

Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody Test

INSTRUCTIONS FOR USE

103750

April 2021

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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.

The clinical applicability of a quantitative or semi-quantitative result is currently unknown and cannot be interpreted as an indication or degree of immunity nor protection from reinfection, nor compared to other SARS-CoV-2 antibody assays.

NAME

Simoa™ Semi-Quantitative SARS-CoV-2 IgG Antibody Test

INTENDED USE

The Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody Test is an automated paramagnetic microbead-based immunoassay intended for qualitative and semi-quantitative detection of IgG antibodies to SARS-CoV-2 in human serum and dipotassium EDTA plasma using the Quanterix HD-X immunoassay system. The Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody Test is intended for use as an aid in identifying individuals with an adaptive immune response to SARS-CoV-2, indicating recent or prior infection. The Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody Test should not be used to diagnose or exclude acute SARS-CoV-2 infection. At this time, it is unknown for how long antibodies persist following infection and if the presence of antibodies confers protective immunity. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C 263a, that meet requirements to perform moderate or high complexity tests.

Results are for the detection of SARS CoV-2 IgG antibodies. IgG antibodies to SARS-CoV-2 are generally detectable in blood several days after initial infection, although the duration of time antibodies are present post-infection is not well characterized. Individuals may have detectable virus present for several weeks following seroconversion.

Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities.

The sensitivity of the Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody Test early after infection is unknown. Negative results do not preclude acute SARS-CoV-2 infection. If acute infection is suspected, direct testing for SARS-CoV-2 is necessary.

False positive results for the Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody Test may occur due to cross-reactivity from pre-existing antibodies or other possible causes.

Samples should only be tested from individuals that are 15 days or more post symptom onset.

The Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody 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) 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, anti-SARS-CoV-2 antibody detection assay detects antibodies to the virus after the host immune response and seroconversion. Classes of antibodies detected by antibody tests are generally IgM and IgG.

PRINCIPLES OF THE PROCEDURE

The Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody Test is a 3-step paramagnetic microbead-based sandwich ELISA that uses single molecule array

(Simoa) technology.² In the first step, sample is drawn from a sample tube or microtiter plate by the instrument pipettor and mixed with COVID-19 spike protein coated paramagnetic capture beads in a reaction cuvette. IgG antibodies in the sample that are specific to COVID-19 spike protein are bound by the capture beads. After an incubation, capture beads are collected with a magnet, and washed. Biotinylated anti-human IgG detector antibodies are then mixed with the capture beads, and the detector antibodies bind to the captured sample IgG. Following a second wash, a conjugate of streptavidin-β-galactosidase (SBG) is mixed with the capture beads. SBG binds to the biotinylated detector antibodies, resulting in enzyme labeling of captured sample IgG. After a third wash, the capture beads are resuspended in a resorufin β-D-galactopyranoside (RGP) substrate solution. 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 anti-spike IgG from the sample has been captured and labeled, the β-galactosidase hydrolyzes the RGP substrate into a fluorescent product that provides the signal for measurement. The concentration of IgG in unknown samples is interpolated 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 2 hours and 45 minutes.

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

REAGENTS

Reagent Kit

Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody Test kit

Reagent Kit	Bead Reagent	1 bottle (4.4 mL)	SARS-CoV-2 spike protein (recombinant) coated capture beads in Tris buffer with a protein stabilizer (bovine) and a surfactant. Preservative: ProClin 300.
	Detector Reagent	1 bottle (12.3 mL)	Biotinylated anti-human IgG antibody (goat polyclonal) in phosphate buffer with a protein stabilizer (bovine) and a surfactant. Preservative: ProClin 300.
	SBG Reagent	1 bottle (12.3 mL)	Conjugate of streptavidin-β-galactosidase (SBG) in phosphate buffer with a protein stabilizer (bovine). Preservative: ProClin 300.
	Sample Diluent	4 bottles (30 mL ea)	Phosphate buffer with a protein stabilizer (bovine), a heterophilic blocker, and a surfactant. Preservative: Sodium azide.
	RGP Reagent	2 bottles (3.4 mL)	Resorufin β-D-galactopyranoside (RGP) in phosphate buffer with a surfactant.
Calibrators	Cals A-H (0 plus 7 levels)	8 vials (1 mL each)	Anti-SARS-CoV/CoV-2 IgG in phosphate buffer with a protein stabilizer (bovine), a surfactant, and sodium azide as a preservative.
Controls	Negative control	1 vial (0.125 mL each)	Chimeric monoclonal IgG antibody in pooled normal human serum.
	Positive control	1 vial (0.125 mL each)	Chimeric monoclonal IgG antibody in pooled normal human serum.

Materials Required but Not Provided

- Simoa HD-X Analyzer, Simoa software v3.1 (Item # 100486)
- Simoa HD-X System Buffer 1 (Item # 101619)
- Simoa HD-X System Buffer 2 (Item # 100487)
- Simoa HD-X Sealing Oil (Item # 100206)
- Simoa HD-X cuvettes (Item # 103346, 500 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)

WARNINGS AND PRECAUTIONS


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

This test has not been FDA cleared or approved, but has been authorized for emergency use by FDA under an EUA for use by laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C 263a, that meet requirements to perform moderate or high complexity tests.

This test has been authorized only for detecting the presence of IgG antibodies against SARS-CoV-2, not for any other viruses or pathogens.

The emergency use of 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 Federal Food, Drug, and Cosmetic 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 2⁴ 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 *Simoa HD-X Analyzer User Guide (EUA Edition)*.
- 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.

Handling Precautions

- The reagents are 1-time use; any remaining material should be discarded after the completion of the assay 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.

Shipping and Storage Instructions

Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody Test reagents are shipped on wet ice. If the reagents arrive at room temperature, reagent integrity may be suspect. Contact Quanterix service.



- Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody Test reagents must be stored at 2–8°C in an upright position.

- Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody Test Calibrators and Controls are shipped on dry ice. If these components arrive in an unfrozen state, their integrity may be suspect. Contact Quanterix service.



- Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody 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

- Insufficient sample processing may cause inaccurate results.
- Serum and plasma specimens should be immediately removed from the sample collection tubes (after centrifugation) and put in a separate tube that can then be aliquoted and frozen for future use.
- For optimal results, specimens should be free of fibrin, red blood cells, or other particulate matter. Do not use grossly hemolyzed specimens.
- Specimens thawed after frozen storage must always be mixed THOROUGHLY by low speed vortexing or inverting 10 times. Visually inspect the specimens. If layering or stratification is observed, continue mixing until specimens are visibly homogeneous.
- Specimens may be stored for up to 24 hours at 2–8°C prior to being tested. If testing will be delayed for more than 24 hours, specimens should be frozen at –20°C or colder.
- Specimen stability in different storage conditions has not been validated for this assay.
- The Simoa HD-X Analyzer does not provide the capability to verify specimen type. It is the responsibility of the operator to verify that the correct specimen type is used in the Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody Test.
- For freshly drawn serum specimens, ensure that complete clot formation has taken place prior to centrifugation. If specimens are centrifuged before a complete clot forms, the presence of fibrin or particulate matter may cause erroneous results.
- Centrifuge all specimens prior to assay. Centrifugation conditions should be sufficient to efficiently remove particulate matter and to clarify the sample, for example 5 minutes at 10,000 g for serum or plasma.
- Centrifuged specimens with a lipid layer on the top should be transferred to a secondary tube. Care must be taken to transfer only the clarified specimen without the lipemic material.
- 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.
- Multiple freeze-thaw cycles of specimens should be avoided.
- Specimens with obvious microbial contamination should not be used.

PROCEDURE

Assay Procedure

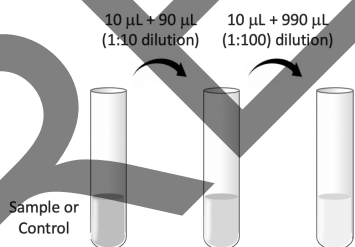
- The Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody Test assay definition must be downloaded from the customer portal and installed on the Simoa HD-X Analyzer prior to performing the assay.
- Calibrators, controls, and samples must be allowed to come to room temperature and mixed thoroughly before loading onto the Simoa HD-X Analyzer.
- Simoa reagents 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 4 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.

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)*).
 - Remove caps from reagent bottles and load the Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody Test reagents (Bead Reagent, Detector Reagent, SBG Reagent) 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 8 hours and one work shift.)
 - In the run set up screen, specify 'neat' protocol. The samples will be diluted off-line prior to running the assay, and the instrument will not perform additional pre-dilutions. Follow the guidelines in the Specimen Dilution Procedure section of this package insert to set up sample dilutions.
 - Replenish consumables and system resources as needed prior to initiating the run, as described in the *Simoa HD-X Analyzer User Guide (EUA Edition)*.
 - Initiate the run.

Specimen Dilution Procedure

- Treat specimens and assay Controls the same way.
- Perform a 1:1000 pre-dilution of specimens and controls with Sample Diluent. Given the large dilution, it is recommended to perform the dilution in two steps to maximize pipetting accuracy. A recommended two-step dilution is to dilute 10 µL of sample to 100 µL, followed by a subsequent dilution of a 10 µL aliquot of diluted sample to 1000 µL:



- If an alternative procedure is followed, volume transfers should be performed with appropriately calibrated pipettors and good pipetting technique to ensure proper accuracy.
- The HD-X system accepts two types of sample inputs: sample tubes or Quanterix-supplied 96-well plates. Both types of sample inputs are valid for calibrators, controls, and samples. Each replicate test consumes 100 µL of diluted sample. Multiple replicate tests of the same sample may be run from the same tube or well. The minimum sample volume in the tube or well depends on the number of replicates to be run and the required dead volume for the tube or well.

- **Note:** 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. As a general guideline, at room temperature and normal humidity, a 60 µL sample will lose approximately 5% of its weight per hour. A 120 µL sample may lose 5% of its weight in approximately 3 hours. If pre-pipetted 96-well plates are to be stored prior to assay, it is recommended to seal the plate as described in the section below (see Quanterix-supplied 96-well plates).

- **Sample tubes.** The minimum sample volume needed may depend on the type and volume of the tube due to different dead volumes. Samples introduced in 5-mL Nalgene Cryogenic sample tubes have a dead volume of 50 µL.
- **Quanterix-supplied 96-well plates.** Quanterix-supplied 96-well plates have a dead volume of 30 µL. The maximum recommended sample volume for sealed Quanterix-supplied 96-well plates is 280 µL. Use of plate seals is necessary 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 Semi-Quantitative SARS-CoV-2 IgG Antibody Test calibration, test Calibrators A through H in duplicate. All levels of Semi-Quantitative SARS-CoV-2 IgG Controls must be tested to evaluate the assay calibration. Ensure that all assay control values are within expected concentration ranges.
- Once a Semi-Quantitative SARS-CoV-2 IgG Antibody Test calibration is accepted and stored, all subsequent samples may be tested without further calibration unless one or more Controls are out of their expected ranges (see Quality Control Procedures) or a reagent kit with a new lot number is used.
- The Semi-Quantitative SARS-CoV-2 IgG Antibody 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.

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.
- Controls should be included in each run and should follow the same dilution scheme selected for samples.

QUALITY CONTROL PROCEDURES

- Follow the specific quality control procedures in your laboratory.
- The Semi-Quantitative SARS-CoV-2 IgG Antibody Test Negative Control and Positive Control should be included in every batch run to assess calibration curve storage integrity and run validity.
- Control values and ranges listed in the lot-specific Certificate of Analysis should be considered only as guides. Each laboratory should 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 the results from one or more of the controls are outside their expected ranges, the stored calibration curve may no longer be valid, and the assay may need to be re-calibrated. The length of time calibration curves may be stored must be validated for each laboratory. Alternatively, calibration can be performed with every batch run. Controls must be run with every calibration to assess calibration accuracy and run validity.

- 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)*.

Interpretation of Results

- The assay clinical cutoff is 0.77 µg/mL.

Result Value	Result Reported	Interpretation
< 0.77 µg/mL	Negative for SARS-CoV-2 IgG antibodies.	SARS CoV-2 IgG antibodies were not detected, or IgG antibodies are below the detection limit of the assay.
≥ 0.77 µg/mL	Positive for SARS-CoV-2 IgG antibodies with the numeric value in µg/mL for semi-quantitative measurements.	SARS-CoV-2 IgG antibodies were detected.

- Results of this assay should always be interpreted in conjunction with the patient's medical history, clinical presentation, and other findings.
- Do not use this assay in patients taking biotin supplements. Testing specimens in patients taking biotin may lead to false results. Therefore, do not report the results with this device unless it is confirmed that the patient is not taking biotin.

Note: Some results may contain information in the Flags field. For a description of the flags that may appear in this field, refer to the *Simoa HD-X Analyzer User Guide (EUA Edition)*.

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.
- The clinical applicability of a quantitative or semi-quantitative result is currently unknown and cannot be interpreted as an indication or degree of immunity nor protection from reinfection, nor compared to other SARS-CoV-2 antibody assays.
- COVID-19 patients may have a delayed antibody response from the time of infection with insufficient levels of antibody to be detectable by the assay.
- This device should not be used to diagnose or exclude acute SARS-CoV-2 infection. Direct testing for SARS-CoV-2 with a molecular assay should be performed to evaluate acute infection in symptomatic individuals.
- Performance has only been established with the specimen types listed in the Intended Use. Other specimen types have not been evaluated and should not be used with this assay.
- Results from antibody testing should not be used as the sole basis for patient management decisions. Test results should be interpreted in conjunction with clinical observations, patient history, epidemiological information, and other laboratory findings.
- A positive result may not indicate previous SARS-CoV-2 infection. Consider other information, including clinical history and local disease prevalence, in assessing the need for a second, but different, serology test to confirm an immune response.
- A negative result means the sample is considered non-reactive for SARS-CoV-2 IgG antibodies by the criteria of the Semi-Quantitative SARS-CoV-2 IgG Antibody Test. Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. A negative result may indicate that IgG antibodies are present in concentrations below the clinical cutoff or detection limit of the assay. This can occur during acute infection prior to seroconversion.
- IgG concentration results from the Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody Test may not be used interchangeably with values obtained with different manufacturers' test methods, i.e., numerical results between different methods are not comparable.
- SARS-CoV-2 IgG antibodies may be below detectable levels in samples collected from patients less than 14 days from a positive polymerase chain

reaction (PCR) result. In addition, the immune response may be depressed in elderly, immunocompromised, or immunosuppressed patients.

- Specimens with direct evidence of antibodies to non-SARS-CoV-2 coronavirus strains such as HKU1, NL63, OC43, or 229E have not been evaluated with this assay.
- Cross-reactivity may occur due to past or present infection with HSV.
- It is not known at this time if the presence of antibodies to SARS-CoV-2 confers immunity to re-infection.
- This test should not be used for screening donated blood.
- Bacterial contamination of specimens may affect the test results.
- Do not use this assay in patients taking biotin supplements. Testing specimens in patients taking biotin may lead to false results. Therefore, do not report the results with this device unless it is confirmed that the patient is not taking biotin.
- The presence of 20 mg/dL of conjugated bilirubin may result in a decrease of the observed value of approximately 16%. The presence of 30 U/mL of rheumatoid factor may result in a decrease or increase of the observed value of approximately 22%. The presence of 12 g/dL or 15 g/dL of protein may result in an increase of the observed value of approximately 17% and 20%, respectively. Do not test icteric samples.
- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with immunoassays. People routinely exposed to animals or animal serum products can be prone to this interference, and anomalous values may be observed.
- The performance of this test has not been established in individuals who have received a COVID-19 vaccine. The clinical significance of a positive or negative antibody result following COVID-19 vaccination has not been established, and the result from this test should not be interpreted as an indication or degree of protection from infection after vaccination.
- The performance of this test was established based on the evaluation of a limited number of clinical specimens. The samples for the negative agreement study were collected in USA prior to December 2019. The samples for the positive percent agreement were collected in Germany in March and April 2020 and in USA between May and July 2020. 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 Semi-Quantitative SARS-CoV-2 IgG Antibody 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 Semi-Quantitative SARS-CoV-2 IgG Antibody Test, must adhere to the Conditions of Authorization indicated in the Letter of Authorization as listed below:

- Authorized laboratories using the Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody Test must include with result reports of the Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody Test, 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 Semi-Quantitative SARS-CoV-2 IgG Antibody Test must use the Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody Test as outlined in the authorized labeling. 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 Semi-Quantitative SARS-CoV-2 IgG Antibody Test are not permitted.
- Authorized laboratories that receive the Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody Test must notify the relevant public health authorities of their intent to run the Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody Test prior to initiating testing.

- Authorized laboratories using the Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody 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 Semi-Quantitative SARS-CoV-2 IgG Antibody Test and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and Quanterix Corporation at www.Quanterix.com any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of the Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody Test of which they become aware.
- All laboratory personnel using the Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody Test must be appropriately trained in automated immunoassay techniques and use appropriate laboratory and personal protective equipment when handling this kit and the Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody Test in accordance with the authorized labeling. All laboratory personnel using the assay must also be trained in and be familiar with the interpretation of results of the product.
- Quanterix Corporation, authorized distributors, and authorized laboratories using the Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody 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, to perform moderate and high complexity tests" as "authorized laboratories."

PERFORMANCE CHARACTERISTICS

Precision

A two-site precision study was conducted in accordance with CLSI Document EPO5-A3.⁶ Samples and assay controls were assayed in duplicate (samples) or triplicate (controls) in 2 runs per day for 3 days using the HD-X system and one lot of Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody Test reagents, calibrators, and controls per site. Since different lots of controls were used at two sites, between-site component for controls was not evaluated. Results from the study are exhibited in the tables below:

	n	Mean IgG (µg/mL)	Repeatability %CV	Between-run %CV	Between-day	Total %CV
Lot 1/Site 1						
Control 1	18	0.454	8.6%	2.4%	0.0%	9.0%
Control 2	18	3.236	3.9%	2.4%	4.6%	6.5%
Lot 2/Site 2						
Control 1	18	0.311	4.8%	5.2%	0.0%	7.1%
Control 2	18	2.855	2.2%	2.5%	2.5%	4.2%

	n	Mean IgG (µg/mL)	Repeatability %CV	Between-run %CV	Between-day	Between-lot/site	Total %CV
Lot 1/Site 1							
Serum 1 (Negative)	12	0.250	10.8%	0.0%	5.2%		12.0%
Serum 2 (Low Positive)	12	1.101	9.9%	0.0%	3.6%		10.6%
Serum 3 (moderate Positive)	12	111.1	8.1%	6.5%	9.7%		14.2%
Lot 2/Site 2							
Serum 1 (Negative)	12	0.223	10.3%	0.0%	4.5%		11.2%
Serum 2 (Low Positive)	12	1.032	3.2%	0.0%	1.4%		3.5%
Serum 3 (moderate Positive)	12	117.8	7.8%	10.0%	0.0%		12.7%
Overall							
Serum 1 (Negative)	24	0.236	10.6%	0.0%	4.7%	7.6%	14.0%
Serum 2 (Low Positive)	24	1.066	7.6%	0.0%	2.8%	4.0%	9.0%
Serum 3 (moderate Positive)	24	114.4	7.9%	8.6%	6.0%	0.0%	13.1%

Analytical Sensitivity

The limit of blank (LoB) and limit of detection (LoD) were estimated according to CLSI Document EP17-A2.⁷ LoB was determined separately with two lots of reagents, calibrators, and controls as the 95% percentile across 6 replicates each of 5 seronegative samples tested over a 3-day period. The greater LoB calculated from the 2 lots was 0.029 µg/mL. LoD was determined with 2 lots of reagents, calibrators, and controls based on the LoB and standard deviation of a population of repeated measurements of 5 low seropositive samples tested over a 3-day period (30 replicates total per lot). The greater LoD calculated from the 2 lots was 0.051 µg/mL.

Limit of Quantitation

The limit of quantitation (LoQ) was estimated according to CLSI EP17-A2.⁷ LoQ was determined with 2 lots of reagents, calibrators, and controls as the lowest concentration of IgG giving $\leq \pm 15\%$ inaccuracy and imprecision of %CV $\leq 20\%$. A panel of 4 seronegative samples supplemented with known increasing concentrations of anti-SARS-CoV-2 IgG were repeatedly tested over 3 days across 2 lots and 2 HD-X instruments (n = 9 replicates/panel member each lot). Bias from expected IgG values and imprecision were evaluated for each panel member. The greater LoQ determined from the 2 lots was 0.213 µg/mL.

Linearity

Dilution linearity was conducted in accordance with CLSI Document EP06-A.⁸ Seropositive serum and/or anti-SARS-CoV-2 IgG were admixed with seronegative serum in different ratios to prepare a dilution series comprised of a minimum of five levels within the linear range of the assay. A linear regression was performed for each dilution series, and the slope, intercept, and R² were calculated. Also, polynomial regression fitting of each dilution series was performed comparing linear and non-linear fits. Deviations from linearity were evaluated by comparing the mean concentrations of four replicates vs a linear fit at each dilution. Linearity was demonstrated for the analytical measuring interval of 0.21 µg/mL to 250 µg/mL with deviations from linearity within 15%. Taking into consideration the estimates of LoB, LoD, LoQ, precision, and linearity, the analytical measuring interval is 0.21 µg/mL to 250 µg/mL.

Recovery

Accuracy of recovery of known quantities of IgG antibody spiked into negative human samples was examined in two studies. In the first study, 3 µg/mL of anti-SARS-CoV-2 IgG were spiked into 3 serum and 2 K2EDTA plasma samples from pre-pandemic donors. Each sample was measured once, and vendor-provided concentrations of the antibody were used for calculations. Mean recovery was 123% and 124% for plasma and serum, respectively. In the second study, 1.7 µg/mL, 4.2 µg/mL, and 17 µg/mL of anti-SARS-CoV-2 IgG were spiked into 3 serum and 3 K2EDTA plasma samples from pre-pandemic donors. Samples were measured in duplicates, and the concentration of the IgG stock solution was value assigned by assaying four dilutions across the assay range and averaging the results. Mean recovery was 98% and 95% for plasma and serum, respectively.

Class Specificity

The anti-human IgG antibody used in the Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody Test demonstrates class-specific reactivity only to human IgG isotypes. No binding interactions were observed to human IgM.

Cross Reactivity

Analytical specificity of the assay was evaluated for potentially cross reacting antibodies. 50 samples from patients with the following viral infections were tested: HIV (20 samples), Hepatitis B (10 samples), Hepatitis C (10 samples), and herpes simplex virus (10 samples). Among these samples 48 were negative, and 2 out of 10 herpes simplex virus samples were false positive with the Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody Test.

Interfering Substances

Potential interference of endogenous substances commonly found in serum and plasma specimens, including hemoglobin, conjugated bilirubin, unconjugated bilirubin, triglycerides, and rheumatoid factor (RF) was

evaluated. Spiked and unspiked serum and plasma samples were tested, and the testing demonstrated the following changes between quantitative results (acceptance criteria were qualitative agreement >95% and <10% bias):

Substance	Substance Concentration	Qualitative Agreement Between Samples with and without Substance	Average Difference Between Samples with and without Substance, %
Triglycerides	1000 mg/dL	100%	9
Hemolysate	500 mg/dL	100%	10
Bilirubin Conjugated	20 mg/dL	100%	16
Bilirubin Unconjugated	20 mg/dL	100%	8
Rheumatoid Factor	30 U/mL	100%	22
Protein (Human Serum Albumin)	15 g/dL	100%	8

Sixteen percent (16%) negative bias was observed in the presence of 20 mg/dL of conjugated bilirubin while a 22% negative or positive bias was observed with 30 U/mL RF, increasing the risk of false results. The average bias observed in the presence of 15 g/dL human serum albumin across three seropositive and three seronegative serum pools was 8%, however the presence of 12 g/dL or 15 g/dL of protein resulted in positive biases of approximately 17% and 20% respectively in individual sample pools. Do not use this assay in patients taking biotin supplements. Testing specimens in patients taking biotin may lead to false results. Therefore, do not report the results with this device unless it is confirmed that the patient is not taking biotin.

Clinical Performance

Positive Percent Agreement (PPA)

A study was performed to determine the clinical performance of the Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody Test. To estimate the positive percent agreement (PPA) between the Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody Test and the polymerase chain reaction (PCR) comparator, 157 serum and plasma samples from 50 symptomatic patients determined to be SARS-CoV-2 positive by RT-PCR were tested. The PPA and the 95% confidence interval (CI) were calculated using the initial sample collected in each of the 3 designated time frames since positive PCR test (i.e. ≤ 7 days, 8-14 days, and 15-30 days), per subject. The performance summary data is illustrated in the table below.

Positive Agreement Days Post RT-PCR Positivity, considering first bleed in each time period

Days from positive PCR test	Number Tested	Reactive	PPA	95% CI
0 - 7	31	14	45.16%	29.16% – 62.23%
8 - 14	16	14	87.50%	63.98% - 96.50%
≥15	28	28	100.00%	87.94% - 100.00%
Total	75			

Note: A positive PCR result confirms the presence of virus, but seroconversion to IgG reactivity follows a latency period of undetermined length. Therefore, samples collected early in the infection are expected to be non-reactive for anti-SARS-CoV-2 IgG.

Negative Percent Agreement (NPA)

496 serum and plasma samples collected from pre-pandemic donors were tested to determine the Negative Percent Agreement (NPA). Out of 496, 4 serum samples were positive with the Simoa Semi-Quantitative SARS-CoV-2 IgG Antibody Test, which translates into a negative percent agreement of 99.19% (492/496), (95% CI: 97.95% - 99.69%).

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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)*.

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April 2021

Simoa HD-X Analyzer®
User Guide (EUA Edition)
Software Version 3.1

USER-0105 03

23 Dec 2020

simOa™



Customer Support

Customer support is available 8 AM to 5 PM, EDT.

email: techsupport@quanterix.com

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email: techsupport@quanterix.com

For use under an Emergency Use Authorization (EUA) for In Vitro Diagnostic Use Only.

