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

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

Prescription Use Only. For In Vitro Diagnostic Use Only.

These quick reference instructions relate to the use of the Simoa SARS-CoV-2 N Protein Antigen Test with saliva samples. 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 infection, 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.

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 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 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

•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 diagnostics 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.

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 **CVDNAgSal** Assay Definition > 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. Do not adjust any other parameters of the Assay Definition.

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 (µL)	Minimum volume per well (µL)
1	not recommended	55
2	230	80
3	prep in separate wells	105

2. Set Up Assay

1. **Solubilize RGP:** Place RGP vial(s) on a temperature-controlled shaker set at 30–37°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 Saliva**: **Specimens must first be frozen prior to testing.**
   a. If specimens were stored at -80°C, 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.

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b. 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.

3. Centrifuge all specimens for 10 minutes at 3,000g to efficiently remove small particulate matter and to clarify the sample

4. 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.

5. 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.

6. Thaw specimens at room temperature.

7. Thawed specimens must always be mixed THOROUGHLY by low speed vortexing or inverting 10 times. Visually inspect the mixed specimens to confirm homogeneity.

8. Centrifuge all specimens for 10 minutes at 3,000g to efficiently remove small particulate matter and to clarify the sample

9. Equilibrate Calibrators and Controls to room temperature, then mix thoroughly by low speed vortexing or inverting 10 times.

10. Determine volume required per well: Each well of the assay plate requires 30 µL dead volume + the volume consumed during testing. Calibrators are run neat, and 100 µL is consumed per test. Controls and Specimens are run with an on-board 4X dilution, and 25 µL is consumed per test.

11. 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.

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.

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 **CVDNAgAN** 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 **CVDNAgAN** 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.

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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.

5. 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.

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).

6. ASSISTANCE

If you have any questions regarding the use of this product or if you want to report a system problem, please contact Quanterix via email: techsupport@quanterix.com **or via phone: 1-877-786-8749.**