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