Prescription Use Only.

The intended use of the Simoa® Semi-Quantitative SARS-CoV-2 IgG Antibody Test and Simoa HD-X Analyzer is for use under the Food and Drug Administration's Emergency Use Authorization (EUA) only. The tests have not been FDA cleared or approved; the tests have been authorized by the FDA under an EUA for use by laboratories certified under CLIA, that meet requirements to perform moderate or high complexity tests. Diagnostic use is authorized while the EUA remains in place.

This product has not been FDA cleared or approved, but has been authorized for emergency use for use by laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C 263a, that meet requirements to perform moderate or high complexity tests.

This product has been authorized for detecting the presence of IgG antibodies against 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 Federal Food, Drug, and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.

This product is protected by US and Foreign patents and patent filings.

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Document Purpose

This document provides information about how to perform Simoa® assays with the Simoa HD-X Analyzer® (termed “the instrument” subsequently). After reading this document, you will know how to operate the instrument, perform assays, maintain the instrument, and obtain help for instrument or assay problems.

Intended Audience

This guide is for anyone who operates or maintains the instrument. Users should be familiar with standard laboratory practices and with computers running the Windows 10 operating system.

Customer Support

Customer support is available 8 AM to 5 PM EST, Monday through Friday.

Email: techsupport@quanterix.com

When you contact Quanterix®, you may be asked to use the Customer Support Tool to generate a support package that will help troubleshoot the problem. See *Quanterix Customer Support Tool User Guide* for instructions.

Log in to the Quanterix portal to view training videos at <http://portal.quanterix.com>.

Purchasing Supplies

For information about purchasing reagent kits, consumables, and instruments, contact Sales at Quanterix:

Quanterix Corporation
 900 Middlesex Turnpike
 Billerica, MA 01821
Phone: 617-301-9400
Email: sales@quanterix.com

Abbreviations Used in This Document

CCD	charge-coupled device (camera imaging system)
ELISA	enzyme-linked immunosorbent assay
GUI	graphical user interface
SBG	Streptavidin-beta-galactosidase
RGP	resorufin-D-galactopyranoside

Notices Used in This Document



Note! This type of notice highlights important information about procedures or provides tips.



CAUTION! This type of notice provides information about minor potential hazards to the instrument or assay performance. Failure to comply with the notice may result in inaccurate results, instrument maintenance problems, or instrument failure.



WARNING! This type of notice provides information about situations that pose a danger to the instrument or to operators. Failure to observe the warnings in this notice may result in instrument damage or a safety hazard for operators.

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Read the Safety Notices

Before you operate the Simoa HD-X Analyzer, familiarize yourself with the safety and regulatory notices in this chapter. If improperly operated, the instrument can pose electrical or mechanical safety hazards to operators. You may also encounter chemical or biological hazards, depending on the assays that you perform with the instrument.

Do not perform any instrument procedures that are not described in this guide without the guidance of Quanterix® Customer Support.

The Simoa HD-X Analyzer must be installed by trained service professionals only. Do not interfere with system electrical grounding or move the instrument after installation without the assistance of Quanterix Customer Support.

General Safety Notices



CAUTION! As the Simoa HD-X Analyzer is designed for measuring biological samples, universal laboratory precautions should be used at all times.



WARNING! Do not install any software on the Simoa HD-X Analyzer computer unless instructed to do so by Quanterix Customer Support. Installing third-party software can result in system failure, job loss, and voiding of your warranty. Contact Technical Support for further details and assistance.



CAUTION! Do not move the instrument after the initial installation.



CAUTION! Do not remove the cover of the instrument.
Label located on pipette rail near cuvette loader.



CAUTION – Mechanical hazard! Do not insert your hands into the reagent bay, the sample bay, or the system bay if the instrument is turned on. Doing so may expose you to risk of personal injury. The instrument contains robotic pipettors that access these areas at times, and they can injure your hands. Label is located on sample bay door, reagent bay door, drawers, waste container drawer (left and right side) and cuvette loader.



CAUTION! Hot surface!
Label is located on LSI unit.



Overheating!
Improper placement of the instrument may cause fire or serious instrument damage in case of overheating.

- Do not block or cover ventilation slots.
- The air shall be able to circulate.

Electrical Safety Notices



CAUTION! The appliance inlet is intended to be used as a disconnecting device in case of an emergency. Set up the instrument so that the appliance inlet is always easily accessible.



Improper connection of mains supply.

Improper connection of the instrument and the peripheral devices to the mains supply can cause serious personal injury with potentially deadly consequences and material damage (e.g. fire).

- Only use grounded connection and extension cables with sufficient capacity (voltage and current) to connect the instrument and any peripheral devices to the mains power supply.
 - Never remove ground connections.
 - Grounding of the instrument and its peripheral devices to the same protective earth potential shall be ensured.
 - The use of a multi-outlet power strip is not allowed!
 - Only use power cables that fulfill the minimum requirements for this instrument.
-

Chemical/Biological Safety Notices



CAUTION! Biohazard materials may be used on this instrument. Proper personal protective equipment (PPE) should be worn at all times to meet the level of biohazard materials used.



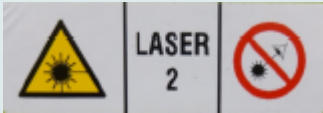




Risk of infection! Monthly maintenance and waste removal procedures put the user in close contact with biohazard materials. Proper personal protective equipment (PPE) should be worn at all times to meet the level of biohazard materials used. Label is located on drawers.

- Strictly follow the local and national provisions, legislation and laboratory regulations.
 - Use appropriate gloves!
 - Use an appropriate lab coat!
 - Use an appropriate eye protection (e.g. protective glasses)!
 - Avoid contact between skin/mucous membrane and samples/test reagents or parts of the instrument.
 - Clean, disinfect and decontaminate the instrument immediately if potentially infectious material has been spilled.
 - Do not use broken or chipped tubes or bottles.
 - Observe the instructions in the package inserts for correct use of reagents.
 - Observe the legal regulations for the handling of infectious material.
 - Never use bio-hazardous liquids for testing the instrument!
-

- The instrument shall be cleaned, disinfected and decontaminated before servicing!

Laser and Electrical Safety Labels

The following regulatory labels are affixed to the Simoa HD-X Analyzer.

Label Image	Description of Label Information
	<p>Laser safety label. The label is located on the side of the reagent bay (right) and the sample bay (left), adjacent to the laser inside of the bay.</p>
	<p>Laser information label. Provides the user with information on the class, wavelength, output power, and regulatory compliance status of the laser. The label is for the barcode readers next to the reagent bay and sample bay.</p>
	<p>Biohazard label. The label is located in five places:</p> <ul style="list-style-type: none"> • The reagent bay door, in front of the reagent bay lanes. • The sample bay door, in front of the sample bay lanes. • The front of liquid waste bottles 1 and 2. • The solid waste intermediate trap door to the waste bin. • The system bay waste drawer, inside rear surface.
	<p>Electrical hazard label. The label is located above and adjacent to the electrical power switch, on the side of the instrument.</p>
	<p>Heavy weight. The label is located on the inside of the reagent and sample bay doors. Do not place heavy objects on the doors. When the system bay drawers are open, do not step on them or apply additional weight.</p>

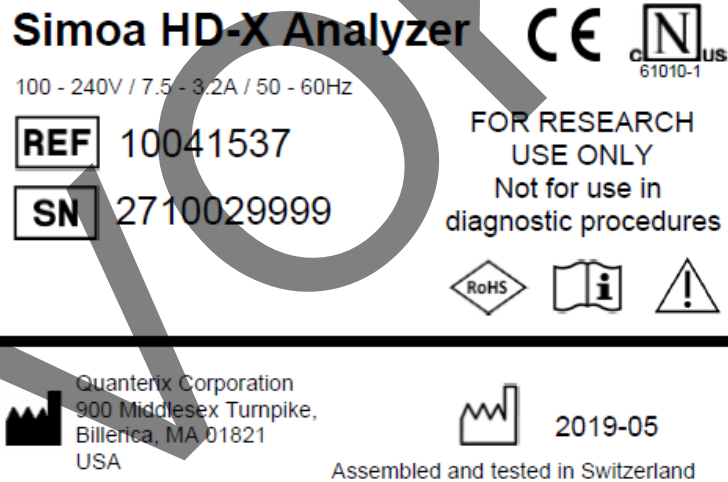
Safety Certifications

This product complies with the EMC standards listed below for class A devices and is suitable for use in commercial environments. In a domestic environment, this product may cause radio interferences, in which case the user may be required to take adequate measures.

- EN ISO 14971:2012 (medical devices – Application of risk management to med devices)
- EN 61010-1 and EN 61326-1 (International) Safety of operation in a laboratory environment

Labeling

The following label is affixed to the instrument:



Restriction of Hazardous Substances (RoHS2)

The instrument system complies with the RoHS2 Directive:

- Directive 2011/65/EU – Restriction of Hazardous Substances in Electrical and Electronic Equipment

3 Overview of the Simoa® HD-X Analyzer

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Introduction

The Simoa HD-X Analyzer is a fully automated instrument that performs ELISA immunoassays. At the heart of the instrument is a precision-engineered, disposable disc with twenty-four ~238,000 femtoliter-sized reaction chambers, termed single molecule arrays (Simoa®), that can isolate and detect single enzyme-labeled protein molecules. Because the array volumes are approximately 2 billion times smaller than those required for conventional ELISA, a rapid buildup of fluorescent product is generated if a labeled protein is present. With diffusion limited, this high local concentration of product can be readily observed.

Only a single molecule is needed to reach the detection limit.

Quanterix provides a wide range of assay kits for the Simoa HD-X Analyzer, containing ready-to-load reagents.

Performing a Simoa assay of any type is simple:

- Load assay reagents into racks in the reagent bay.
- Load plates or tubes of samples and a fluorescent substrate (RGP) into racks in the sample bay.
- Initiate the assay with the instrument’s touch-screen software.

- View and analyze assay results in the instrument software or export them to analysis packages or a laboratory information management system (LIMS).

Key Instrument Parts

External View



Reagent bay showing four reagent rack lanes



Lane 1 2 3 4

Loading a reagent rack into reagent bay lane 4



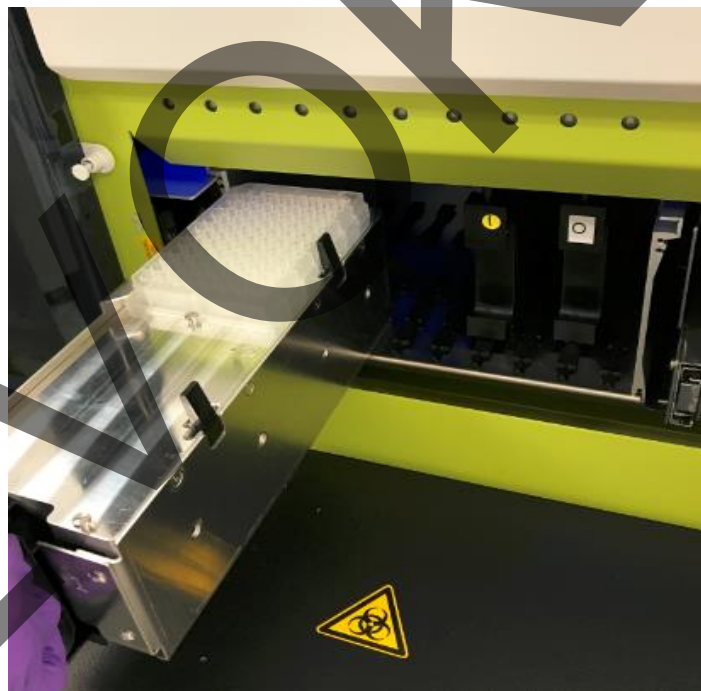
Sample Bay

Sample bay, showing eight lanes for plate racks or tube racks and two lanes for RGP racks

Two tips/disc drawers

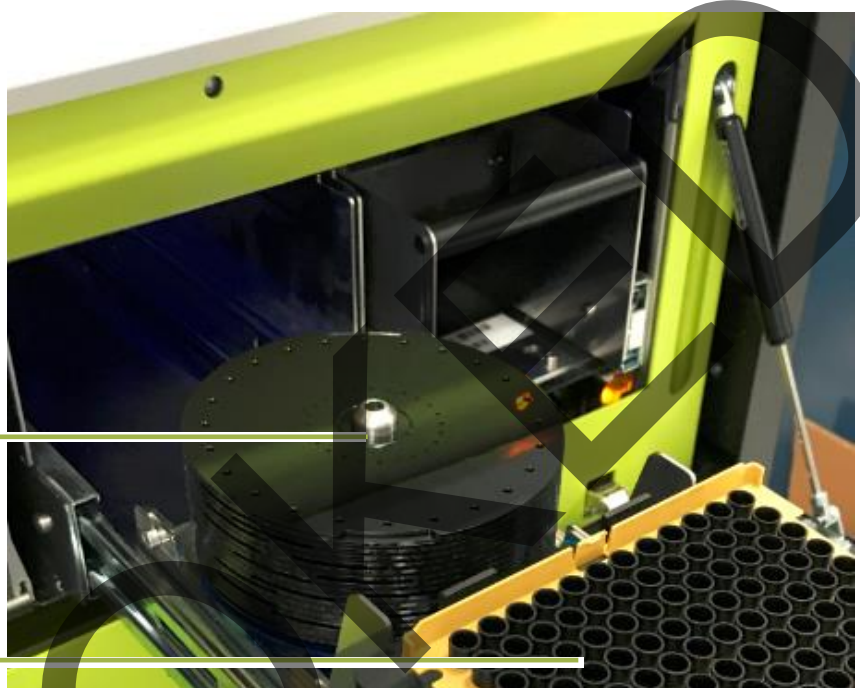


Loaded plate rack being loaded into sample bay lanes 1-4 and RGP rack loaded into RGP lane 1



Stack of Simoa discs on mounting pole

Disposable pipettor tip rack



REVIEW

System Bay



System fluid containers, page 14

Solid waste container, page 15

Liquid waste containers, page 15



Labeling for System fluid (left) and Liquid waste containers (right)

System Fluid Containers

The system uses three system fluids:

- Deionized water (system fluid and needle wash)
- Wash buffer 1 (main assay wash buffer)
- Wash buffer 2 (bead wash pre-loading)

The deionized water and wash buffer 1 each have a secondary container that feeds into a primary container. The instrument draws fluid from the primary containers, which in turn replenish themselves passively from their secondary containers. The container caps connect to tubing that delivers system fluids where needed in the instrument. The secondary containers connect to the primary containers via a plug.

Each secondary system fluid container feeds into a primary container via a short connector. The instrument monitors fluid levels in the containers. The software notifies operators when fluids need replenishing via color-coded icons.



System Waste Containers

The instrument discards used cuvettes, tips, and Simoa Discs into the solid waste container and the effluent from sample washing and incubation into the liquid waste container.

The caps on the liquid waste containers hold liquid-level sensors that allow the instrument to determine when the containers are full and need to be emptied.

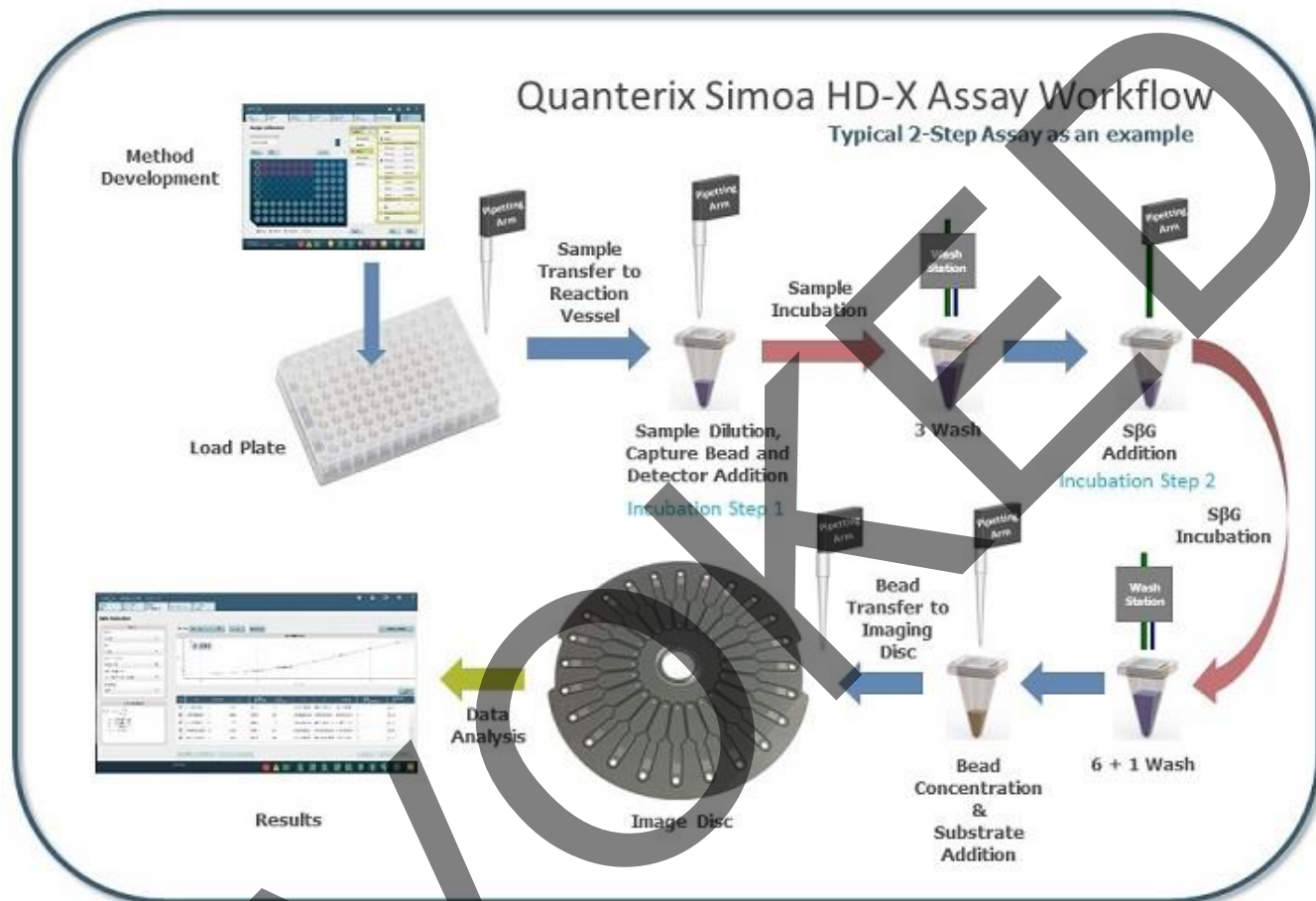


Solid waste container



Liquid waste containers

Overview of Key Instrument Operations



- **Method Development** – Set up your assay using the HD-X touch screen.
- **Load Plate** – Load reagents, samples, and consumables.
- **Sample Transfer to Reaction Vessel** – Robotic pipettors deliver samples into the cuvettes.
- **Sample Dilution, Capture Bead and Detector Addition** – Samples are diluted; capture beads and detector reagents are added to the cuvette.
- **Sample Incubation** – The system implements a one-, two-, or three-step incubation series by moving the cuvette within the washer/incubator ring per the details of the user-specified assay protocol.
- **3 Washes/6+1 Washes** – The washer/incubator ring moves the cuvettes through a series of pelleting, mixing, and washing, according to the protocol. All washes, except the final wash, are performed using Wash Buffer 1. Wash Buffer 2 is used for the final wash preceding substrate addition.

- **SBG Addition / SBG Incubation** – SBG is added to the cuvette and the washer/incubator ring implements the protocol.
- **Bead Concentration & Substrate Addition** – Beads are pelleted, wash buffer is removed and beads are re-suspended via mixing with enzyme substrate, RGP.
- **Bead Transfer to Imaging Disc** – The disposable tip pipettor transfers the mixture of beads and RGP into the sample inlet of a microarray on the Simoa Disc. The instrument loads and seals individual beads into microwells within the array. Loose beads that have not settled into microwells are flushed away.
- **Image Disc** – The instrument moves the disc to the imaging station, where a camera images the sealed wells, capturing the growth of fluorescent signal generated by enzyme labeled beads.
- **Data Analysis/Results** – The Simoa software analyzes the image, determines the average enzymes per bead (AEB), and generates a curve.

4 Quick Start Guide

This quick start guide walks you through performing an assay run on the Simoa® HD-X Analyzer. Be sure to read the entire user guide before you perform your first run.

After that, you can use this guide as a ready reference and to locate explanations of instrument procedures.

Log in to the Quanterix customer portal to view training videos for additional detail at <http://portal.quanterix.com/>.



Note! The Instrument and the computer must be power-cycled once every 24 hours to permit internal systems to reinitialize.

1. Turn on the Simoa HD-X Analyzer computer.	Chapter 5, page 23
2. If the instrument is not running, turn it on. If the instrument has been running continuously overnight, turn it off, wait 15 seconds, and turn it on again.	Chapter 5, page 24
3. Log in to the Simoa software.	Chapter 5, page 25
4. If performing the first run of the day:	
<ul style="list-style-type: none"> • Perform the End of Day task if it was not performed the previous night. 	Chapter 10, page 129
<ul style="list-style-type: none"> • Perform the Start of Day task. 	Chapter 10, page 128
5. Prepare reagents, samples, and calibrators.	Chapter 7, page 66
6. If needed:	
<ul style="list-style-type: none"> • Empty the solid and liquid waste containers. 	Chapter 5, page 52
<ul style="list-style-type: none"> • Refill system fluid containers (deionized water, system wash buffer 1 and 2). 	Chapter 5, page 47
<ul style="list-style-type: none"> • Load cuvettes, disposable pipettor tips, and Simoa discs. 	Chapter 5, page 37

7.	Mix the bead reagent according to the kit instructions. Re-mix the bead reagent if it sits for >5 minutes before being loaded.	Chapter 7, page 70
8.	Set up the software for sample plates or sample tubes.	Chapter 11, page 142
9.	Load bead, detector, and SBG reagents into the instrument.	Chapter 7, page 70
	<ul style="list-style-type: none"> Place bead, detector, and SBG in a reagent rack. 	
	<ul style="list-style-type: none"> If using the handheld barcode scanner: Identify the rack position of each reagent bottle and the lane into which you plan to load them. Scan the bottle barcodes and insert the reagent rack. 	
	<ul style="list-style-type: none"> If using the on-board scanner: Enable the on-board scanner in the Load Reagents screen and select the lane into which you will load the reagent rack. Insert the rack. 	
10.	Load RGP reagent into the instrument.	Chapter 7, page 74
	<ul style="list-style-type: none"> Place RGP reagent in the RGP rack. 	
	<ul style="list-style-type: none"> If using the handheld barcode scanner: Identify the rack position of the RGP reagent and the lane into which you plan to load it. Scan the RGP bottle barcode and insert the RGP rack. 	
	<ul style="list-style-type: none"> If using the on-board scanner: Enable the on-board scanner and select the lane into which you will load the RGP rack. Insert the rack. 	
11.	Load calibrators and samples:	
	<ul style="list-style-type: none"> Assign calibrators in the software. 	Chapter 7, page 85
	<ul style="list-style-type: none"> Assign samples in the software. 	Chapter 7, page 93
	<ul style="list-style-type: none"> Insert calibrator and sample plates or tubes. 	Chapter 7, page 99

12. Start the run.

Chapter 7, page 100

13. Monitor the assay in the Current Run tab.

Chapter 7, page 104

REVOKED

5 Operating the Simoa HD-X Analyzer®

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Connecting the Simoa HD-X Analyzer to Electrical Power

The Simoa HD-X Analyzer must be connected to a dedicated electrical outlet that conforms to the instrument specifications for electrical power (see *Simoa HD-X Analyzer Site Requirements*). Your instrument will be equipped with the correct type of electrical plug for your country.



CAUTION! If you must use an extension cord to connect the instrument to its dedicated electrical outlet, use only a cord with a three-prong connector on both ends. Using a two-prong extension cord defeats the grounding circuit of the outlet, creating a hazard of shock.



CAUTION! Quanterix recommends that the dedicated outlet that you use for the instrument be equipped with a ground fault circuit interrupter.



Note! Do not disconnect the instrument from its electrical power source when the instrument is running an assay. Doing this will cause the instrument to discard the Simoa Disc and the cuvettes for the assay when you reconnect it to power.

Turning the Instrument Computer On and Off

The instrument computer is inside of the system bay. Complete all assay operations and log out of the Simoa software before you turn off the computer.

❖ To turn the computer on

Press the on/off power switch at the top of the computer, wait three minutes, power up the instrument (see “Turning the Instrument On and Off” on page 24), and open the HD-X software (see “Logging In to and Out of the Simoa Software” on page 25).

❖ To turn the computer off

Close the software and shut down the computer through Windows Shut down.



Turning the Instrument On and Off

❖ To turn the instrument on

Move the power switch to the on position: Push down the end of the switch with the dash (—). The instrument initializes itself over the next three minutes.

❖ To turn the instrument off

Move the power switch to the off position: Push down the end of the switch with the circle (○).



Note! Do not turn off the instrument during an assay run. Doing this will cause the instrument to discard the Simoa Disc and the cuvettes for the assay when you reconnect it to power.



On Off



Note! You must turn the instrument on and off once a day to power cycle instrument systems. During power cycling, the instrument performs a series of hardware and software initialization processes. Wait 15 seconds after you power down before powering up again.

If the instrument has not been power cycled in over 24 hours, the software will prompt you to turn it off with the following message:



Select “Yes” to close the software and shut down the instrument computer. You must then manually turn off the instrument by moving the power switch to the off position.

