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The Science of Precision Health

# Simoa® HD-1/HD-X Analyzer Quick Start Guide

## 1. Prepare Instrument

**Note:** Instrument must be power-cycled once every 24 hours to permit internal systems to reinitialize.

1. **Turn on the HD-1/HD-X Analyzer:**
   Turn on the PC > Start the HD-1/HD-X Analyzer (switch on the right side of instrument) > Open the Simoa® software. The instrument **initialization** begins when the Simoa software is launched. When this step is complete (about 3 minutes), 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** (about 10 minutes).
3. **Import the Assay Definition:**
   **Custom Assay** Tab > click **Import** and select the XML file from the saved location.

## 2. Set Up Assay

1. **Prepare Calibrators:**
   Depending on the kit, calibrators may be pre-diluted or supplied as a concentrate. For calibrator concentrate, refer to the Certificate of Analysis (CoA) for the stock concentration. Refer to kit instructions (on the customer portal) to prepare serial dilutions of the calibrator using the calibrator diluent. Calibrators are run using the neat protocol.
2. **Prepare QC Controls:**
   Depending on the kit, QC controls may be pre-diluted or supplied as a concentrate. Refer to kit instructions to prepare dilutions.
3. **Prepare Samples:**
   Quanterix® supplied 96-well plates have a dead volume of 30 µL. By using the table in the kit instructions, calculate the required volume of sample to be added per well.

**Example:** aspiration volume/replicate for PSA Assay

| | |
|---|---|
| Calibrator volume | 100 µL / replicate |
| Sample & Control Dilution factor | 4x |
| Pre-diluted sample volume | 100 µL / replicate |
| Undiluted sample & control volume | 25 µL / replicate |
| Total tests per kit | 96 |
| Does not account for dead volumes | |

**Calculate Required Volume of Sample per Well**

= Dead volume + (number of replicates × protocol aspiration volume)

**Example a:** For the Neat Protocol, with 3 Replicates:
Required volume = 30 µL + (3 × 100 µL) = 330 µL/well.
(For 4X dilution: Prepare 82.5 µL sample in 247.5 µL Sample diluent.)

**Example b:** For On-board Sample Dilution with Standard 4x Protocol and 3 Replicates:
Required volume = 30 µL + (3 × 25 µL undiluted sample) = 105 µL/well.

**Note:** All samples should be centrifuged at 10,000xg for 5 min to clear any debris

4. **Prepare Plate:**
   Pipette required volumes of calibrators, controls and samples into the 96-well plates.

## 3. Load Instrument and Run Assay

1. **Vortex Beads:**
   Vortex Beads for about 30 seconds before loading. Don't let beads sit idle for > 5 minutes. If this happens, vortex again.
2. **Load Reagents:**
   **a. Load Bead, Detector and SBG reagents into Reagent Rack:**
   Make sure beads are in one of the three shaking positions to continue mixing beads.
   Select **Load Reagent** Tab > Select reagent lane > Make sure on-board barcode scanner is enabled (HD-X only) or use handheld scanner to assign positions of reagents in reagent rack > Insert reagent rack.

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**b. Load RGP**:
Select an RGP lane > Make sure on-board barcode scanner is enabled (HD-X only) or use the handheld scanner to assign RGP > Insert RGP rack (rack marked with an O) > Touch **Done Loading Reagents** Tab.

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 a single well > Select **Assay** > Select calibrator from the **Select Calibrator** pop up > Click **Ascending/Descending** to populate the remaining calibrators > Select the **Replicates per well**.
   **c. Assign Samples:** Select **Assign Sample** Tab > Highlight all wells that contain controls/samples > Select **Assay** > Select the **Replicates per well**.
   **d.** When setup is complete, click on **List View** to confirm selections. Insert plate rack or tubes. Touch **Done with Setup**.
4. **Fill Liquid and Solid Consumables and Empty Waste if Necessary:**
   **a. Load Liquid Consumables**:
   Fill the DI Water and Wash Buffer 1 secondary containers and the Wash Buffer 2 container (primary for HD-X and secondary for HD-1).
   **b. Load Cuvettes, Tips, and Discs, if Required:**
   - Load Cuvettes:
     Cuvettes are added by placing a full stack of 50 in the cuvette chute.
     **Note:** Additional stacks must be loaded only when the system says **Ready**.
   - Load Tips:
     **System Resource** Tab > Select **Solid Resources** > Click **Unlock Drawers** > Load Tips > In the software, tap twice in the positions where you loaded new tip racks. The tip positions in the rack diagram turn light blue.
   - Load Discs:
     **System Resource** Tab > Select **Solid Resources** > Click **Unlock Drawers** > Use Barcode Scanner to scan the barcode on the wrapper > Remove blue base plate (from the old stack) from the disc pole > Load the new stack on an empty disc pole and remove the wrapper and the top disc with the Quanterix logo.

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

1. **Remove Sample Plate, Reagents and RGP**
2. **Review the Results in One of Three Ways:**

*Refer to Simoa HD-1 Data Analysis Guide or Simoa HD-X Data Analysis Guide available on the customer portal (http://portal.quanterix.com/).*

   a. **Export the Data as a CSV File for External Analysis:**
      **History & Reports** Tab > click **Run History** Tab > filter (tap on +) by **Batch ID** > Click **Select All Results** Tab > Click the **Export** Tab > Export the data as a CSV file for external analysis.
   **b. Analyze the Calibrators in the Data Reduction Tab.**
   **c. Export a Report:**
   From the **History & Reports** Tab, click **Reports** Tab > click **Batch Calibration Report** > select a Batch > generate and **Export** a report as a PDF file.
3. **Post Run Maintenance:**
   **Maintenance** Tab > Check the **End of Day** task > Click **Run Task** (about 15 minutes).
4. **Shut down:**
   Shut down software > Turn off instrument > Shut down PC.

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