Logging In to and Out of the Simoa Software

To log in to the software, you need a user name and a password. Only a user with administrative privileges can add users to the system. For information on managing users, see “Managing Users” on page 149.

❖ To log in to the software

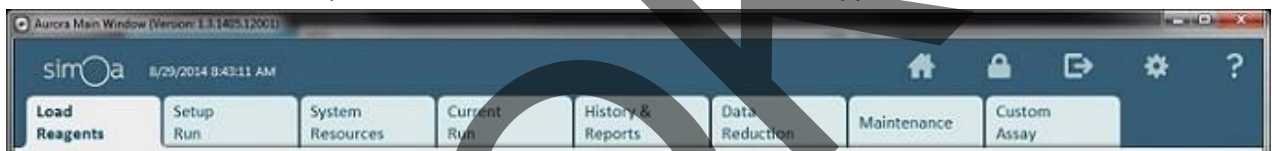
- 1 Double-touch the Simoa software icon on the instrument desktop.



- 2 Enter your user name and password > touch **Enter**.
- 3 Touch **Accept** in the Terms of Use dialog. Depending on how your system is configured, the first time you open the software, you may see the startup screen.

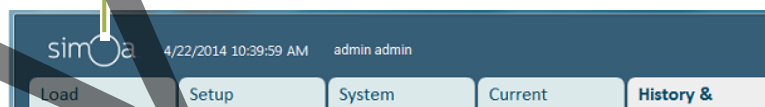


If you check the “Do not show this screen next time” checkbox, the next time you open the software, the tabbed user interface appears.



4 To minimize the software GUI, double-touch the letter O in simOa.

Double-touch here



❖ To Log Out of the software

1 Touch the Logout icon at the top right of the software screen.



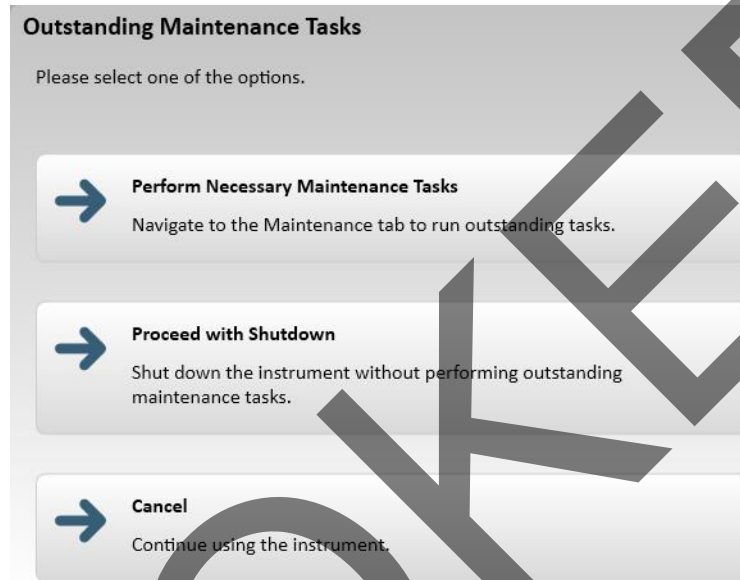
Windows 7



Windows 10

Logout icon

- 2 Click Logout to log out the current user (without closing the software) or Shutdown to log out and exit the software. Click the lock to lock the screen.
- 3 Perform maintenance tasks, exit, or cancel the log out.



Note! Do not shut down the software during an assay run or while data analysis is in progress, as this will result in run failure or the cancellation of data analysis.

Preparing the Instrument for the First Run of the Day

Before the first run of the day:

- Power cycle the instrument if it was not turned off the preceding night by turning the instrument off, waiting for 15 seconds, and turning it on again (see page 24).
- If the End of Day task was not performed the preceding night, perform it now (see page 129).
- Run the Start of Day task (see page 128).
- Refill the system fluid containers if necessary (see page 47).
- Empty the waste containers if necessary (see page 52).

Shutting Down the Instrument After the Last Run of the Day

After the last run of the day:

- Remove any leftover reagents and samples from the instrument. Recap the reagents and store at 2–8° C or as instructed in the assay kit instructions.
- Empty the solid and liquid system waste containers (see page 52).
- Perform the End of Day task (see page 129).

Operating the Barcode Scanners

You have the option to use the handheld or on-board barcode scanners to scan reagent label barcodes.

Using the Handheld Scanner

The handheld scanner is used to scan reagent, RGP, and Simoa disc barcodes.

The barcode scanner is connected to the instrument computer by default. If you need to connect it, plug it into one of the USB ports on the front of the instrument computer, which is in the system bay. The scanner beeps once when it becomes active.

When not in use, store the scanner in the holster that is mounted on the right side of the instrument.

To operate the scanner, point it at the barcode and press the scan button. The scanner transmits the barcode data to the computer, which displays it in the software.



Using the On-Board Scanner

You can use the on-board scanner as an alternative to the handheld device for scanning reagent, RGP, and sample barcodes. It cannot be used to scan Simoa discs.

The on-board scanner is located on the inside of the reagent and sample bays and reads the barcoded reagents, RGP and samples as you insert the racks into the instrument. To use the on-board scanner, you must enable it by turning on the “Enable On-Board Scanner” toggle in the Load Reagents or Setup Run tab. For more information about using the on-board scanner, see Loading Reagents and RGP into the Instrument on page 68.



Note! The on-board barcode scanner will only work if the red scanner light is visible on the inside of the reagent or sample bay. Make sure that the red light is visible before you load barcoded items into the instrument.



Red scanner light in sample bay

Opening and Closing the Instrument Bay Drawers

Pull the door/drawers to open the reagent, sample, and system bays. Keep all instrument drawers closed except when you need to open them for access into the instrument or the system bay.



Inserting and Removing a Reagent Rack

Each of the four lanes in the reagent bay can hold one reagent rack. Each reagent rack can hold six 15-mL bottles of assay reagents, for a maximum of 24 reagent bottles.

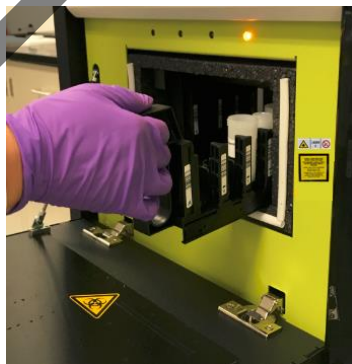
The first three positions in the reagent racks oscillate during runs and are intended to hold reagents that must be held in suspension (for example, capture bead reagent).



❖ To insert and remove a reagent rack

To insert a rack into a reagent bay lane, line it up between the lane guides and push the rack into the instrument until the catch engages.

To remove a rack, grasp the handle, push the rack in slightly to release the catch, and pull the rack straight out.



Inserting and Removing a Plate Rack

Lanes 1 to 8 in the sample bay can hold two plate racks. Each plate rack can hold two 96-well plates, for a maximum of four loaded plates. Plate rack 1 goes in lanes 1–4, and plate rack 2 in lanes 5–8.

If you are loading only one plate, place it in position 1 in plate rack 1.



CAUTION! Use only Quanterox-supplied sample plates. These plates have been verified to be nonreactive with Simoa assay reagents and to be correctly sized to work with the instrument pipettors. Quanterox cannot guarantee system performance if you use any other type of plate. For information on ordering plates, see “Purchasing Supplies” on page 2.

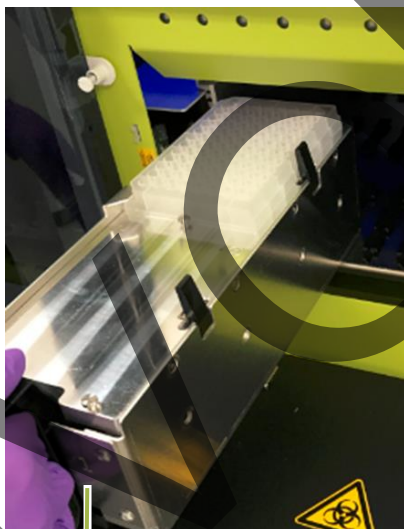
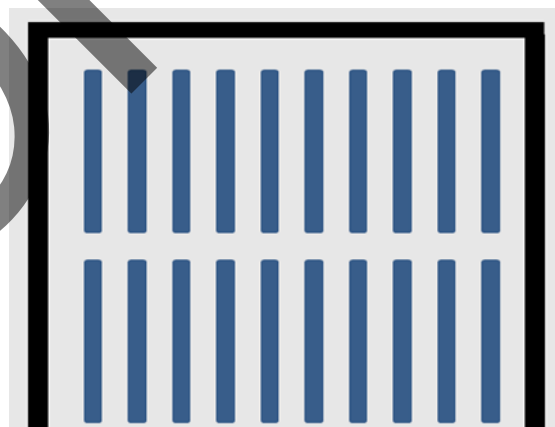


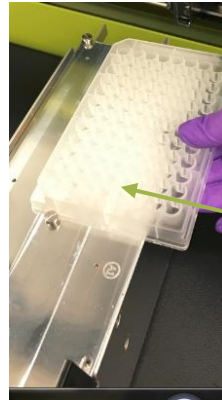
Plate rack number engraved here



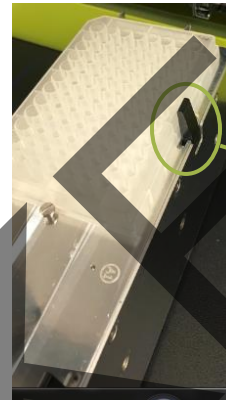
1 2 3 4 5 6 7 8 RGP
 Plate rack 1 Plate rack 2

❖ To load a plate onto a rack

- 1 Slide the plate onto the rack.
- 2 Ensure the lip of the plate sits below the head of both positioning screws.
- 3 Lift the tabs to secure the plate.



Slide plate
onto rack



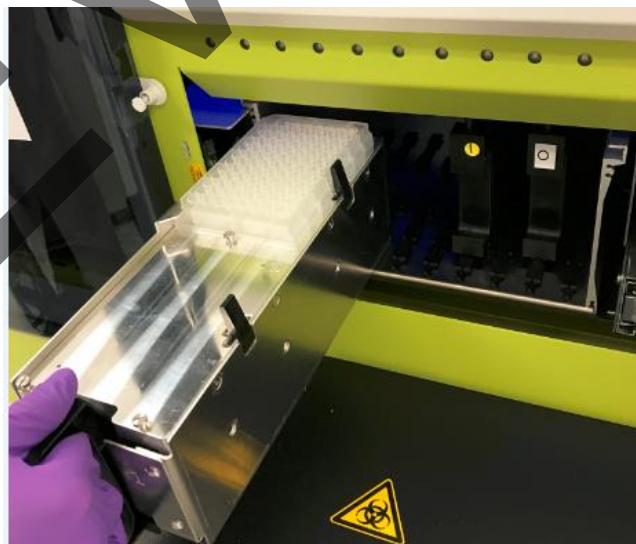
Lift tabs

❖ To insert and remove a plate rack

The plate racks are numbered and must be inserted into the instrument in a specific position. Plate rack 1 is inserted in lanes 1 to 4, and plate rack 2 in lanes 5 to 8.

To insert a plate rack, line it up over four lane guide rails in the sample bay and push the rack into the instrument until the catch engages.

To remove a plate rack, grasp the handle, push the rack in slightly to release the catch, and pull the rack straight out.

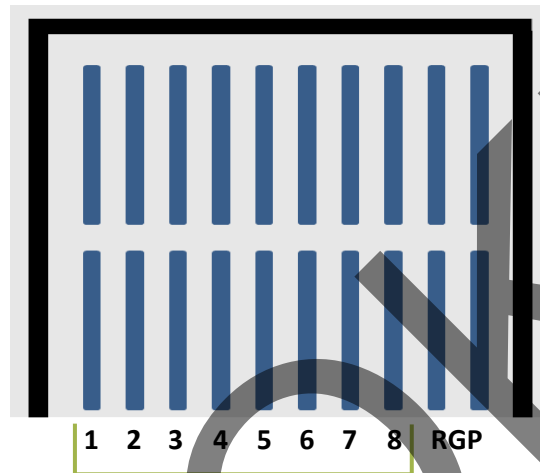


Inserting and Removing a Tube Rack

Lanes 1 to 8 in the sample bay can hold tube racks.

One tube rack can hold 12 tubes, for a maximum of 96 tubes in the sample bay. The tube racks accommodate tubes up to 100 mm tall and 10 to 16 mm in diameter.

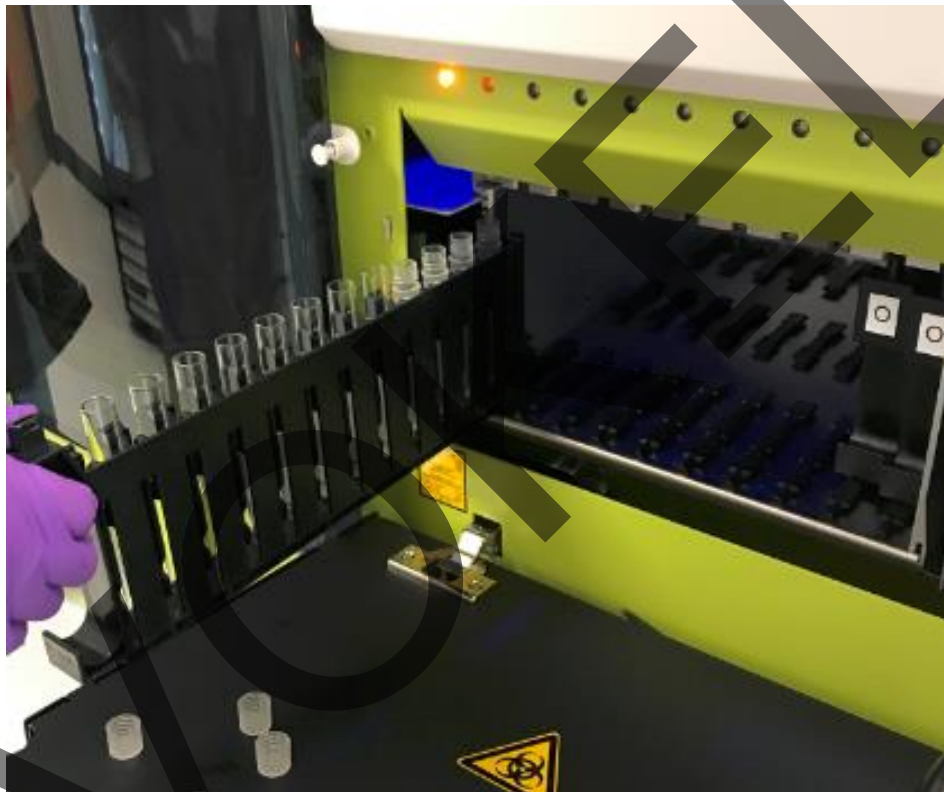
1 tube rack per lane



❖ To insert and remove a tube rack

To insert a tube rack, center the rack over a lane guide rail in the sample bay and push the rack into the instrument until the catch engages.

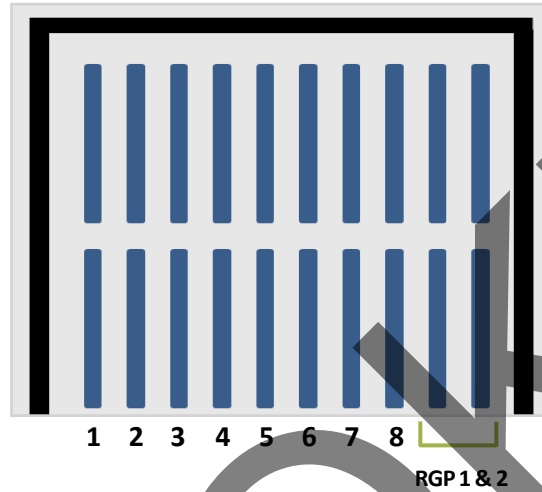
To remove a tube rack, grasp the handle, push the rack in slightly to release the catch, and pull the rack straight out.



Inserting and Removing an RGP Rack

The RGP racks are similar to tube racks. They have been customized to hold the short RGP reagent vials.

The RGP racks must be inserted into lanes RGP 1 and RGP 2 only (also termed the “substrate lanes”).



RGP racks are inserted and removed in the same manner as the tube racks (see “Inserting and Removing a Tube Rack” on page 34).



Loading Instrument Consumables



CAUTION! Use only Simoa cuvettes and Simoa disposable pipettor tips. Using any other type of cuvette or disposable pipettor tip may cause unreliable assay results or damage to the instrument. Quanterix cannot guarantee system performance if you use any other type of cuvette or disposable tip. For information on ordering consumables, see page 2.

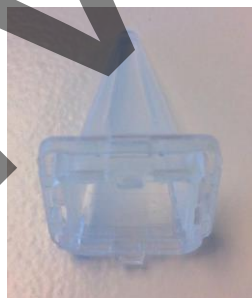
Loading Cuvettes

The instrument uses 1-mL plastic cuvettes as reaction vessels. Cuvettes are snapped together into stacks of 50 to make them easy to load.

The cuvette holder has 10 chutes into which you insert stacked cuvettes. The instrument monitors the number of cuvettes in each chute. When a chute contains only five cuvettes, that chute will be considered empty. This chute will be refilled the next time by rotating to the front of the instrument when the cuvettes are loaded.



Note! Always load full stacks of 50 cuvettes. The instrument counts partial stacks as a single cuvette.



Single Cuvette



Cuvette Stack

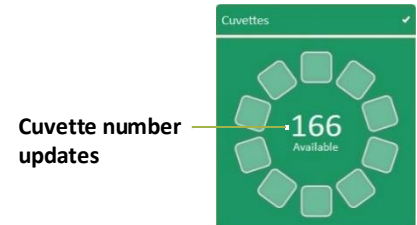
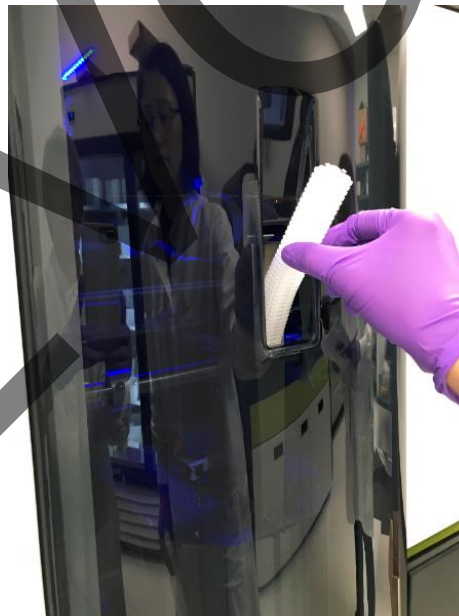
❖ To determine whether you need to load cuvettes

Touch the System Resources tab. If there are insufficient cuvettes, the cuvette diagram is red.



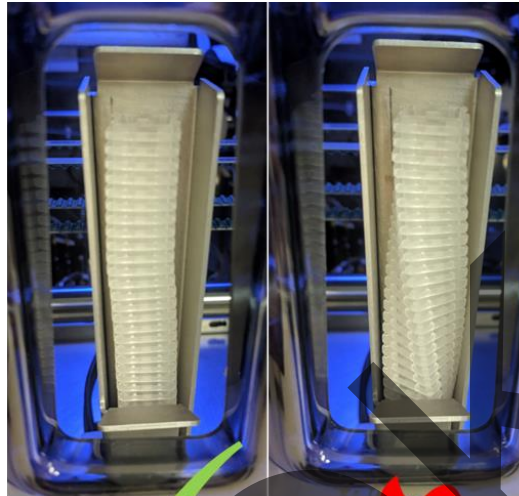
❖ To load cuvettes

- 1 Touch the System Resources tab > touch the cuvette diagram. The Solid Resources screen appears. For information on reading this screen, see page 103.
- 2 Ensure the instrument status in the lower left corner shows the system is in a “Ready” state.
- 3 Unwrap a stack of cuvettes and insert it into the cuvette holder, as illustrated below, with the point down. The cuvette number in the Solid Resources screen will update.





CAUTION! When loading the chute, ensure the stack does not become twisted (see below). Twisting can occur if the stack is dropped too quickly into the chute and may cause a jam in the cuvette loader.



- 4 The cuvette loader rotates and the cuvette stack drops into the loader. “Maintenance” appears in the lower left corner of the screen. **Wait until the rotation is finished and the system status changes from “Maintenance” to “Ready” before loading a new stack.**

Loading Disposable Pipettor Tips

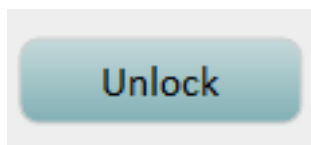
Each of the two System Resources drawers holds up to three disposable pipette tip racks. A full rack holds 96 tips, so the maximum number of tips that may be loaded at one time is 576, or 6 full racks.

Use only Quanterix-supplied tips to ensure proper instrument functioning and transfer of liquids. The instrument uses two tips per sample – one when the sample is picked up for processing and another when the sample is picked up for imaging.

When you load tips, you indicate in the software the position of each loaded tip rack. Doing so specifies the number of loaded tips and allows the software to count down from this number and to notify you if you need to load more tips.

❖ To determine whether you need to load disposable pipettor tips

- 1 Touch the System Resources tab > touch one of the Tip diagrams to view the Solid Resources screen (for information on reading the Solid Resources screen, see page 103.)
- 2 If the System Resources drawer is locked, touch the **Unlock** button located under the System Resources Drawer diagram (see page 103).



- 3 Pull out both System Resources drawers and verify that enough tips are loaded to complete the next planned assay run.
- 4 Double the total number of tests to be run in the assay to determine the number of tips required.

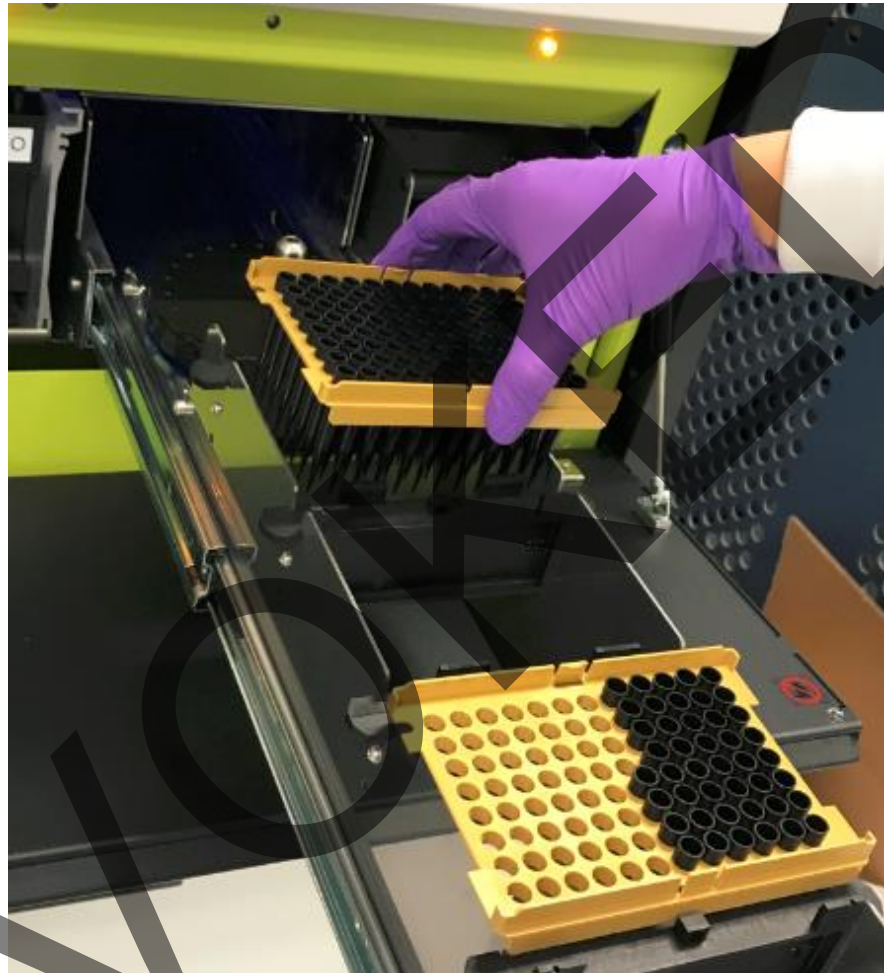
❖ To load disposable pipettor tips



CAUTION! The software only allows loading of full tip racks to prevent contamination that could result from handling tips.

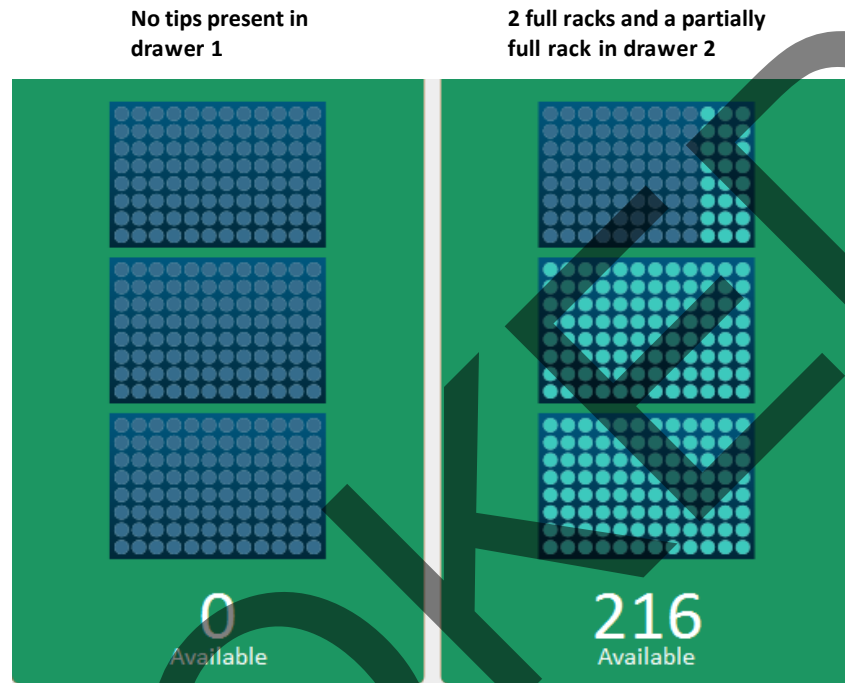
- 1 Open the System Resources drawers > remove empty tip racks.

- 2 Remove the cover from new tip racks and place them in the drawer. Fill all rack positions.



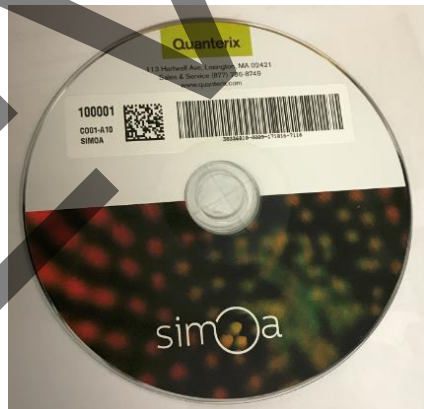
- 3 In the System Resources tab, touch one of the tip diagrams to open the Solid Resources screen.

- 4 In the Solid Resources screen, tap twice in the positions where you loaded new tip racks. The tip positions in the rack diagram turn light blue.



Loading Simoa Discs

Simoa Discs are loaded onto mounting poles at the rear of the System Resources drawers. One disc stack contains 16 discs. The maximum disc capacity is 32.



When you load a stack of discs, you scan the package barcode, which tells the software that you have loaded 16 discs. The instrument counts down from this number every time you consume a disc and notifies you if you need to load more discs.



CAUTION! Only load whole packages of Simoa Discs. Do not attempt to top off a partially used stack with new discs. To prevent disc contamination, do not remove the plastic wrapper from a stack of discs before you load the stack into the instrument.

❖ **To calculate the number of discs needed for a run**

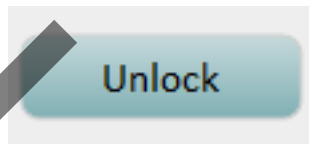
Each disc has 24 arrays, and one array is required per job. Therefore, divide the number of jobs in the run by 24 and then round up to the nearest whole disc. For example: $96 \text{ jobs} / 24 = 4 \text{ discs}$; $78 \text{ jobs} / 24 = 3.25$, round up to 4 discs.

To calculate the number of jobs in the run, multiply the number of samples by the number of replicates per sample.

- 1 Touch the System Resources tab > touch one of the disc diagrams to view the Solid Resources screen (for information on reading the Solid Resources screen, see page 103).
- 2 View the Solid Resources screen to determine if there are enough discs for your run.

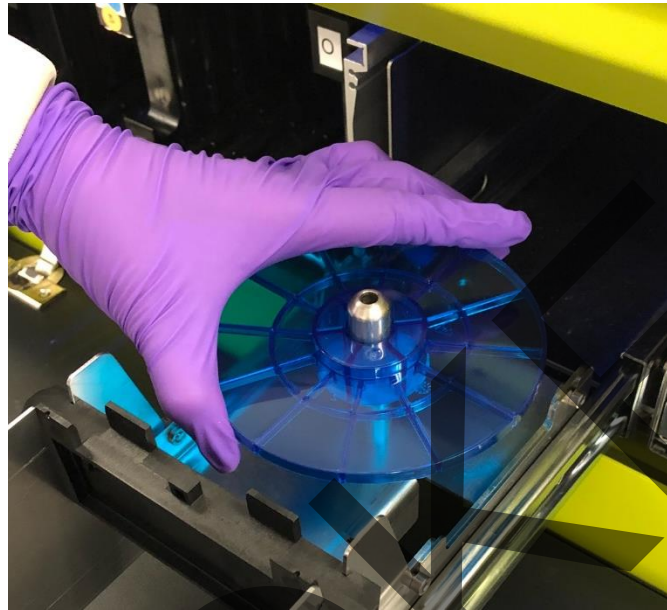
❖ **To load Simoa Discs onto an empty disc pole**

- 1 Obtain one package of discs.
- 2 When the Solid Resources screen indicates there are zero discs available, touch **Unlock** to open the appropriate System Resource drawer.



- 3 Pull out the System Resources drawer.

- 4 Remove the blue base plate from the disc pole. This plate sits at the bottom of every package of discs.



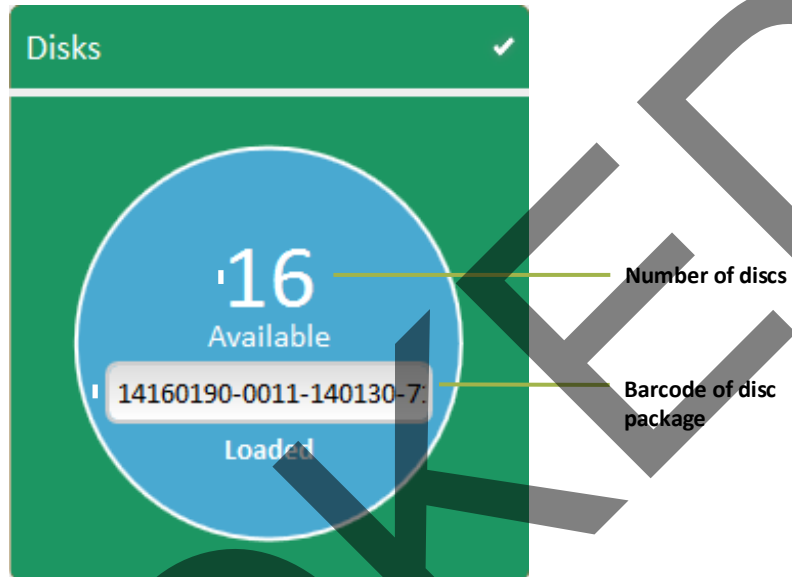
- 5 Load one package of discs:



Note! Only load a complete package of discs onto an empty disc pole. Do not add discs from the other stack.

- a Tap the disc barcode box.
- b If an existing barcode appears in the box, delete it.

- c Scan the package barcode with the barcode scanner. The barcode and the number of discs in the package (16) appear in the disc diagram.



- d Press the package onto the mounting pole. The package label should be on top.



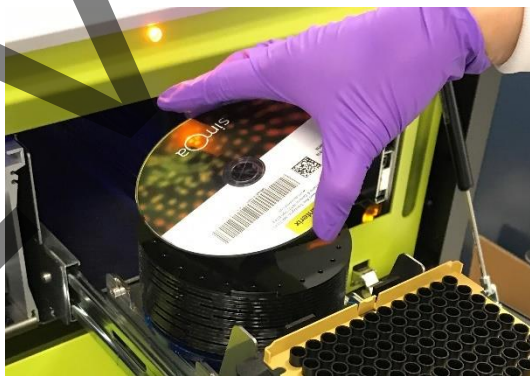
- e Line up the perforation with the top of the empty disc pole, slide the disc stack onto the pole, and orient the red pull tab toward the front.



- f Pull the red tear strip and remove the plastic disc covering completely.



- g Remove the top disc imprinted with the Simoa logo from the spindle.



Note! Take care not to shift discs against each other as they are very sensitive to scratching, which can affect image quality and data.

- 6 Push the drawer into the instrument until it catches.
- 7 Verify that the Resources screen reports the correct number of discs in both drawers.
- 8 Repeat “To load Simoa discs onto an empty disc pole” on page 43 if you need to load a second package of discs.

Refilling the System Fluid Containers

Refill the secondary system fluid containers for wash buffer 1 and deionized water and the system fluid container for wash buffer 2 before the first run of the day and whenever the container alert icons at the bottom of the software screen turn red.



“System Liquid” is deionized water

Note that for wash buffer 1 and the deionized water, you only need to fill the secondary containers, not the primary containers. The primary containers passively refill themselves from the secondary containers. Wash buffer 2 only has one container, and it must be refilled.



CAUTION! Be sure to refill the system fluid containers with the correct solution. Filling with the wrong solution may require multiple flushes with water and/or the appropriate fluid in order to clean the lines.

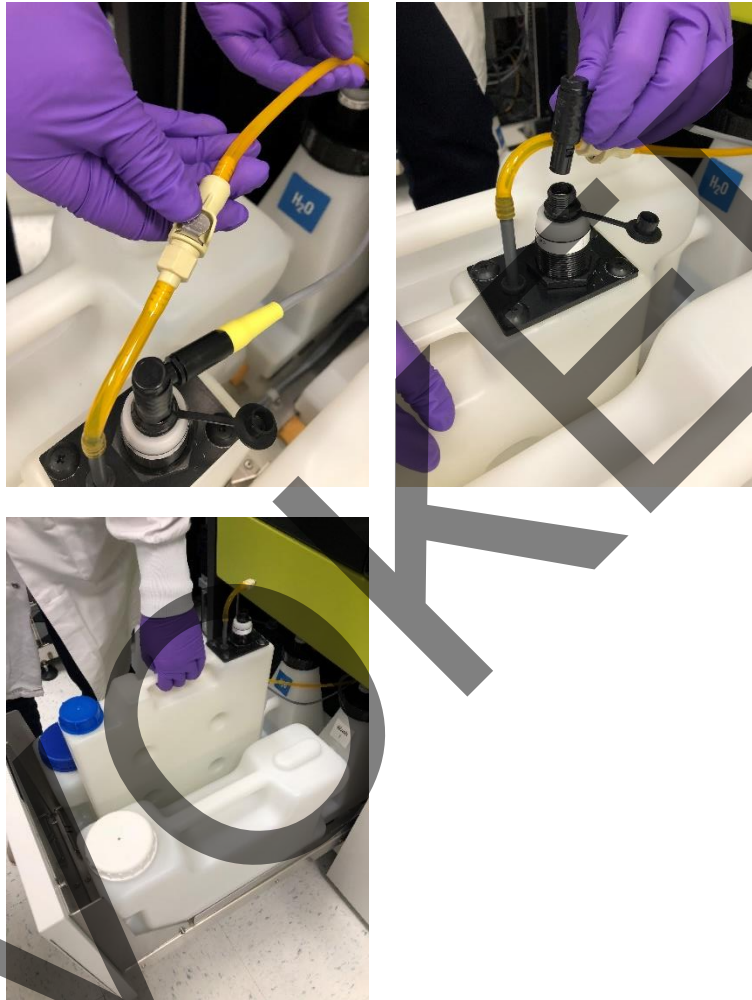
❖ To refill a secondary system fluid container or wash buffer 2 container

1 Remove the container as described below.

- To remove the secondary containers for the deionized water and the system wash buffer 1, lift the containers out of their seats. As you lift the front end of the container, the plug connecting it to the primary container disconnects.



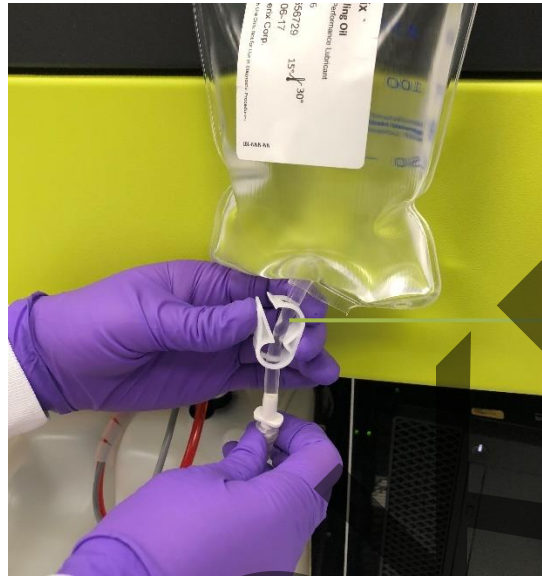
- To remove the system wash buffer 2 container, disconnect the sensor and fluid port and lift the container.



- 2 Unscrew the cap and refill the container with the correct fluid. Screw the container cap back on when you are finished.
- 3 Replace the container, as described below. **Do not force a container into its seat.** The connectors should mate easily. If you experience resistance, remove the container and try again.
 - To replace the deionized water and the system wash buffer 1 secondary containers, tip the connector end of the container down slightly and insert it into the seat.
 - To replace the system wash buffer 2 container, place it in the seat between the secondary deionized water and secondary wash buffer 1 containers. Reconnect the sensor and fluid port.

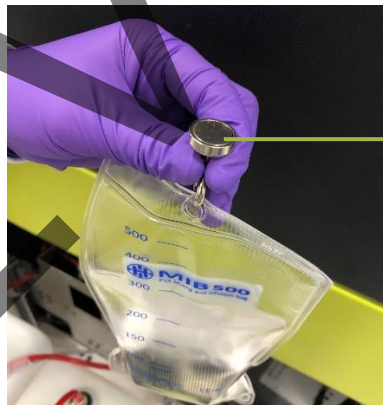
❖ To change the sealing oil bag

- 1 Close the tubing clamp just below the bag.



Tubing clamp

- 2 Unscrew the oil tubing from the bag.
- 3 Unhook the bag and remove from the system bay. Dispose of the bag according to the waste disposal policies of your organization.
- 4 Connect the hook to the new bag by piercing through the perforated circle at the top of the bag. The magnetic hook may be temporarily removed from the system bay ceiling to accomplish this.



Magnetic hook

- 5 Confirm that the tubing clamp is closed on the new bag, then unscrew the yellow cap.



- 6 Connect the oil tubing to the new bag. Before connecting, twist the connectors in the opposite direction to allow “self-threading.”
- 7 Open the tubing clamp that is below the bag.
- 8 Touch the Maintenance tab > touch the Replenish Oil task checkbox > touch **Run Task**. The task takes approximately 15 minutes to complete. During the task, the instrument software is inactive. When the software screen reactivates, the task is complete.

Emptying the Solid Waste Container

Empty the solid waste container whenever the solid waste alert icon at the bottom of the software screen turns yellow or red.



The solid waste container holds discarded cuvettes, disposable pipettor tips, and Simoa Discs.

❖ To empty the solid waste container

- 1 Pull the solid waste drawer out of the system bay.



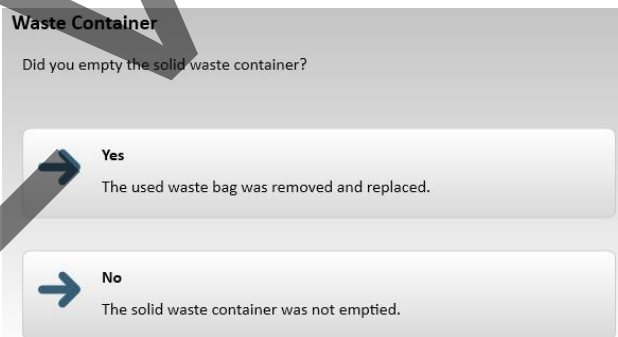
CAUTION! Do not place your hand in the opening in the shelf above the solid waste container when you pull the container drawer open. As the drawer slides out, it carries the container with it. If your hand is in the opening, your fingers or hand could be pinched between the edge of the opening and the rear top edge of the container.

- 2 Remove the waste bag > insert a new waste bag. Dispose of the waste bag according to the waste disposal policies of your organization.



CAUTION! The bag contains biohazard waste materials and should be handled with care.

- 3 Touch the System Resources tab > touch one of the disc diagrams to view the Solid Resources screen (for information on reading the Solid Resources screen, see page 103).
- 4 Push the solid waste drawer into the system bay.
- 5 Select “Yes” when you see the following dialog box to confirm that you emptied the solid waste container.



Emptying the Liquid Waste Containers

The instrument discards liquids into only one liquid waste container at a time. When a container is in use, a light in its cap turns on.

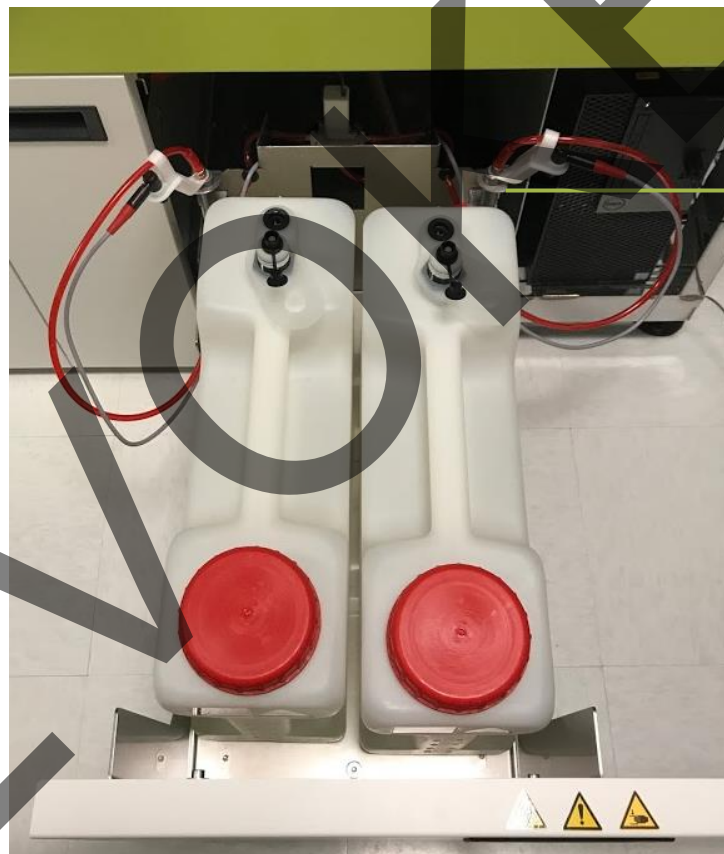
Empty both liquid waste containers before the first run of the day.

During a run, the instrument senses the fluid level in the containers with a floating sensor inside the containers.

To ensure decontamination of liquid waste, follow the instructions below to clean the waste container with the cleaner specified in your in-house decontamination procedures.

❖ **To empty the liquid waste container**

- 1 Disconnect the fluid tubing and the container sensor by pulling them straight up.
- 2 Once disconnected, place the container sensor cables into the holsters behind the waste bottles.



- 3 Remove the container and dispose of the liquid waste according to the waste disposal policies of your organization.



CAUTION! The liquid waste container holds biohazard waste and should be handled with care.

- 4 Place cleaner into the container as specified by your in-house cleaning procedures and place it back in the system bay.
- 5 Reconnect the tubing and the container sensor. When done correctly, you will hear a click when you reinsert each sensor.



Monitoring the Instrument's Internal Temperature

The instrument's internal temperature is displayed in the upper left corner of the System Resources screen. See Understanding the System Resources Screen on page 102 for more information.

The recommended internal temperature range is between 18.0°C (64.4°F) and 25.0°C (77.0°F). The system will notify you if the temperature falls outside of this range. If this happens, read the notification and follow the instructions on the screen to restore the instrument to an acceptable temperature.



CAUTION! Do not start a run while the internal instrument temperature is above or below the recommended range. Assay data may be compromised and any individual jobs processed while the temperature is out of range will be flagged.

6 Importing Assay Definitions

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Understanding Assay Definitions

The system software provides a range of pre-defined 1-, 2-, and 3-step incubation protocols that specify reagents, calibrator levels and concentrations, reagent volumes, and incubation times. (For information on the protocol steps, see the instructions in your assay kit.)

An **assay definition** is an XML document that specifies the pre-defined protocol that a given Simoa assay uses and all of the settings for the protocol. It also includes information on curve fitting and concentration result generation.

To perform a Simoa assay, you must import an assay definition in the system software.

Checking Imported Assay Definitions

Before you set up a run, make sure that the assay definition you need has been imported on your Simoa HD-X Analyzer.

❖ To check imported assay definitions

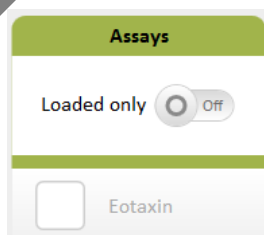
- Touch the **Custom Assay tab** > touch the **Assay** field under **General**. The **Select Assay** dialog opens, showing all assays that have been defined for the system.



Note! For this specific application, enter SARS-CoV-2 IgG for the assay shown below. This applies to subsequent images in this chapter that reflect IL-10.



- Alternatively, you can check imported assay definitions in the **Setup Run tab**. Touch the **Setup Run tab** > turn off the **Assays Loaded only** toggle to display all assay definitions that have already been imported into the system. Note that the **Loaded only** toggle is set to off by default.



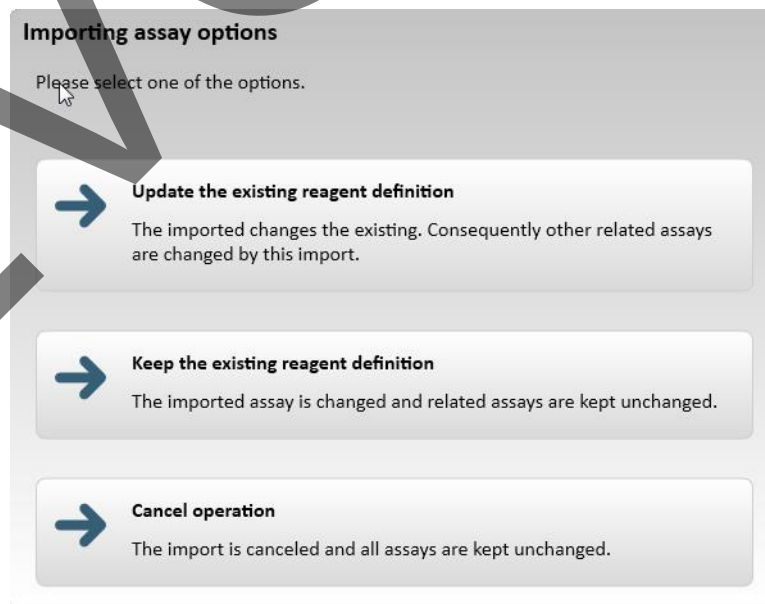
Importing an Assay Definition

You can download assay definitions from the Quanterix Customer Support website (<http://portal.quanterix.com>).

If you are importing an assay definition that shares reagents with existing assays, you can replace the existing reagent definition with the imported assay definition, or you can leave the existing definition for those shared reagents unchanged.

❖ To import an assay definition

- 1 Touch the Custom Assay tab.
- 2 In the Assay Overview screen, touch **Import**.
- 3 Navigate to the assay definition you want to import and touch **Open**.
- 4 Choose the importing options:
 - a Select **Update the existing reagent definition** to replace existing shared reagent definitions with the values specified in the imported assay definition.
 - b Select **Keep the existing reagent definition** to leave existing shared reagent definitions unchanged. The imported assay uses the values of the previously specified reagents instead of the ones in the imported assay definition.
 - c Select **Cancel** to cancel the import.



- Optionally, edit the Assay Name or the Assay Short Name.

- Touch **Save**.

Configure Assay Settings

You can specify the following assay settings that control how assays are displayed and accessed:

- Read-Only
- Visible Only to Me
- Archive

Read-Only

Depending on how roles and features are configured for your system, you may or may not see a Read-Only button on the Custom Assay screen.

Typically, administrators hide this button from users with non-administrator roles.

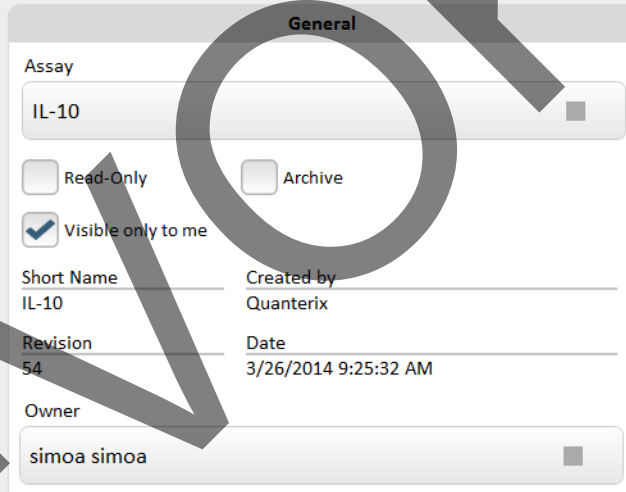
By default, imported assays are read-only and cannot be edited. Administrators can disable the Read-Only button to allow editing of assay definition values.

For more information, see “Managing Roles and Features” on page 151.

Visible Only to Me

Your assay definitions are associated with your user profile to prevent other users from accidentally modifying them and to limit the number of assays displayed on the Setup Run tab.

To protect your assays, check the Visible Only to Me checkbox.



The screenshot shows the 'General' configuration page for an assay. At the top, the assay name 'IL-10' is displayed in a search bar. Below this, there are two unchecked checkboxes: 'Read-Only' and 'Archive'. The 'Visible only to me' checkbox is checked. The 'Short Name' is 'IL-10', 'Created by' is 'Quanterix', 'Revision' is '54', and 'Date' is '3/26/2014 9:25:32 AM'. The 'Owner' is 'simoa simoa'.

When you log in, you will see all assays that you defined and all assays created by others, except for those configured as Visible Only to Me when created by other users.

Archive

Archive assays that are not currently in use to limit the number of assays displayed in the other tabs. Check the **Archive** checkbox to hide an assay or uncheck it to display it again. When you archive an assay, it is stored in the database but is not visible in the **Setup Run** tab under **Assays**.

General

Assay
IL-10

Read-Only Archive
 Visible only to me

Short Name: IL-10 Created by: Quanterix
Revision: 56 Date: 3/26/2014 9:25:32 AM
Owner: simoa simoa

7 Performing an Assay Run

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REVOKED

Steps in an Assay Run

Although the examples in this chapter show plates of samples and calibrators, the instructions apply to tubes as well.

Performing a Simoa assay run involves the following general steps:

- 1 Prepare reagents, samples, and calibrators (if you will be running them).
- 2 Select Rack or Plate mode for samples. Prepare the instrument for the run.
- 3 Place bead, detector, and SBG reagents into a reagent rack, identify the reagents by position and barcode in the software, and load the rack into the instrument.
- 4 Place one or more vials of RGP into an RGP rack, identify the vials by position and barcode in the software, and load the rack into the instrument.
- 5 Place samples, calibrators, and controls in plate or tube racks, select an assay to run on them, name the run, name the rack or plate, identify the samples, calibrators, and controls in the software, and load the racks into the instrument.
- 6 Start the run and monitor it.

When the run finishes, the results appear in the Run History table in the History & Reports tab. For information on analyzing results, see “Analyzing Run Data” on page 107.

The Role of Barcodes in an Assay Run

The labels on all assay reagents and Simoa Discs have barcodes that provide information such as reagent or item name, fill volume (as specified in the reagent definition), lot number, and expiration date. When you load barcoded reagents or RGP into the instrument, you can scan them with the handheld scanner or use the on-board scanner. When loading barcoded Simoa Discs, you must manually scan their barcodes using the handheld device.

The software uses the scanned barcode data to:

- Identify loaded reagents in screens.
- Monitor expiration dates.
- Calculate reagent and consumable consumption during an assay run and notify you when more reagents or consumables should be loaded.

Be sure to follow instructions in this guide regarding barcode scanning. If barcodes are not scanned, the instrument will not be able to perform the assay.

Manually Entering a Reagent Barcode

In the event that a reagent label is damaged and cannot be scanned, you will need

Use Approved Sample Plates and Sample Tubes

Use only Quanterix®-supplied sample plates. These plates have been verified to be nonreactive with Simoa assay reagents and to be correctly sized to work with the instrument pipettors. For information on ordering plates, see page 2.

Please contact Quanterix Technical Support for information regarding which sample tubes you may use with your HD-X instrument.



CAUTION! Quanterix cannot guarantee system performance if you use any type of plate other than the ones supplied by Quanterix.

Preparing Reagents, Calibrators, Controls and Samples

Follow the relevant test kit Instructions for Use to prepare reagents, calibrators, controls and samples.

Preparing the Instrument for the Run

For information on how to load reagent racks, plate and tube racks, and RGP racks, see pages 31 to 36.

❖ To prepare the instrument

- 1 If this is the first run of the day, perform the instrument startup procedure (see page 27).
- 2 Load Simoa cuvettes, Simoa disposable pipettor tips, and Simoa Discs into the instrument as needed. For information on how to load these consumables, see page 37.
- 3 Set up the software for sample plates or sample tubes:
 - a Touch the Settings icon at the top right of the software to open the System Settings screen.
 - b Touch **Plate Mode** or **Rack Mode** > touch **Done**.
- 4 Obtain enough reagent racks, RGP racks, and either tube racks or plate racks to perform the assay.
- 5 Make sure that the necessary assay definition is imported on the instrument. For information on checking this, see page 57.

Loading Reagents and RGP into the Instrument

Loading reagents and RGP involves:

- Placing reagent bottles into a reagent rack.
- Enabling the on-board barcode scanner or scanning reagent label barcodes with the handheld scanner.
- Inserting the rack.

Load as follows:

- Insert the rack into any lane of the reagent bay.
- Load detector and SBG reagents into any position in the rack.
- Load beads into the first three (shaking) positions.
- Load RGP into lanes labeled RGP 1 or RGP 2.

Using the Barcode Scanners

You can use the on-board or handheld scanners to scan reagent and RGP barcodes. When the on-board barcode scanner is enabled, it automatically reads the reagent/RGP barcodes and associates each barcoded bottle with its position in the rack. When the on-board scanner is turned off, you must identify the rack position of the reagent bottles in the Load Reagents screen and then scan their barcodes using the handheld scanner.

You can manually enter one or more reagent barcodes (either by using the handheld scanner or by typing it) and still use the built-in scanner for the remainder of reagents on the rack.

When the on-board scanner is turned on, it reads all reagent barcodes in the rack, including those that have already been scanned with the handheld device or typed in. If a barcode detected by the built-in scanner does not match the previously entered barcode, then the barcode and all associated reagent information will be overwritten by the on-board scanner.

The software associates the barcodes with their positions in the rack so that the instrument pipettors can be directed to the correct bottles in the loaded rack. The barcode data also allows the software to calculate running reagent volume and count down to reagent expiration dates.

Occasionally, a barcode may not be scannable because the label has been damaged in some way. When this happens, manually enter the barcode into the appropriate software field (see page 65).

Understanding the Load Reagents Screen

By default, the screen shows a reagent rack diagram, as shown below. When you touch one of the RGP lanes, the screen changes to show an RGP rack diagram. To view an example of the RGP rack diagram, see page 74.

On-board scanner toggle

**Diagram of reagent bay lanes 1 to 4
Green box shows selected lane**

Diagram of RGP 1 and RGP 2 lanes

Touch to view screen information as a graphic or table

Displays barcode number and data for the reagent in the highlighted position in the reagent rack diagram.

Graphic View **List View**

Reagent Barcode

Reagent Options

Type

Lot

Expiration

Date On-Board Stability

Volume Info

Total Volume [μ l]

Available Volume [μ l]

Dead Volume [μ l]

Reserved

Load Reagents

Enable On-Board Scanner Off

Loading Empty Empty Empty

Unknown Avail. Vol. [μ l] Unknown Avail. Vol. [μ l] Unknown Avail. Vol. [μ l] Unknown Avail. Vol. [μ l] Unknown Avail. Vol. [μ l] Unknown Avail. Vol. [μ l]

6 5 4 3 2 1

Load this end into instrument →

Assigned Not Assigned Selected

Done Loading Reagents

**Reagent rack diagram
Green box shows selected rack position**

**Rack position numbers
Position 1 is farthest from rack handle**

Complete and open Setup Run tab

Loading Reagents

Loading reagents involves placing the bead, detector, SBG, and sample diluent reagents in the reagent rack and inserting the rack into the reagent bay.

❖ To load reagents using the handheld barcode scanner

- 1 Mix the bead reagent according to instructions in the assay kit. If the bead reagent is left sitting stationary for more than five minutes before you load it, repeat this step.
- 2 Remove and set aside the caps from the reagent bottles.
- 3 Insert the bead reagent into reagent rack position 1, 2, or 3.



Note! You must insert the bead reagent in rack position 1, 2, or 3. These positions shake during the assay run to keep the beads suspended.

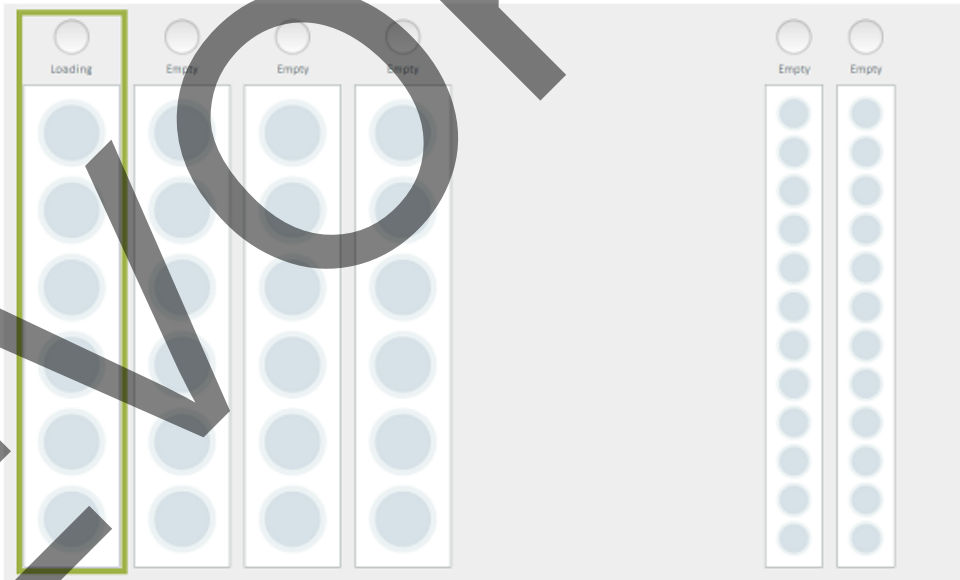
- 4 Insert the other reagents into any other reagent rack position, including unoccupied shaking positions (1, 2, or 3).

- Turn the bottles so that the reagent label barcodes are centered in the notch of the bottle holder. The barcodes must be unobstructed so that they can be scanned accurately.



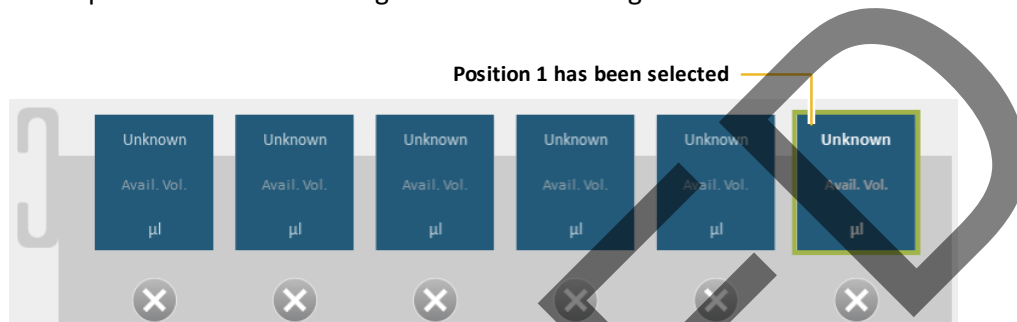
- Touch the Load Reagents tab > touch a reagent bay lane in the screen. This step identifies the lane into which you will load the reagent rack.

Reagent Bay position 1 has been selected



7 Scan the bottle barcodes:

- a In the reagent rack diagram, touch the position that corresponds to the position of the bead reagent bottle in the reagent rack.



- b Touch the reagent barcode field and scan the bead reagent barcode with the handheld scanner. The barcode and data about the bead reagent appear in the green box in the screen. If a barcode does not scan, enter it manually (see “Manually Entering a Reagent Barcode” on page 65).
- c Repeat these two steps for the rest of the reagents in the rack.

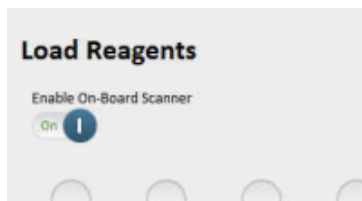


Note! If you have not inserted the bead reagent into position 1, 2, or 3, the software displays a warning, and you cannot proceed until you move the bottle into one of these positions.

- 8 Insert the rack in the reagent bay lane that you selected in step 6 until it clicks into place. The rack diagram updates to show the short name of each loaded reagent in its identified rack position. The lane diagram updates to show the positions of reagents in the selected lane. The Reagent Barcode box shows barcode data for the position that is highlighted in the reagent rack diagram.

❖ To load reagents using the on-board barcode scanner

- 1 Perform **Steps 1 through 5** as described in the previous section, “To load reagents using the handheld barcode scanner”.
- 2 Touch the Load Reagents tab. On the Load Reagents screen, turn on the “Enable On-Board Scanner” toggle.



- 3 Touch a reagent bay lane in the Load Reagents screen. After you select a lane, the on-board scanner focuses its laser on the lane and a message appears to notify you that the barcode scanner is focusing. Wait until focusing completes and the message disappears before proceeding to the next step.

Barcode scanner is focusing...

- 4 Insert the loaded rack in the reagent bay lane that you selected in step 3 until it clicks into place. As you load it, the on-board scanner reads the barcodes of each reagent bottle in the rack. The rack diagram updates to show the short name of each loaded reagent in its identified rack position. The lane diagram updates to show the positions of reagents in the selected lane. The Reagent Barcode box shows barcode data for the position that is highlighted in the reagent rack diagram.



Note! The on-board scanner will only work if the red scanner light is visible while you are loading the reagent rack. It appears on the left side of the reagent bay when the scanner is turned on.



Red scanner light

Loading RGP

RGP must be loaded into an RGP rack and placed in sample bay RGP 1 or RGP 2 only (the substrate lanes).

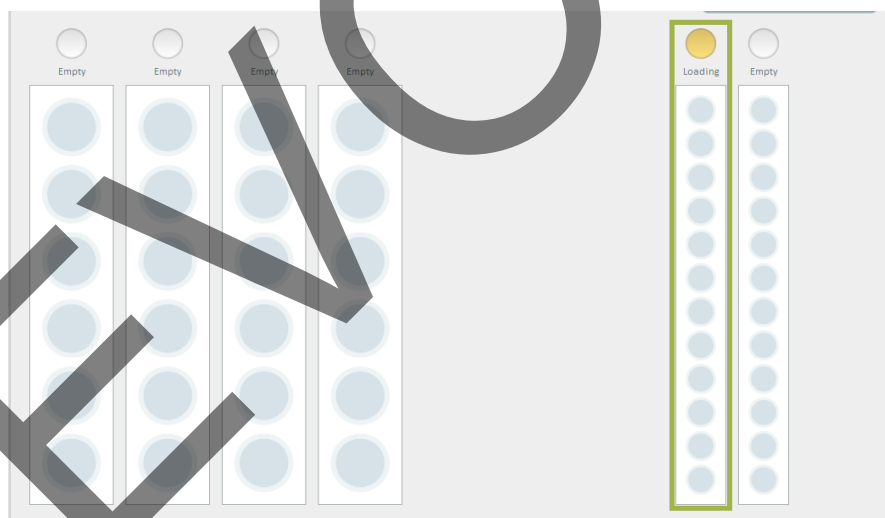
The RGP racks are customized to hold the short RGP reagent vials at optimum position for the instrument pipettors.



CAUTION! Do not load RGP into a sample tube rack. Always use an RGP rack. If you load RGP into a sample rack, the pipettor will strike the bottom of the loaded vials, possibly damaging the instrument.

❖ To load RGP using the handheld barcode scanner

- 1 Prepare RGP according to the assay kit instructions.
- 2 Remove the caps of one or more RGP vials > insert the RGP vials into the RGP rack.
- 3 Touch the Load Reagents tab > touch lane RGP 1 or RGP 2 in the screen. This step identifies the lane into which you load the RGP rack. The Load Reagents screen shows an RGP rack diagram.



Sample bay lane
RGP 1 has been
selected

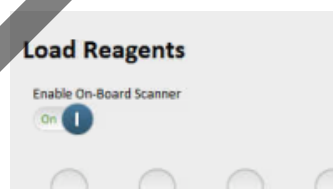
- 4 Scan the vial barcodes:
 - a In the RGP rack diagram, touch the position that corresponds to the position of the RGP vial in the reagent rack.



- b Scan an RGP vial barcode with the handheld scanner and insert the vial in position 1 of the rack. The barcode and data about the scanned vial appear in the Load Reagents screen.
 - c Repeat these two steps for the rest of the RGP vials.
- 5 Insert the rack into the lane that you selected in step 3 until it clicks into place. The RGP rack diagram updates to show each RGP vial in its identified rack position. The lane diagram updates to show the RGP vial positions in the selected lane.

❖ **To load RGP using the on-board barcode scanner**

- 1 Prepare RGP according to the assay kit instructions.
- 2 Remove the caps from one or more RGP vials > insert the RGP vials into the RGP rack.
- 3 Turn the RGP vials in the rack so that the barcodes are centered in the notch of the bottle holder. The barcodes must be unobstructed so that they can be scanned accurately.
- 4 Touch the Load Reagents tab. Turn on the “Enable On-Board Scanner” toggle in the upper left part of the screen.



- 5 Touch lane RGP 1 or RGP 2 in the Load Reagents screen. Once you have selected an RGP lane, the on-board barcode scanner focuses its laser on the lane and a message appears to notify you that the scanner is focusing. Wait until the message disappears before proceeding to the next step.

Barcode scanner is focusing...

- 6 Insert the rack into the lane that you selected in step 4 until it clicks into place. As you insert the rack, the on-board barcode scanner reads the barcode for each RGP bottle. The RGP rack diagram updates to show each RGP vial in its identified rack position. The lane diagram updates to show the RGP vial positions in the selected lane.



Note! The on-board scanner will only work if the red scanner light is visible while you load the RGP rack. The red light appears on the right side of the sample bay when the scanner is turned on.



Red scanner light

Setting Up a Run

Setting up the run involves identifying calibrators and samples in the Setup Run tab. In this tab, you can toggle between the Assign Calibrators and Assign Samples screen.


In this chapter, the examples describe a single plate that contains all of the calibrators. You can also set up your assay calibrators on multiple plates.

Once you have set up a run, you can export the assignments to a file and import the mappings the next time you run the assay.

If an experiment requires multiple assay runs, you can assign a **Batch Name** to all the plates or tube racks that you run for the experiment. This procedure allows you to group the experiment's results in the Run History screen.

Assigning calibrators and samples involves:

- Selecting an assay.
- Identifying in the software the physical location of calibrators and samples in the plates or tubes that you will be loading into the instrument.
- Associating each calibrator with one of the calibrator concentrations in the assay definition.
- Selecting the number of replicates to run. See “**Error! Reference source not found.**” on page **Error! Bookmark not defined.** for a complete explanation.
- Naming each sample, selecting the number of replicates to run, and, depending on the assay, selecting a dilution factor for the sample.
- Selecting the sample processing order (Rows or Columns).

 **Note!** You will find it easier to assign calibrators and samples if you put them into plate racks or tube racks and then place the racks in the sample bay before you begin. Do not fully insert the racks; simply place them into the bay. The procedures for assigning calibrators and samples include directions to do this.

Understanding the Assign Calibrators Screen

The Assign Calibrators screen shows a plate diagram or a tube diagram depending on how you have set up the instrument (see “Preparing the Instrument for the Run” on page 67).

For information on using the well selection tools, see “Selecting Plate Wells and Removing Assignments” on page 82.

Use Multi Select to assign replicates for multiple calibrators.

The Assign Calibrators screen in plate view appears below.

Batch name and plate barcode batch

Process in row or column order

View screen information As a graphic or table

Multi-select non-adjacent wells

Plate position diagram. See page 82 for explanation

Displays ID, concentration, and replicates for highlighted wells

Slide to view all assay definitions or loaded definitions only

Select number of Replicates per well

Touch to select a Calibrator value (assay must be selected first)

Touch one to automatically assign all calibrators

Displays sample volume for highlighted wells

Import saved calibrator and sample plate assignments

Touch to switch between Assign Calibrators and Assign Samples views

Assign Calibrators

2019-04-17 Run 1

Plate Barcode: 2019-04-17 Run 1

Plate 1

Well Processing Order: Row Column

Clear Rack Clear Selection Clear Contents Multi Select

Graphic View List View

Assays: loaded only ON

Replicates: 3

Calibrator: Pin Concentration

Assign all Calibrators: Ascending Descending

Required Sample Volume [µl]

Assign Calibrators Done with Setup Assign Samples

Legend: Sample Calibrator Empty Selected Assay Assigned Scheduled or Processed Warning

The Assign Calibrators screen in tube rack view appears below. It is identical to the screen for plates except that it shows a tube rack diagram, a location diagram for the eight sample bay lanes, and the Enable On-Board Scanner toggle.

Tube position diagram (sample bay rows 1-8)
Green box shows selected lane

On-board barcode scanner toggle

Touch to specify replicates



Tube rack diagram for selected lane

Tracks volume of calibrator used

Understanding the Assign Samples Screen

The Assign Samples screen in **plate view** appears below.

The screenshot shows the 'Assign Samples' interface. On the left, there are input fields for 'Batch name and plate barcode fields' (2019-04-17 Run 1, Plate Barcode: 2019-04-17 Run 1, Plate 1) and buttons for 'Clear Rack', 'Clear Selection', and 'Clear Contents'. A 'Multi Select' toggle is set to 'Off'. The main area is a 12x8 grid of wells. A 'Plate position diagram' is shown above the grid, with callouts for 'Process in row or column order' and 'Multi-select non-adjacent wells'. A 'Graphic View' button is also present. On the right, a list of assays is shown, with a 'Slide to view all assay definitions or loaded definitions only' callout. Below the assays is a 'General' panel with 'Replicates per Well' and a 'Dilutions' table. A 'Required Sample Volume [µl]' field is also present. At the bottom right, there is a 'Generate Sequential IDs' section with 'Profiles' and 'Start Number' fields, and 'Row Order' and 'Column Order' radio buttons. A legend at the bottom left identifies well states: Sample, Calibrator, Empty, Selected, Assay Assigned, Scheduled or Processed, and Warning. At the bottom right, there are buttons for 'Export Layout', 'Assign Calibrators', 'Done with Setup', and 'Assign Samples'. A large 'DRAFT' watermark is overlaid on the screen.

Batch name and plate barcode fields

Process in row or column order

Multi-select non-adjacent wells

Graphic View

List View

Plate position diagram
See page 82 for explanation

Touch to view screen information as a graphic or table

Slide to view all assay definitions or loaded definitions only

Touch to select sample replicates and dilutions

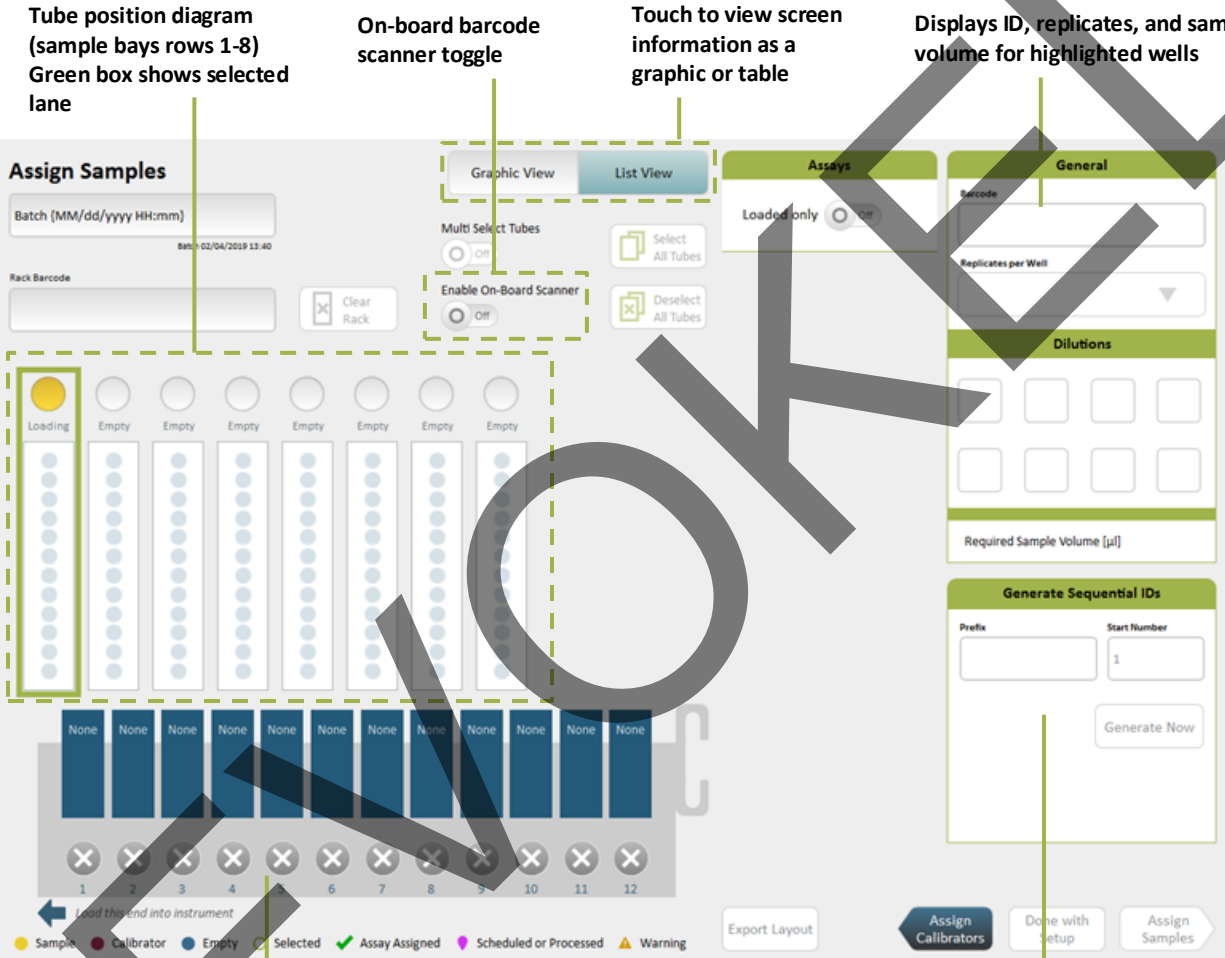
Displays sample volume for highlighted wells

Autoname tool

Export calibrator and sample assignments

Touch to switch between Assign Calibrators and Assign Samples view

The Assign Samples screen in tube rack view appears below. It is identical to the Assign Samples screen for plates except that it shows a tube rack diagram, a location diagram for the eight sample bay lanes, and the Enable On-Board Scanner toggle.



Tube position diagram (sample bays rows 1-8) Green box shows selected lane

On-board barcode scanner toggle

Touch to view screen information as a graphic or table

Displays ID, replicates, and sample volume for highlighted wells

Tube rack diagram for selected lane

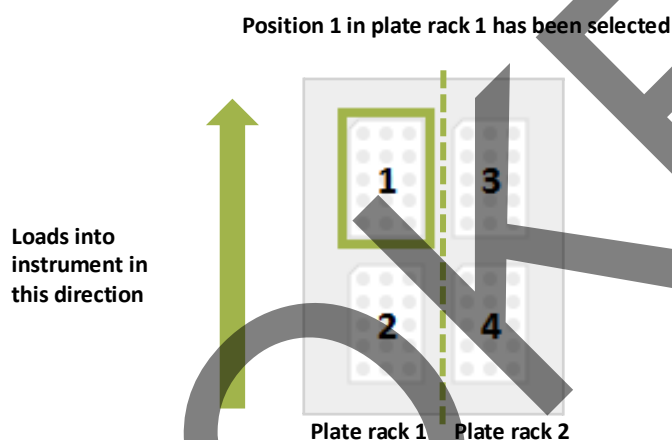
Autoname tool

DRAFT

Understanding the Plate Position Diagram

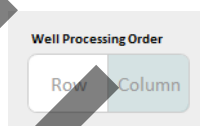
The plate position diagram identifies the lanes into which a plate is loaded and the position of a plate in those lanes. Plate rack 1 may only be loaded into lanes 1–4, and plate rack 2 into lanes 5–8. There are two plate positions for each plate rack.

Selections in the diagram apply to both the Assign Calibrators and the Assign Samples screens. For example, if you select plate 2 in the Assign Calibrators screen and then switch to the Assign Samples screen, the plate remains selected.



Selecting Processing Order

You can process your calibrators and samples by Row (left to right) or Column (top to bottom). This is a batch-wide setting that applies to all jobs in a run, even if on separate plates. If you have multiple plates, they are processed in ascending numerical order from 1 to 4.



Selecting Plate Wells and Removing Assignments

You must select plate wells to assign calibrators or samples to them. After you have made assignments, you can deselect or select wells without changing their assignments.

- To select a block of wells, touch the plate and draw a marquee around the wells.
- To select individual, nonadjacent wells, turn on **Multi Select** > touch the wells.

- To remove specific selections, touch the plate and draw a marquee around the wells > touch **Clear Selected**.
- To remove all selections and assignments on a plate, touch **Clear Rack**.
- To remove assignments from specific wells, select the wells > touch **Clear Contents**.

Understanding the Required Sample Volume Field

The Required Sample Volume field reports the total amount of sample that will be consumed by all assignments that you have made. This field can help you monitor sample consumption so that you do not assign more sample volume than you have placed in a plate or a tube.

Understanding the Autaname Tool

Use the Autaname tool to quickly name a range of plated samples. This tool creates names of the following format:

[alphanumeric prefix up to nine characters long][first number in series]

For example: PSA02Mar1, PSA02Mar2, PSA02Mar3

Autanames are applied to a set of selected samples in row order or column order.

❖ To generate sequential IDs

- 1 Enter a text or numeric prefix in the Prefix field.
- 2 Touch **Row Order** or **Column Order**.
- 3 Optionally, change the Start Number.
- 4 Touch **Generate Now**.

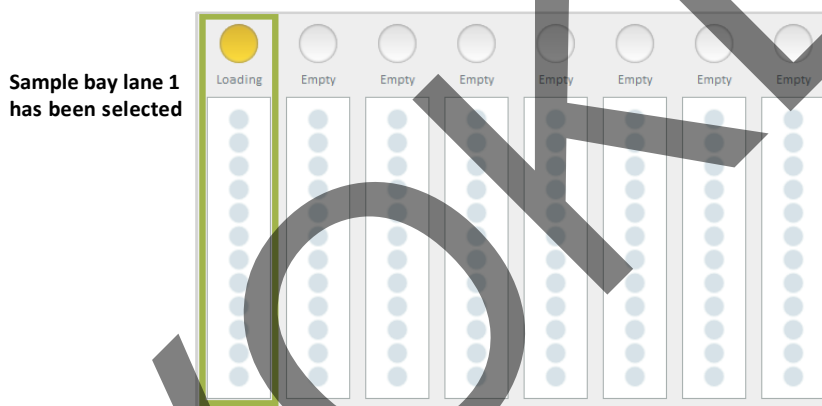
Scanning Sample Barcodes

When loading samples in tube racks, you can use the handheld or on-board scanners to enter sample barcode information.

Note that if you are using the on-board scanner, you must assign all sample properties (including assay definition, replicates, and dilutions) prior to loading the barcoded sample tube rack. These properties cannot be modified once you insert the rack.

❖ To scan samples with the handheld scanner

1. In the **Setup Run tab > Assign Samples** screen, select the lane into which you plan to load the sample tube rack.



2. Touch the position of the sample tube in the tube rack diagram.



3. Scan the sample barcode with the handheld scanner.

❖ To scan samples with the on-board scanner

1. In the **Setup Run tab > Assign Samples** screen, turn on the **Enable On-Board Scanner** toggle.
2. Select the lane into which you plan to insert the tube rack.

3. Wait for the on-board scanner to focus its laser on the lane. You will see a notification informing you that the laser is focusing. Do not navigate to a new tab while the scanner is focusing, as this will halt the process.

Barcode scanner is focusing...

4. Insert the rack into the sample bay lane that you selected. The on-board scanner will read the sample barcodes as you insert the rack.

Assigning Calibrators

To assign calibrators, you identify in the software the physical location of all calibrators in a plate or tube rack, and then you associate each calibrator with a calibrator concentration in the assay definition.

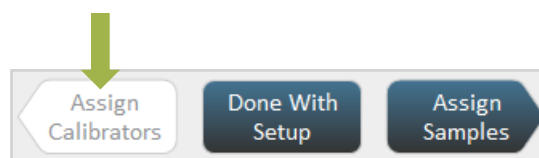


Note! You can import a saved map of calibrator and sample assignments. For information about this option, see “Importing and Exporting a Layout” on page 100. The Import button appears on the Assign Calibrators screen. The Export button appears on the Assign Samples screen.

Assigning Calibrators in Plates

Make sure that you have plated the calibrators correctly. You can assign multiple calibrators to a plate up to the capacity of the plate well. See “Preparing Reagents, Calibrators, Controls, and Samples” on page 66.

1. Open the Assign Calibrators screen:
 - If you are in the Assign Samples screen, touch **Assign Calibrators**.
 - If you are not in the Assign Samples screen, touch the Setup Run tab > touch **Assign Calibrators**.



2. Recommended: Place prepared plates of calibrators and samples in plate racks and put the racks into the sample bay, but do not fully insert them.

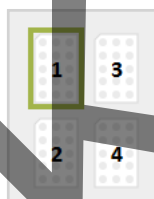


Note! If you choose to insert the plate racks after you finish assigning calibrators and samples, **you must make sure** that the plate containing the calibrators is loaded in the position that you identify for it in the next step of this procedure. Placing the racks into the sample bay now makes it easier to correctly identify plate position.

3 Name the plate that contains the calibrators:

- a Touch the plate position that matches the location of the plate that contains the calibrators. For information on reading the plate position diagram, see page 82.

Plate 1 in plate rack 1 has been selected



- b Enter a name for the batch and a plate name > press **Enter**. You can use any combination of letters and numbers.

Batch name

Assign Calibrators

Exp 230

Plate Barcode

Plate name

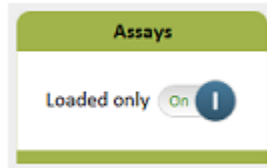
PSA27Jan14

4 Perform the following steps to associate each set of calibrator replicates with the calibrator values in the assay definition, in ascending or descending order.

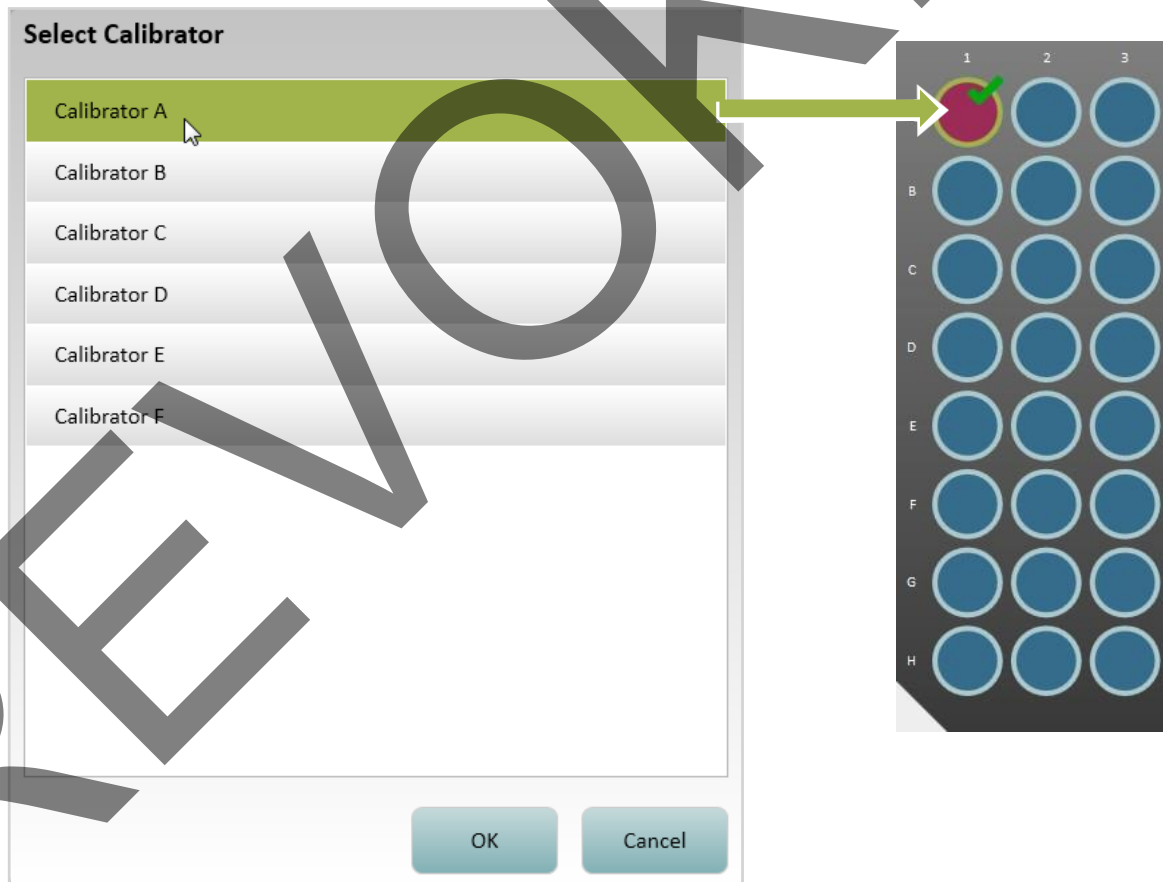
- a Identify the first of the plated calibrators:
 - ♦ **If you have plated only one set of calibrators:** Touch the well that contains the first calibrator in the set.
 - ♦ **If you have plated two or more replicates:** Touch the first calibrator for one of the replicates. (You will repeat the entire assignment procedure to assign the remaining replicates.)

b Identify the assay:

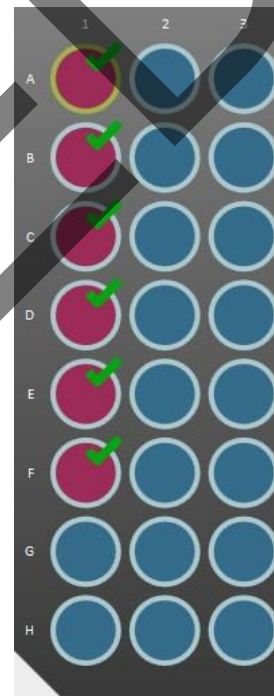
- ◆ **If you have already loaded reagents:** Set the Assays Loaded only button to **On** so that the screen only displays assay definitions that contain the loaded reagents. Select your assay.



- c Touch the highest or lowest calibrator > touch **OK**. The well that you selected turns red and a checkmark appears on it, signifying that an assay has been assigned to the well.



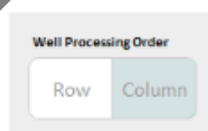
- d Touch **Ascending** or **Descending** under Assign All Calibrators. The software assigns all calibrators that are defined for the assay in the specified order, beginning with the selected well. The plate diagram shows the assignments. To view the concentration that is assigned to a well, touch the well and view the Calibrator box.
- e Repeat to assign all sets of calibrator replicates.



5 Select the processing order for the plates. This is a batch-wide setting that applies to all jobs in the run, even if on separate plates.

- Select **Row** to process the calibrators from left to right
- Select **Column** to process the calibrators from top to bottom.

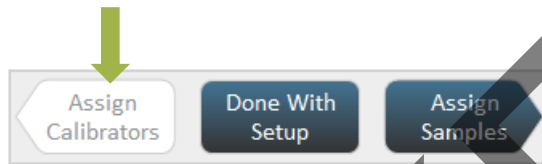
If you have multiple plates, they are processed in ascending numerical order from 1 to 4.



6 If the plates contain samples, assign them now. See “Assigning Samples in Plates” on page 85. If you are finished, see “Loading Calibrators/Samples into the Instrument” on page 99.

Assigning Calibrators in Tubes

- 1 Open the Assign Calibrators screen:
 - If you are in the Assign Samples screen, touch **Assign Calibrators**.
 - If you are not in the Assign Samples screen, touch the Setup Run tab > touch **Assign Calibrators**.



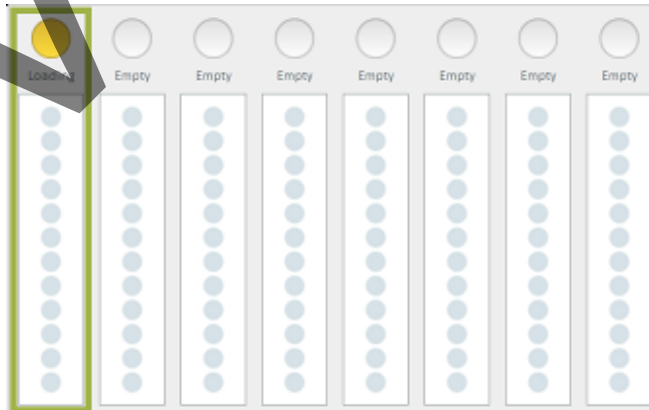
- 2 Recommended: Place tubes of prepared calibrators and samples in tube racks and put the racks into the sample bay, but do not fully insert them.



Note! If you choose to insert the tube racks after you finish assigning calibrators and samples, **you must make sure** that tubes containing the calibrators are loaded in the positions that you identify for them in the next step of this procedure. Placing the racks into the sample bay now makes it easier to correctly identify tube positions.

- 3 In the tube position diagram, touch the lane that contains the calibrators.

Sample bay lane 1 has been selected



- 4 Enter a batch name and a tube rack name > touch **Enter**. You can use any combination of letters and numbers.

Assign Calibrators

Batch name

Rack Barcode

Rack name

- 5 Perform the following steps to associate tubes of calibrators with the calibrator values in the assay definition, in ascending or descending order.

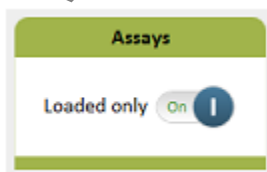
a In the tube rack diagram, touch the position that holds the first calibrator in the rack.

Tube 1 has been selected



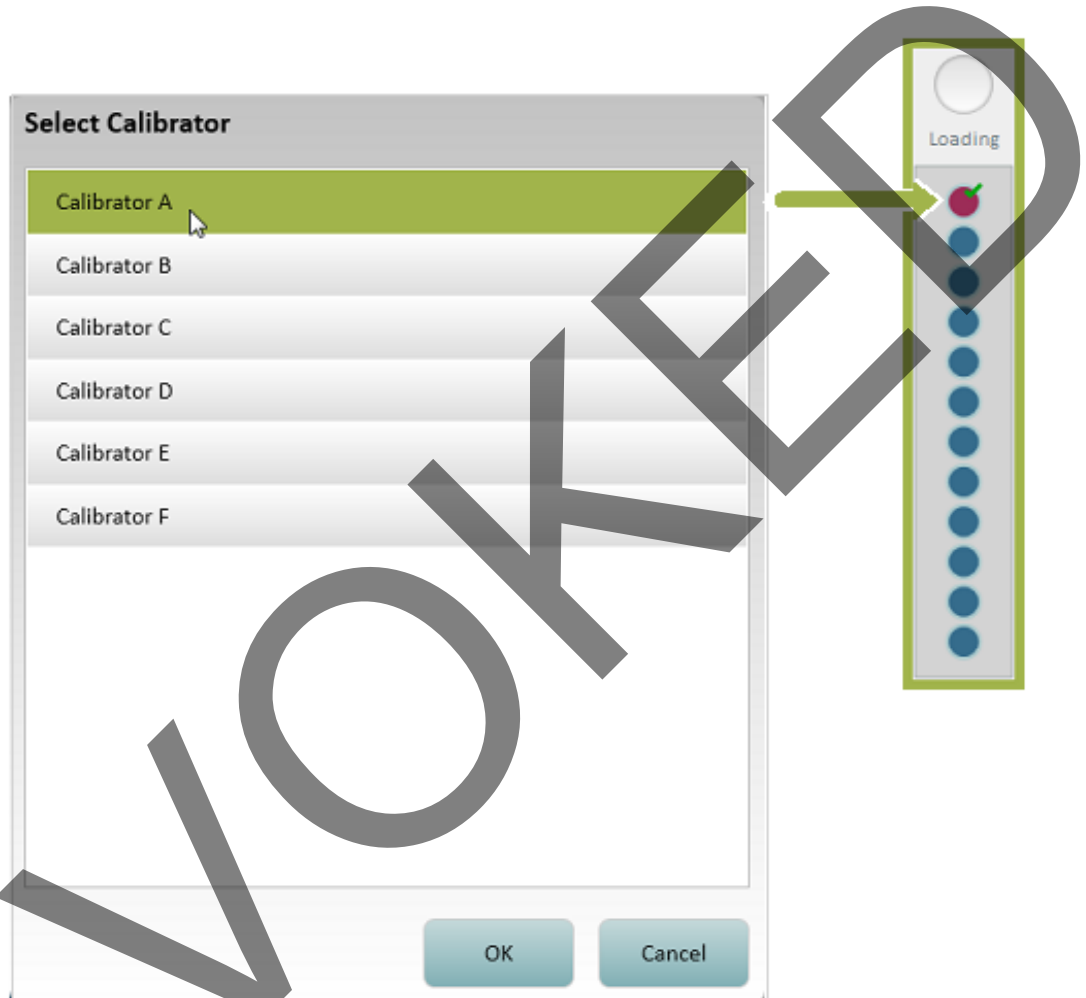
b Identify the assay:

- ◆ **If you have already loaded reagents:** Set the Assays Loaded only button to **On** so that the screen only displays assay definitions that contain the loaded reagents. Select your assay.



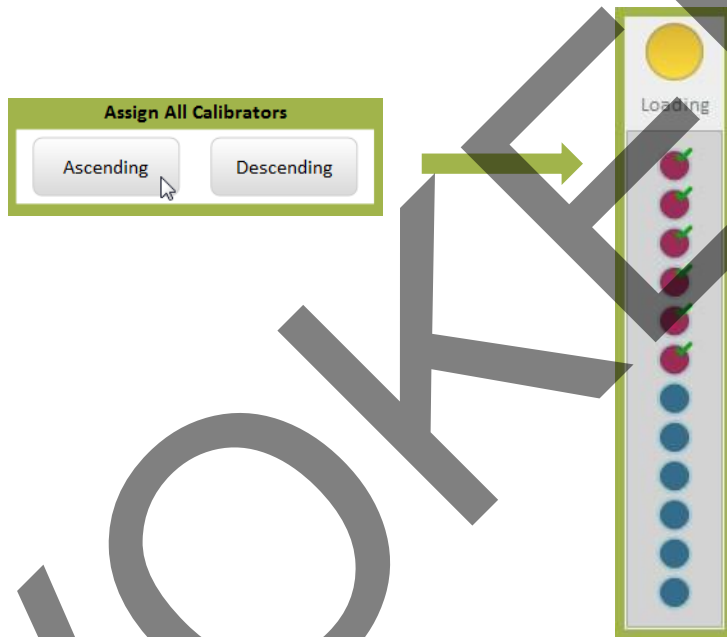
c In the Select Calibrator dialog, touch the position that contains the highest or lowest calibrator > touch **OK**.

In the rack position diagram, the tube position that you selected turns red and a checkmark appears on it, signifying that an assay has been assigned to the tube.



- d Touch **Ascending** or **Descending** under Assign All Calibrators. The software assigns all calibrators that are defined for the assay in the specified order, beginning with the selected tube position. The tube position diagram shows the assignments.

To view a calibrator assignment, touch a tube in the tube rack diagram. The calibrator and concentration appear in the Calibrator box.



- 6 If you have loaded tubes of samples, assign them now. See “Assigning Samples in Tubes” on page 97. If you are finished, see “Loading Calibrators/Samples into the Instrument” on page 99.

Assigning Samples

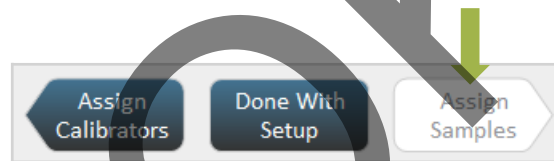
To assign samples, you identify the physical location of all samples in a plate or tube rack, and then you specify the number of replicates to run for each sample and dilutions (if the assay definition allows).



Note! You can also import a saved layout of sample (and calibrator) assignments. For information about this option, see “Importing and Exporting a Layout” on page 100.

Assigning Samples in Plates

- 1 Open the Assign Samples screen:
 - If you are in the Assign Calibrators screen, touch **Assign Samples**.
 - If you are not in the Assign Calibrators screen, touch the Setup Run tab > touch **Assign Samples**.



- 2 Recommended: Place prepared plates of calibrators and samples in plate racks and put the racks into the sample bay, but do not fully insert them.



Note! If you choose to insert the plate racks after you finish assigning calibrators and samples, **you must make sure** that plates containing the samples are loaded in the positions that you identify for them in the next step of this procedure. Placing the racks into the sample bay now makes it easier to correctly identify plate positions.

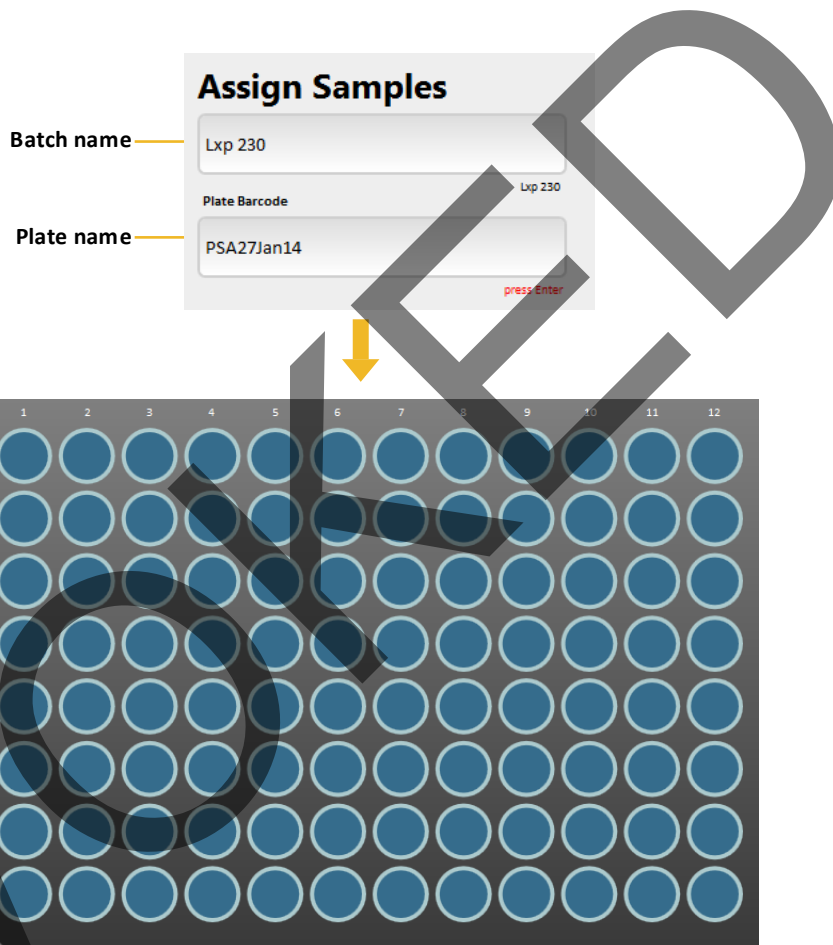
- 3 In the plate position diagram, touch a plate that contains samples. (For information on reading the plate position diagram, see page 82.)

Plate 1 in plate rack 1 has been selected



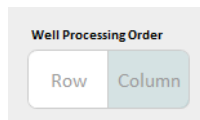
- a If a name appears in the Plate Barcode field, the plate is already named, and you can skip to step 4. This is true if you touch a plate that contains calibrators that you have previously assigned.

- b If the Plate Barcode field is empty, enter a batch name and a plate name > touch **Enter**. You can use any combination of letters and numbers.



- 4 Select the processing order for the plates. This is a batch-wide setting that applies to all jobs in the run, even if on separate plates.
- Select **Row** to process the samples from left to right.
 - Select **Column** to process the samples from top to bottom.

If you have multiple plates, they are processed in ascending numerical order from 1 to 4.



- 5 Assign an ID to the samples in the selected plate (see page 83 for information on autonames):
- a Select a range of wells (for information on selecting wells, see page 82).

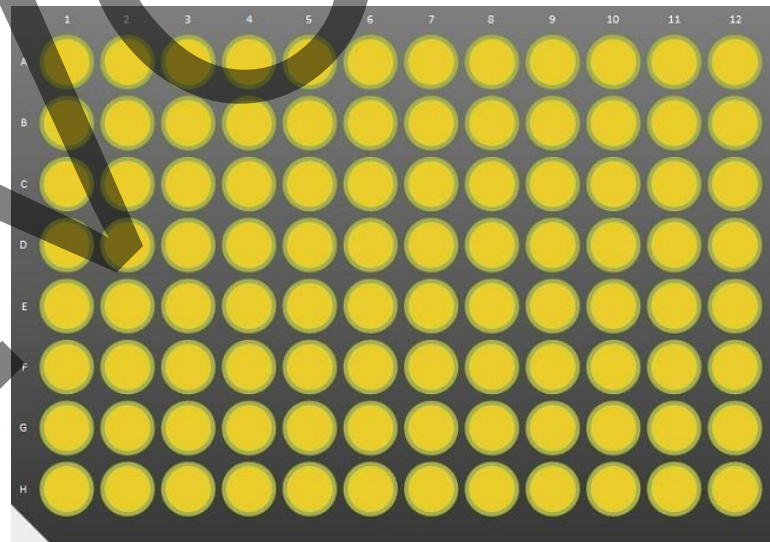
- b In the Autaname tool, enter a prefix string, which will apply to every name, and a starting number.
- c Touch **Row Order** or **Column Order**.
- d Touch **Generate Now**. The software assigns a name to the selected wells and they turn yellow.

Generate Sequential IDs

Prefix

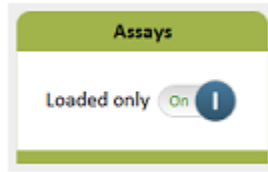
Start Number

Row Order Column Order



- 6 Specify an assay, replicates, and dilutions for samples:
 - a Select plate wells.
 - b Specify an assay (checkmarks appear on the selected wells after you do this):

- ◆ **If you have already loaded reagents:** Set the Assays Loaded only button to **On** so that the screen only displays assay definitions that contain the loaded reagents. Select your assay.



c Specify replicates and dilutions:

- ◆ Select a replicate number from the drop-down list.
- ◆ Touch the dilution that is specified in the assay kit instructions.
- ◆ Touch any other dilution that has a checkmark to remove the checkmark.

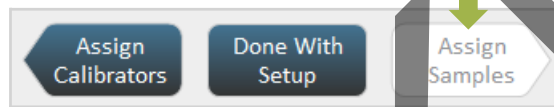


- 7 If you have more than one plate of samples, repeat steps 3 to 5 to assign all the samples.

- 8 If you need to assign calibrators, see “Assigning Calibrators in Plates” on page 85. If you are finished, see “Loading Calibrators/Samples into the Instrument” on page 99.

Assigning Samples in Tubes

- 1 Open the Assign Samples screen:
 - If you are in the Assign Calibrators screen, touch **Assign Samples**.
 - If you are not in the Assign Calibrators screen, touch the Setup Run tab > touch **Assign Samples**.



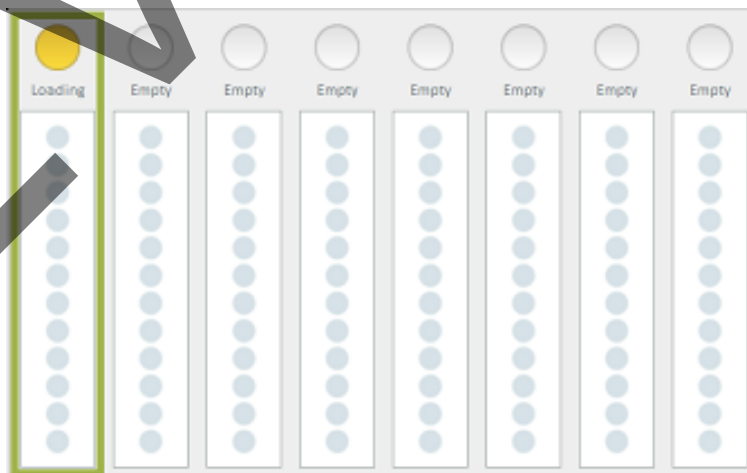
- 2 Recommended: Place prepared tubes of calibrators and samples in tube racks and put the racks into the sample bay, but do not fully insert them.



Note! If you choose to insert the tube racks after you finish assigning calibrators and samples, **you must make sure** that tubes containing the samples are loaded in the positions that you identify for them in the next step of this procedure. Placing the racks into the sample bay now makes it easier to correctly identify tube positions.

- 3 Touch a lane that holds a rack containing sample tubes.

Sample bay lane 1 has been selected



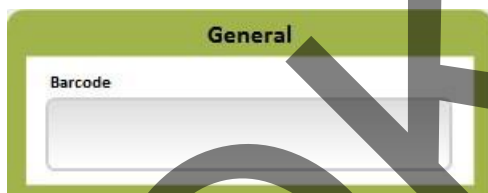
4 Assign an ID to the samples in the selected rack (see page 83 for information on autonaming):

a In the tube rack diagram, select a tube position.

Tube 1 has been selected



b Enter a Barcode ID for the tube. This step is not necessary if you already assigned an ID to the samples, as specified in Step 4 above.

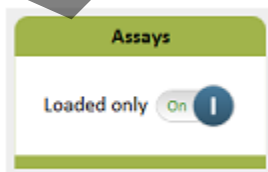


c Physically load the rack. See “Loading Calibrators/Samples into the Instrument” on page 99.

d Repeat these steps to name all tubes in the rack.

5 Select an assay for the selected tube:

- ◆ **If you have already loaded reagents:** Set the Assays Loaded only button to **On** so that the screen only displays assay definitions that contain the loaded reagents. Select your assay.



6 Specify replicates and dilutions for the selected tube:

a Select a replicate number from the drop-down list.

b Touch the dilution that is specified in the assay kit instructions.

- c Touch any other dilution that has a checkmark to remove the checkmark.

The screenshot shows a software interface for setting assay parameters. At the top, there is a dropdown menu labeled 'Replicates per Well' with the value '1' selected. Below this is a section titled 'Dilutions' containing a 2x4 grid of buttons. The first button is labeled 'Neat', the second '4x', and the others are unlabeled. The '4x' button has a blue checkmark. At the bottom of the interface, there is a field labeled 'Required Sample Volume' with the value '25'.

- 7 Repeat steps 3 to 6 to assign all tubes of samples.
- 8 If you need to assign calibrators, assign them now. See “Assigning Calibrators in Tubes” on page 89. If you are finished, see “Loading Calibrators/Samples into the Instrument” on page 99.

Loading Calibrators/Samples into the Instrument

The final step in assigning calibrators and samples is to load them into the instrument so that the software accepts the assignments.

❖ To load assigned calibrators and samples

- 1 Optional: Save the layout. In the Assigned Samples screen, touch **Export Layout** > save the file in the Save As dialog.
- 2 If you have not already done so, place the plate racks or tube racks into the sample bay.

Note! Place the racks in the sample bay according to the assignments that you have made. If you do not put plates or tubes in the positions that you identified for them, the instrument will not be able to locate the calibrators or samples correctly, and your assay results will be undefined.

- 3 Push the plate racks or tube racks into the sample bay until they latch into place.
- 4 Recommended: Touch **List View** to see a list of everything you have loaded. This allows you to check for the correct number of replicates, dilutions, and other consumables.

- 5 Touch **Done With Setup**. The All Resources screen in the System Resources tab opens. See “Starting a Run” on page 100 to initiate the run.



Importing and Exporting a Layout

You can export a finished layout of calibrator and sample assignments to a file and then re-use it the next time you run the assay.

❖ To export a layout

In the Assign Samples screen, touch **Export Layout** > name and save the file. Do not change the specified file extension (.csv).

Export Layout

❖ To import a layout

In the Assign Calibrators screen, touch **Import Layout** > open the file.

Import Layout

Starting a Run

You can begin a run immediately after you assign calibrators and samples if sufficient system resources are available and no mandatory maintenance tasks are pending.

When you finish assigning calibrators and samples, the software calculates the system resources needed to run the assay and posts resource status on the System Resources screen. If enough consumables are available to run the loaded calibrators and samples, the supplies of wash buffer are sufficient, the liquid waste container is not full, and no maintenance tasks are pending, you can start the run.

❖ To start a run

- 1 Touch **Done With Setup** in the Assign Calibrators or Assign Samples screen. The System Resources tab opens.
- 2 If the button is active, touch **Start Run**.



If the button is inactive, touch **Resource Details** to display a complete readiness status (including status of beads, detectors, SBG, and RGP). You may need to perform some of the following tasks to activate the **Start Run** button again:

- If the Maintenance task icon is red, touch the Maintenance icon and perform any pending mandatory tasks. See Chapter 10, “Maintaining the Simoa HD-X Analyzer,” on page 123.
- If the cuvettes, tips, or discs diagrams are red, add more of those resources. See “Loading Instrument Consumables” on page 37.
- If the system liquid diagram or either wash buffer diagrams are red, refill the container. See “Refilling the System Fluid Containers” on page 47.
- If the liquid waste diagram is red, empty the liquid waste container. See “Emptying the Liquid Waste Containers” on page 54.
- If the solid waste diagram is red, empty the solid waste container. See “Emptying the Solid Waste Container” on page 52.
- If the sealing oil diagram is red, replace the sealing oil bag. See “Changing the Sealing Oil Bag” on page 50.

Understanding the System Resources Screen

Diagram color indicates status:

- **Green:** Adequate amounts
- **Yellow:** Low amounts
- **Red:** Empty, must be be refilled, or container is full and must be emptied.

The screenshot shows the 'All Resources' screen with the following components and callouts:

- Internal temperature display:** Shows a thermometer with 'max 25.0 °C', 'current 25.4 °C', and 'min 18.0 °C'.
- Cuvette status:** Points to the 'Cuvettes' resource icon.
- Disc status:** Points to the 'Discs' resource icons.
- Select type of resource and detail level:** Points to the navigation tabs: 'All Resources', 'Solid Resources', 'Fluid Resources', and 'Resource Details'.
- Disposable pipettor tip status:** Points to the 'Tips' resource icons.
- Fill status of deionized water container:** Points to the 'System Liquid' resource.
- Fill status of wash buffer containers:** Points to 'Wash Buffer 2' and 'Wash Buffer 1' resources.
- Fill status of solid waste container:** Points to the 'Solid Waste' resource.
- Fill status of liquid waste container:** Points to the 'Liquid Waste' resource.
- Fill status of sealing oil bag:** Points to the 'Oil' resource.
- Touch to start a run:** Points to the 'Start Run' button.
- Overall Resource Status Legend:**
 - Red square: Unable to Run
 - Yellow square: Low
 - Green square: OK to Run
 - Orange square: Out of Range

Understanding the Solid Resources Screen

Touch the System Resources tab > touch the cuvette diagram or one of the disposable tip or disc diagrams. The Solid Resources screen opens.

Solid Resources

All Resources | Solid Resources | Fluid Resources | Resource Details

Number of loaded cuvettes: 291 Available

A cuvette chute in the holder

Number of discs on each mounting pole: 0 Available, 11 Available

Select type of resource and detail level

Barcode for disc package: 22985380-0007-150511-7, 22985380-0004-150511-7

System resource drawer 1: 0 Available

System resource drawer 2: 248 Available

Number of disposable pipettor tips present in the drawer

Touch Unlock to open a locked resource drawer

Overall Resource Status
 Unable to Run (Red), Low (Yellow), OK to Run (Green)

Disk Status
 Partial (Light Blue), Empty (Dark Blue)

Tip Status
 Full (Light Green), Empty (Dark Green)

Solid Waste

PREVIEW

Monitoring a Run

Touch the Current Run tab to observe the status of sample processing during the run. Each loaded plate or tube is graphically represented on this tab. As samples are processed, they change color. See the color key at the bottom of the screen for an explanation of the meaning of each color.

Sample In-Process Run screens for plates and tubes appear below.



CAUTION! Do not attempt to open the system resource drawers; remove or insert a reagent rack, a sample rack, or an RGP rack; or change the software settings once the run has begun. Doing any of these things may cause the instrument to halt the run, with subsequent loss of results. You can refill system liquids and empty one liquid waste container at a time during the run.

Understanding the In-Process Run Screen for Plates

The screenshot shows the 'Processing' screen with the following elements and annotations:

- View screen information as a graphic or table:** A callout box points to the 'Graphic View' and 'List View' toggle buttons.
- Countdown display:** A callout box points to the 'Time Remaining' section showing '1:49'.
- Plate Barcode:** The barcode 'SQTSEALD' is displayed above the plate grid.
- Plate Grid:** An 8x12 grid of wells. The first four columns (1-4) show various colors (yellow, orange, blue, cyan) representing different processing statuses. The remaining columns (5-12) are empty (blue).
- Selected Panel:** A panel on the right titled 'Selected' with fields for 'Sample Barcode', 'Status', and 'Assays'. A callout box points to a well in the grid with the text 'Select a well and click to display the associated Event Log'.
- Show Related Flags and Events:** A callout box points to a button at the bottom right of the screen.
- Color Key:** Located at the bottom of the screen, it defines the following statuses: Completed (grey), Scheduled (cyan), Processing (yellow), Flagged (red), Empty (blue), Assigned (black), and Selected (green).

Processing status, see the color key below the plate

Select a well and click to display the associated Event Log

Understanding the In-Process Run Screen for Tubes

Connected

Graphic View List View

Time Remaining

01:00

Selected

Sample Barcode

Status

Assays

Show related Flags and Events

Completed Scheduled Processing Flagged Empty Assigned Selected

Touch to view screen information as graphic or table

Countdown display

Processing status, see the color key below the plate

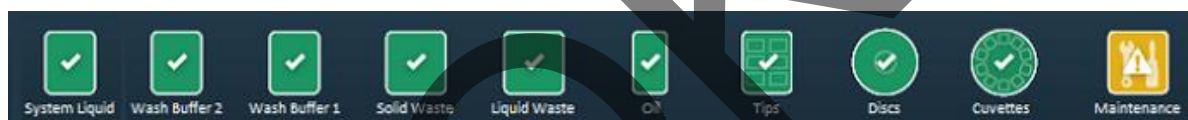
Checking Instrument Status

During the run, periodically check the instrument status indicators at the bottom of the software to determine whether you need to refill any system liquids or empty any system waste containers. The icons for system fluids, consumables, and instrument waste containers change color when any of these components need attention:

- **Green:** Adequate amounts
- **Yellow:** Low amounts
- **Red:** Empty, must be refilled, or container is full and must be emptied

For information about refilling system fluid containers or emptying system waste containers, see Chapter 10, “Maintaining the Simoa HD-X Analyzer,” on page 123.

When the run finishes, the countdown display says 00:00 and the instrument state notice changes to Ready.



Viewing the Event Log

Samples that cannot be processed for some reason are flagged red in the Current Run tab. To determine the reason for the flag, you can view the Event Log.

❖ To view the Event Log

Touch the History & Reports tab > touch **Event Log**. For more information, see Understanding the Event Log Screen on page 118.

8 Analyzing Run Data

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Data Analysis Overview

When the assay run is complete, results appear on the Run History screen. You can perform data analysis on all of the results or a subset.

Results can be exported to an external analysis package or a LIMS system, or you can view and analyze results in the instrument's software. The following options are available:


- Export a CSV file for external analysis
- Export a PDF or Excel file for external analysis
- Analyze the results in the instrument's Data Reduction screen

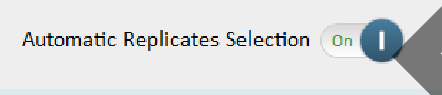
Working with Run History

The Run History screen provides options that allow you to select the data and columns to include in the analysis, export the data to a CSV file, and move analysis data between multiple instruments.

The Run History view calculates replicate statistics only for samples (including calibrators) that come from the same well or tube (for each group of replicates that originate from the same location).

The following table explains how to use the Run History screen.

Element	Definition
1 Add New Filter 	Adds filter criteria that determine what results appear in the table. Multiple filters are allowed. Touch the Add New Filter (+) button to add a filter. Touch the X on the filter button to remove the filter from the table. Loading a large amount of data into the results table can decrease responsiveness. To avoid this, add a new filter before removing unwanted ones.
2 Configure Columns	Opens the Configure Columns window that allows you to configure column display and ordering.

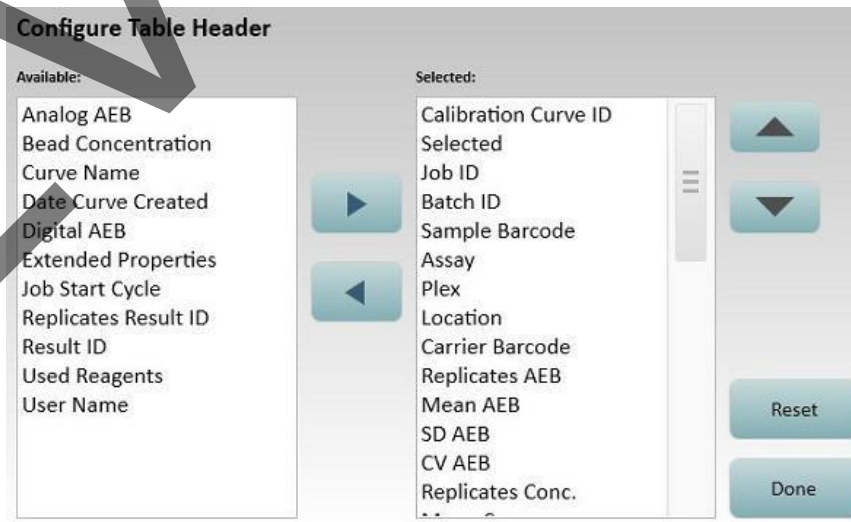
Element	Definition
3 Automatic Replicates Selection	<p>When enabled, the software automatically selects all related replicates when one of the replicate results is selected.</p> 
4 Select all Results	Selects all currently displayed results, as determined by data filters, if any are in place.
5 Deselect all Selected Results	Clears all selections made on results in the table.
6 Exclude Selected Results from Analysis	<p>Marks selected results for exclusion in analysis. Only results that have been automatically included in analysis can be excluded manually. Single is not recalculated when excluding results in the Run History table.</p> <p>Note: Ensure the Automatic Replicates Selection button is turned off > select the replicates to be excluded from analysis, then touch Exclude Selected Results from Analysis button for result.</p>
7 Include Selected Results Into Analysis	Marks selected results for inclusion in analysis. Only results that have been excluded automatically from analysis can be included manually.
8 Show Related Flags and Events	Displays all flags and event messages for selected results.
9 Recalculate with Different Curve	Allows you to recalculate concentration results by manipulating the default calibration curve or to select a new calibration curve.
10 Export	Exports displayed results to a comma separated value (CSV) file. All columns displayed in the table are exported. If you have not selected a column with Configure Columns, it is not exported. Additionally, if you applied data filters to the table, only results matching the filter criteria are exported.
11 Archive/Restore	Provides ability to move result data between two computers running the Simoa HD-X software. Results archived (exported) to an XML file can be restored (imported) into the software running on another computer.

Exporting Results in CSV Format

Export a CSV file from the Run History screen to create a file for external analysis. To export data for troubleshooting analysis, include the Extended Properties column.

❖ To export results in CSV format

- 1 In the History & Reports tab, touch **RunHistory**.
- 2 Clear all filters on the results table.
- 3 Touch the **Add New Filter** button > select a filter from the drop-down list > touch **Next**.
- 4 Specify the filter criteria. Some filters provide drop-down lists and others provide a text entry field that sets the filter.
- 5 Touch **OK**. The results are filtered and the filter name appears above the results table.
- 6 Touch **Configure Columns**.
- 7 Move columns between the Available and Selected lists and change their order with the up and down arrow key buttons. The columns you select identify the data that is exported See *Appendix A – Configure Columns Definitions* for more information about each column.
- 8 Touch **Done**.



- 9 Touch **Select All**.
- 10 Touch **Export** > specify a file name > touch **Save**.

Exporting Results in PDF or Microsoft Excel Format

Export a file in PDF or Microsoft Excel format from the Reports window to create a file for external analysis.

❖ To export results in PDF or Microsoft Excel format

- 1 In the History & Reports tab, touch **Reports**.
- 2 Touch a report in the displayed list.
- 3 Select individual reports or touch **Select All** for all reports.
- 4 Touch **Preview Report** > touch **Export**.
- 5 Save the data in PDF or Excel format.

Analyzing Results in the Data Reduction Tab

The instrument plots a Calibration Curve representing the selected results on the Data Reduction tab.

Curves are batch-based. All the samples in the batch have the same calibration curve applied to them, regardless of processing order.

The calibration data table contains all calibration data points used in curve fitting. The table includes aggregated replicates data. If calibrators were run as replicates, their averages are used.

Curves are specific to the assay. All samples of the same assay and batch will initially have the same calibration curve, but they can be assigned to a different curve, if calibration data points are included in the batch or Curve Selection Strategy is set to None. Following is a sample Data Reduction tab and a table that explains its components.

❖ To review results in the Data Reduction tab

- 1 In the Data Reduction tab, select the relevant fields in Assay, Plex, Calibration Curve, Curve Fit Algorithm, and Weighting.
- 2 Use the tools described in the following table to analyze the data.

The following screen shows a calibration curve calculated with the default settings. Some default settings, such as fitting algorithm, come from plex definition.

Data Reduction

1 Selection

Assay: PSA
 Plex: PSA
 Calibration Curve: Measured
 Curve Fit Algorithm: 4PL
 Weighting: 1/y²

2 Plot Style: Log - Log
3 Pan Zoom
4 Reset Axes
5 Curve Residuals

Measured

R² 0.9998

6 Calibration Means Calibration Replicates **7** Configure Columns

8	Include	AEB	Concentrati	Fitted-X	Residuals (X - Fitted)	% Diff (Residuals/)	% CV	SD	Weighting	Custom Weighting Multipl	Concentrati Unit
	<input checked="" type="checkbox"/>	0	0	0	0	NaN	0	0	13091	1	pg/mL
	<input checked="" type="checkbox"/>	0	0.1	0.091	0.009	9	0	0	778	1	pg/mL
	<input checked="" type="checkbox"/>	0	0.3	0.357	-0.057	-19	0	0	83	1	pg/mL
	<input checked="" type="checkbox"/>	0	1	0.952	0.048	5	0	0	14	1	pg/mL
	<input checked="" type="checkbox"/>	1	3	3.02	-0.02	-1	0	0	2	1	pg/mL

9 Recalculate and View Results
10 Cancel Recalculate Results
11 Save As
12 Cancel Curve Changes

The following table explains how to use the Data Reduction tab.

Element	Definition										
1 Selection	Controls which calibration curve is displayed and the fitting algorithm and weighting used to generate the curve. Below are the recommended minimum number of data points needed for the various curve formulas.										
	<table border="0"> <tr> <td>Points Needed</td> <td>Curve Formula</td> </tr> <tr> <td>2</td> <td>Piecewise Linear, Linear, Exponential, Log-Log</td> </tr> <tr> <td>3</td> <td>Quadratic</td> </tr> <tr> <td>4</td> <td>Cubic, 4PL</td> </tr> <tr> <td>5</td> <td>5PL</td> </tr> </table>	Points Needed	Curve Formula	2	Piecewise Linear, Linear, Exponential, Log-Log	3	Quadratic	4	Cubic, 4PL	5	5PL
Points Needed	Curve Formula										
2	Piecewise Linear, Linear, Exponential, Log-Log										
3	Quadratic										
4	Cubic, 4PL										
5	5PL										

Element	Definition
2 Plot Style	Controls how the data is plotted.
3 Pan/Zoom	Pan across the graph or zoom in on a selected area for more detail.
4 Reset Axes	Use this to reset the graph axes to their original values.
5 Curve/Residuals	Use the Curve and Residuals buttons to toggle between the calibration curve and fitting errors.
6 Calibration Means/ Calibration Replicates	Calibration Means shows the replicates' summarized information in the main table. Calibration Replicates shows individual replicate concentrations.
7 Configure Columns	Select the columns to include in the calibration data table.
8 Include	To exclude individual replicate results from analysis, uncheck the Include checkbox in the row you want to exclude.
9 Recalculate and View Results	After selecting a different calibration curve, recalculates results and displays them in the Run History table.
10 Cancel Recalculate Results	Cancel recalculation and return to the History and Reports tab.
11 Save As	Saves the calibration curve. When you modify an existing curve, you must save it before it can be used to recalculate the results.
12 Cancel Curve Changes	Resets any changes made to the existing calibration curve.

REVOCKED

9 Using Reports, Run History, and the Event Log

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The History and Reports Tab

The History and Reports tab provides access to information about your assay runs from the following screens:

- Reports
- Run History
- Event Log

Understanding the Reports Screen

A report is a PDF file of run data that you can print or export to a computer. See “Creating a Report” on page 121 for an explanation of each report.

The screenshot shows the 'Reports' interface. On the left is a 'Report Types' list with 'Sample Results Report' selected. The main area is a table with columns: Selected, Sample Barcode, Sample Type, Location, Assay, Plex (Analyte), Batch Name, and Used Reagents. At the bottom are 'Select All', 'Deselect All', 'Selected Grouping' (set to 'None'), and 'Preview Report' buttons. A large 'REPORT' watermark is overlaid on the table.

Touch a report to view its fields

Touch to add a new filter

Column headers vary with the report

Touch to choose displayed columns

Select to include/exclude all results for report

Select report grouping

Preview report before printing or saving to file

Understanding the Run History Screen

This tab is most frequently used to export the raw data for archival use or inclusion in the database. The Run History view calculates replicate statistics only for samples (including calibrators) that come from the same well or tube (for each group of replicates that originate from the same location).

Run History

Assay Contains: psa **Filter results**

Number of decimal places controlled in System Settings

Choose columns to display

Selected	Sample Barcode	Assay	Plex	Location	Carrier Barcode	Replicates AEB	Mean AEB	SD AEB	CV AEB	Replicates Coor.
<input checked="" type="checkbox"/>	PSA Calibrator A	PSA	PSA Kit Assay plex id=5	Lane: 1 - Well: 1	RI	0.0101243920206295				0
<input checked="" type="checkbox"/>	PSA Calibrator A	PSA	PSA Kit Assay plex id=5	Lane: 1 - Well: 1	RI	0.0200974777815112				0
<input checked="" type="checkbox"/>	PSA Calibrator A	PSA	PSA Kit Assay plex id=5	Lane: 1 - Well: 1	RI	0.0211416546448138				0
<input type="checkbox"/>	PSA Calibrator A	PSA	PSA Kit Assay plex id=5	Lane: 1 - Well: 1	RI	0.0194545081489848	0.00208438413186732	0.107141445874903		
<input type="checkbox"/>	PSA Calibrator B	PSA	PSA Kit Assay plex id=5	Lane: 1 - Well: 2	RI	0.0335304985516174				0.1
<input type="checkbox"/>	PSA Calibrator B	PSA	PSA Kit Assay plex id=5	Lane: 1 - Well: 2	RI	0.0331392657389118				0.1
<input type="checkbox"/>	PSA Calibrator B	PSA	PSA Kit Assay plex id=5	Lane: 1 - Well: 2	RI	0.0260605604070937				0.1

Automatic Replicates Selection On | Number of Selected Results: 6 out of 20

L2DR Result Replicate Result Flagged Result

Selects all currently displayed results

Automatically select all replicates when one is selected

Marks results for exclusion from analysis

Clears selected results

Marks results for inclusion in analysis

Displays all event messages and flags

Recalculate concentration results with a new or modified curve

Exports displayed results to a CSV file

Archives to or restores batch data from an XML file

Understanding the Event Log Screen

The Event Log tracks significant events that occur during software operation. Event log information is helpful for troubleshooting problems.

Icons in the Type column specify the severity of the message. These icons also appear at the bottom of every tab in the software. A number in the center of these icons signifies how many messages of that type are listed in the log.

To view the Event Log, touch the **History & Reports** tab > touch **Event Log**.

Using the Configure Columns button, you may customize the columns displayed (page 119). When exporting the Event Log, however, column headings are automatically selected to ensure the best export format (columns fit on a page). You may select either PDF (.pdf) or an Excel file (.xls). See “Exporting or Printing a Report” on page 122.

Touch to add a new filter

Touch to view, print, or save data reports

Touch to view the run results

Touch to display the Event Log

Touch to choose displayed columns

Event Log

The screenshot shows the Event Log interface. At the top, there are tabs for Reports, Run History, and Event Log. A plus sign icon is used to add filters. A 'Configure Columns' button is located in the top right. The main area is a table with columns: Type, Category, Creation Date, Severity, Event Text ID, and Local. The table contains several rows of event data. At the bottom, there are 'Pause' and 'Preview Report' buttons.

	Message		Problem		System Halted
	Warning		Processing Error		Software Failure

Touch to pause the display of events (prevents auto-scrolling)

Preview report before printing or saving to file

Customizing the Table View in the History & Reports Tab

Changing Columns

To change the columns for any table in the History & Report tab, touch **Configure Columns** > touch the arrows to move column names between the Available and the Selected boxes > touch **Done**.

Configure Columns



Column headers vary among the Reports, Run History, and Event Log screens

Configure Table Header

Available:

- Analog AEB
- Analysis Mode
- Batch ID
- Batch Name
- Calibration Curve Id
- Curve Name
- CV AEB
- CV Conc.
- Date Curve Created
- Date Finished
- Digital AEB
- Dilution Factor
- Estimated Time To Result
- Extended Properties

Selected:

- Carrier Barcode
- Assay
- Plex
- Dilution Description
- Sample Type
- Replicates AEB
- Mean AEB
- Replicates Conc.
- Mean Conc.
- Unit
- Flags
- Job Status

Reset

Done

Changing Column Order

To move a column in any table in the History & Reports tab, use the up and down arrow keys, or touch the column header and drag it to the new position. In table view, you can also reorder the columns by dragging.

Sorting Rows

To sort any table in the History & Reports tab in ascending or descending alphabetical order for a specific column, touch the column header.

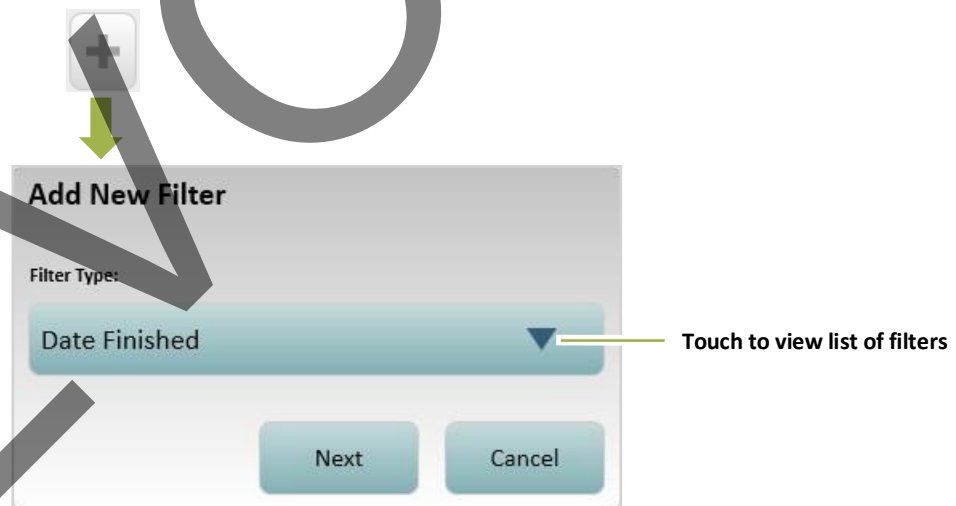
Adding a Filter

A filter modifies the contents of the Run History, the Event Log, or a report to show only data that matches the filter requirements. For example, you can filter the Event Log to show only warning messages.

You can add multiple filters to any table. Available filters vary with the table type.

❖ To add a filter

- 1 Touch the **Add New Filter** button > select a filter type from the drop-down list > touch **Next**.
- 2 Specify the filter criteria. Some filters provide drop-down lists for this, and others provide a text entry field in which you enter terms that set the filter.
- 3 Touch **OK**. The filter appears above the table.



❖ To remove a filter

Touch the large X in the filter.



Creating a Report

A report is a templated summary of run data that can be exported to a user-specified location. The following types of reports are available:

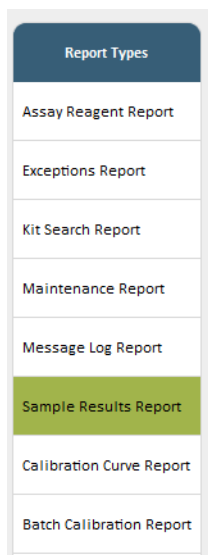
Assay Reagent Report	Reagents used in selected assay runs
Exceptions Report	List of exception (error) messages occurring during the selected runs
Kit Search Report	List of samples associated with a specific kit reagent lot
Maintenance Report	List of completed instrument maintenance tasks
Message Log Report	List of all messages generated by the software during a specified selected time interval
Sample Results Report	Results for selected assay runs
Calibration Curve Report	Calibration curve information for selected run (fitting algorithm, weighting factor, style, etc.)
Batch Calibration Report	Includes calibration curves for all assays and all samples processed for a selected batch. Organized by assay plexes.

To customize reports, change the report table (see “Customizing the Table View in the History & Reports Tab” on page 119.)

To create a report

- 1 Touch the History & Reports tab > touch **Reports**.

2 Touch a report type.



Exporting or Printing a Report

You can preview a report, export it to a file, or print it.

❖ To export or print a report

- 1 Touch **Preview Report** to view the formatted report. After a few moments, the report appears.
- 2 Touch **Export** or **Print**.
 - **Export:** Opens a Save As window. Enter a filename > select a file type > navigate to a storage location > touch **Save**. You can export a report as a PDF (.pdf) or an Excel file (.xls).
 - **Print:** Opens a Save As window. Enter a filename > navigate to a storage location > touch **Save**. You can export a report as a .xps file for printing on a non-system PC connected to a printer.
- 3 Touch **Done**.

Customizing the Logo for a Report

See “Managing Report Settings” on page 152.

10 Maintaining the Simoa® HD-X Analyzer

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Performing Maintenance Tasks

Perform instrument maintenance tasks according to the following schedule table. These tasks are listed in the Maintenance tab.



Note! Check the Quanterix® customer portal at <http://portal.quanterix.com/> for updates to monthly maintenance procedures.

Task	Default Interval	Description
Start of Day	After initialization	Prepares instrument systems to start a run. See “Performing the Start of Day Task” on page 128.
End of Day	Daily after the last run of the day	Cleans the system at the end of the day.
Idle Fluid Prime	After 240 idle minutes	Primes the system fluids and resets the idle time counter.
Monthly Fluid Prime	Monthly	Primes the system fluids three times the normal length, approximately 30 minutes.
Replenish Oil	When the sealing oil is empty	Primes sealing oil through the entire line.
Database Clean	Database size limit reached, at least weekly	Cleans up the database. See “Performing the Database Clean Task” on page 131.
Computer Memory Management	Monthly	Removes temporary files and performs disk utility that improves data access speeds.



Note! The quality of system fluids may degrade over time due to limited shelf life, cross-contamination, salt buildup, etc. You must perform the monthly maintenance procedures described in this chapter to maintain optimal performance of the instrument. As a check, you can run the Simoa Qualification Test (SQT) to measure the precision of the system upon completion of the maintenance tasks.

Understanding the Maintenance Screen

The Maintenance screen shows status information about each maintenance task.

Add task filter
Customer Service only

Blocking behavior
See "Setting and Viewing Task Details" on page 126

Touch to show log

Touch to choose columns

View task parameters

Print the task

Run the selected task

Selected	Name	Interval	Last Run Date	Next Run	Blocks Sample Processing	Blocks shutdown when done	Status of Last Run
<input type="checkbox"/>	Start of Day	After initialization	--	--	Yes	No	Pending
<input type="checkbox"/>	End of Day	Daily	--	--	No	Yes	Pending
<input type="checkbox"/>	Idle Fluid Prime	After 240 idle minutes	--	--	Yes	No	Finished
<input type="checkbox"/>	Monthly Fluid Prime	Monthly	--	--	No	No	Pending
<input type="checkbox"/>	Replenish Oil	Seal oil empty	--	--	No	No	Pending
<input type="checkbox"/>	Database Clean	Database size limit reached	--	--	Yes	No	Finished
<input type="checkbox"/>	Computer Memory Management	Monthly	--	--	No	No	Pending

Determining Whether a Maintenance Task Was Performed

The Maintenance screen shows status information about each maintenance task. Two methods are available to do this:

- Check the Last Run column in the Maintenance tab, which reports the date and time when a task was last performed.
- In the Maintenance tab, touch **Show Log** to view a log that lists all completed maintenance tasks.

Setting and Viewing Task Details

Use the Task Details screen to set and display additional task settings.

❖ To view task details

Touch the checkbox for a task > touch **View Details**.

View Details

Name: Start of Day

Procedure: Start of Day

Description: This task must be executed after initializing the instrument. Running this task prepares the instrument to process batches.

Alert user before due date: 0

Blocks sample processing: Yes No

Interval: After initialization

Resets idle time: Yes No

Time:

Blocks shutdown when due: Yes No

Dependent Tasks: Dependent Tasks (0)

Save Cancel

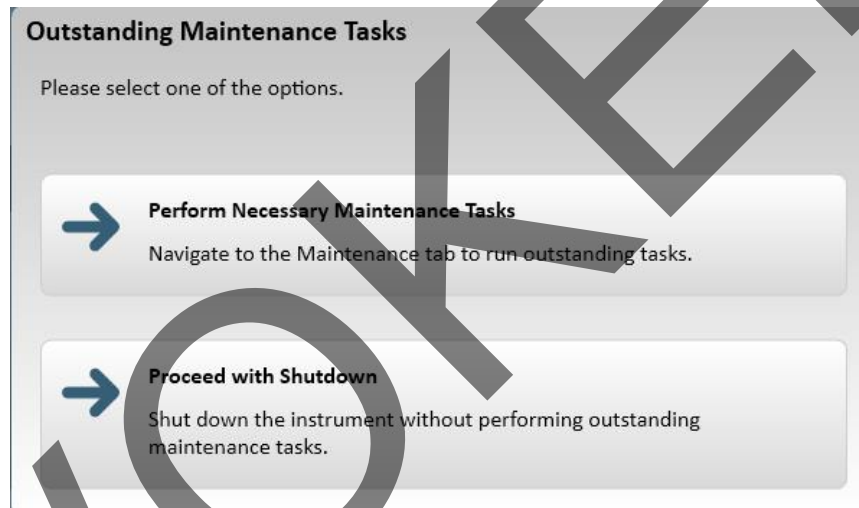
- **Interval** – The frequency of the task.
- **Time** – Time of day the task is performed.
- **Dependent Tasks** – Run specified tasks immediately after completion of the current task.

Select Dependent Tasks

Name	Selected
Start of Day	<input checked="" type="checkbox"/>
End of Day	<input type="checkbox"/>
Idle Fluid Prime	<input type="checkbox"/>
Monthly Fluid Prime	<input type="checkbox"/>
Replenish Oil	<input type="checkbox"/>
Database Clean	<input type="checkbox"/>

OK Cancel

- **Alert user before due date** – Number of days prior to the due date that the software provides a notification prompting you to complete the task. This is only available for the Monthly Fluid Prime and Computer Memory Management tasks.
- **Blocks sample processing** – Prevents/allows running assays when the task is not complete.
- **Resets idle time** – Resets/does not reset counter after task is performed.
- **Blocks shutdown when due** – When enabled, the following prompt appears when you shut down the software.



Changing Columns

To change the table columns, touch **Configure Columns** > touch the arrows to move column names between the Available and the Selected boxes.



Performing the Start of Day Task

This task sets up the instrument for a new assay run as follows:

- Primes oil line.
- Flushes the pipettor with deionized water, cleans it with system wash buffer 1, and flushes it again with deionized water.
- Primes tubes with wash buffer fluid, then discards the cuvettes.
- Loads 10 cuvettes into the Wash/Incubator rings.

After you perform the End of Day Task, you must perform the Start of Day Task before performing a run.

❖ To perform the Start of Day task

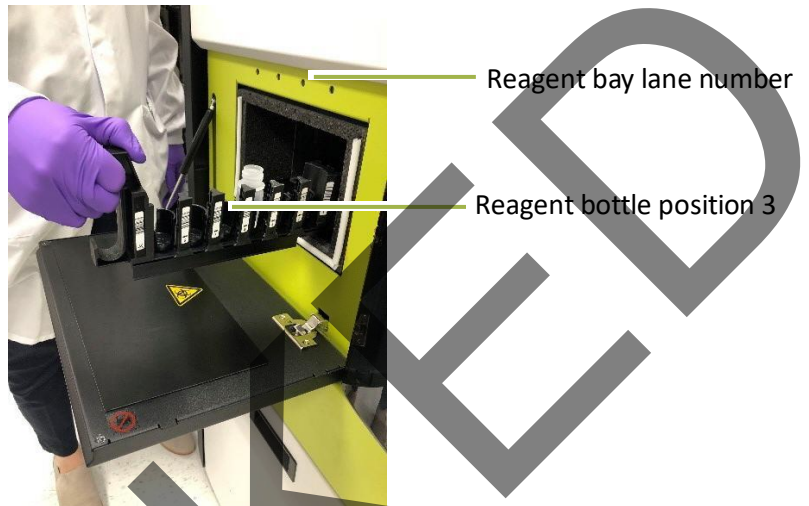
- Fill a clean reagent bottle a little more than half way with system wash buffer 1. Make sure there are no bubbles in the bottle.
- Touch the Maintenance Tab > touch the Start of Day task checkbox > touch **Run Task**.

Maintenance

Selected	Name	Interval	Last Run Date	Next Run	Blocks Sample Processing	Blocks shutdown when due	Status of Last Run
<input checked="" type="checkbox"/>	Start of Day	After Initialization	--	--	Yes	No	Pending
<input type="checkbox"/>	End of Day	Daily	--	--	No	Yes	Pending
<input type="checkbox"/>	Idle Fluid Prime	After 240 idle minutes	--	--	Yes	No	Pending
<input type="checkbox"/>	Monthly Fluid Prime	Monthly	--	--	No	No	Pending



- c When prompted, place the reagent bottle into position 3 on a reagent rack (fourth position from the handle) and put the rack in the rightmost reagent bay position (lane 4).



- d After loading the reagent rack into the instrument, select “next step” to continue with the Start of Day task.
- e Allow approximately 20 minutes for completion. When the task is complete, touch **Close**, remove the reagent rack and dispose of the reagent bottle.

Performing the End of Day Task

The End of Day task flushes the pipettor that aspirates reagents and all pipettor and system fluid tubings to prevent the buildup of salts, which can clog the pipettor and instrument tubings.

The task performs these actions:

- Flushes the pipettor with deionized water, cleans it with system wash buffer 1, and flushes it again with deionized water.
- Primes the tubing for system wash buffer 1 and 2 with deionized water.

After you perform the End of Day Task, you must perform the Start of Day Task before performing a run. Quanterix recommends setting a dependent task to automate this process, as described in Setting and Viewing Task Details.

❖ To perform the End of Day task

- 1 Fill a clean reagent bottle a little more than half way with system wash buffer 1. Make sure that there are no bubbles in the bottle.
- 2 Touch the Maintenance tab > touch the End of Day task checkbox > touch **Run Task**.

Selected	Name	Interval	Last Run Date	Next Run	Blocks Sample Processing	Blocks shutdown when due	Status of Last Run
<input type="checkbox"/>	Start of Day	After Initialization	--	--	Yes	No	Pending
<input checked="" type="checkbox"/>	End of Day	Daily	--	--	No	Yes	Pending
<input type="checkbox"/>	Idle Fluid Prime	After 240 idle minutes	--	--	Yes	No	Pending
<input type="checkbox"/>	Monthly Fluid Prime	Monthly	--	--	No	No	Pending
<input type="checkbox"/>	Replenish Oil	Seal Oil Empty	--	--	No	No	Pending
<input type="checkbox"/>	Database Clean	Database size limit reached	--	--	Yes	No	Finished
<input type="checkbox"/>	Temporary Files Clean	Monthly	--	--	No	No	Pending

Run Task

- 3 When prompted, place the bottle into position 3 on a reagent rack (fourth position from the handle) and put the rack in the rightmost reagent bay position (lane 4). See the photo in the section above.
- 4 Allow approximately 20 minutes for completion. When the task is complete, touch **Close**, and remove the reagent rack and dispose of the reagent bottle.

Performing the Idle Fluid Prime Task

Prime the system fluids after 240 idle minutes. Idle time is the amount of time since the instrument finished a run, or the time since a task that resets the idle counter was run.

- 1 In the Maintenance tab, touch the Idle Fluid Prime checkbox > touch **Run Task**.
- 2 The system fluids are primed and the idle time counter is set to zero.

Selected	Name	Interval	Last Run Date	Next Run	Blocks Sample Processing	Blocks shutdown when due	Status of Last Run
<input type="checkbox"/>	Start of Day	After Initialization	--	--	Yes	No	Pending
<input type="checkbox"/>	End of Day	Daily	--	--	No	Yes	Pending
<input checked="" type="checkbox"/>	Idle Fluid Prime	After 240 idle minutes	--	--	Yes	No	Pending
<input type="checkbox"/>	Monthly Fluid Prime	Monthly	--	--	No	No	Pending
<input type="checkbox"/>	Replenish Oil	Seal Oil Empty	--	--	No	No	Pending
<input type="checkbox"/>	Database Clean	Database size limit reached	--	--	Yes	No	Finished
<input type="checkbox"/>	Temporary Files Clean	Monthly	--	--	No	No	Pending

Run Task

Performing the Database Clean Task

The Simoa HD-X Analyzer generates a lot of data for each sample—approximately 115 MB per result—mostly consisting of raw camera images. For example, a batch with 96 samples results in approximately 11 GB of data. This data is stored in a database on the instrument computer.

Each *week*, you must perform the Database Clean task to purge old data so that new data can be stored. The task removes all data for runs older than 30 days from the first time that you perform the task.

If you want to retain data older than 30 days or to protect data against potential disk failure, it is *your responsibility* to back up the database prior to running the Database Clean task (at least monthly).

The Database Clean task permanently deletes data, so it is highly recommended that you back up the database before performing this task, especially if retention of raw sample images is required.

Backup Strategy

Contact Customer Support to discuss best practices regarding database backup and to obtain sample scripts that automate the backup process.

Do not store database backups on the instrument computer, for two reasons:

- Data can be lost if the disk fails.
- Database backups are very large and consume disk space that is needed to operate the instrument.

If the backup strategy you select results in backup files being stored on the instrument hard drive, you must move them off the disk to an external hard drive, network drive, or similar destination.

You can perform a partial backup that contains all of the data in the database except for the raw IPL image files or a complete database backup that includes the raw IPL image files.

To perform a database backup

See the *Simoa HD-X Analyzer Quanterix Customer Support Tool 2.0 User Guide* for information on performing a full backup, or follow the instructions in *Simoa HD-X Analyzer IT Requirements Guide* to make a partial backup.

❖ To perform the Database Clean task



Note! Be sure to back up the database before you perform this task to avoid permanently losing data.

In the Maintenance tab, touch the Database Clean checkbox > touch **Run Task**. After the Database Clean task completes, exit the HD-X software and reboot the PC.

Cleaning Exterior Instrument Surfaces

Clean exterior instrument surfaces whenever necessary (at least once per month) by wiping with a soft, clean cloth moistened with water or 10% ethanol.

Cleaning the Touchscreen

Use any glass cleaner that does not contain ammonia.

Wet a paper towel with glass cleaner and then gently wipe down the touchscreen. Do not spray glass cleaner directly onto the touchscreen.

Cleaning the System Resources Drawers

Once a month, clean the system resource drawers.



CAUTION! Do not attempt to clean the system resource drawers when an assay is running. If you open a drawer during a run, the instrument halts the run and you lose any accumulated data.

Completely pull out both drawers from the instrument and wipe the metal frame beneath the drawers with water and then with 70% ethanol.

Cleaning the Reagent and Sample Bays

Once a month, clean the inside surfaces of the reagent and sample bays.



WARNING! Turn off the instrument before you perform any interior surface cleaning. The robotic pipettors inside the instrument can injure your hands. Do not attempt to clean the incubator or the washer rings and do not insert your hands into the sample bay or the reagent bay if the instrument is turned on.

❖ To clean the reagent and sample bays

- 1 Turn off the instrument (see “Turning the Instrument On and Off” on page 24).
- 2 Remove all reagent racks, plate racks, tube racks, and RGP racks.
- 3 Wipe the walls, ceiling, and base of each bay first with water and then with 70% ethanol.
- 4 Replace the racks.

Cleaning the Liquid Waste Containers

Once a month, clean liquid waste containers and the platform on which they sit.

During this procedure, discard all fluids and rinsates according to your company’s waste disposal policies.

❖ To clean the liquid waste containers

- 1 Power off the instrument and remove both liquid waste containers by disconnecting the container sensor. If the instrument is not powered off, it will halt when both liquid waste containers are removed.
- 2 Wipe the platform on which the containers sit first with water and then with 70% ethanol.
- 3 Rinse the containers by performing the following procedure three times on each container:
 - a Fill the container at least one-third full of deionized water.
 - b Shake to rinse inner surfaces.
 - c Discard the rinsate.
- 4 Replace the containers and reconnect the container sensors. Align the tab in the sensor with the cutout in the container. When done correctly, you will hear a click when you reinsert each sensor.

Cleaning the System Fluid Containers and Tube Rinsing

Once a month, the fluid resource containers must be cleaned. The tubing from the containers into the system must be primed with water for 30 minutes before they are primed with buffer for another 30 minutes.

During this procedure, discard all fluids and rinsates according to your company's waste disposal policies.

The DI water and wash buffer 1 each have two containers, which are defined as follows:

- **Secondary Container** – This is the removable container with a screw cap where new fluid is added. The wash buffer 1 and DI water secondary containers are identical in size.
- **Primary Container** – This is the pyramidal-shaped container behind the secondary containers. The liquid level sensor and tubing are connected through a screw cap on top.

Wash buffer 2 only has one container, which sits between the DI water and wash buffer 1 secondary containers. It has a screw cap for adding new fluid, a liquid level sensor, and a fluid port that connects directly to the instrument tubing.

The tubing leading to the sensor cap is color coded as follows:

- **Blue** – DI water
- **White** – Wash buffer 1
- **Yellow** – Wash buffer 2

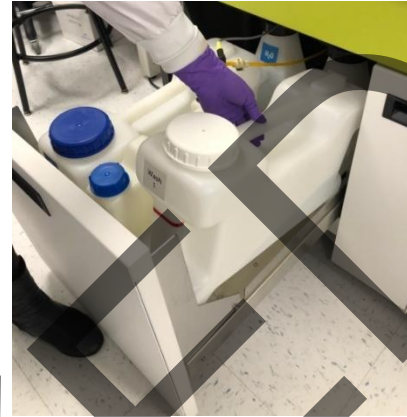
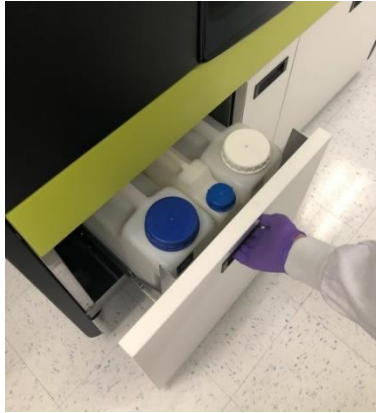
Note! Do not attempt to perform this procedure when an assay is running. The procedure involves disconnecting the fluid containers, which will prevent the instrument from obtaining the system fluids that are required to perform the assay.

❖ **To clean the secondary and primary containers**

- 1 Close the Simoa HD-X Analyzer software and shut down the instrument.
- 2 Remove the secondary containers and wash buffer 2 container.

Note! Do not reuse the fluids after emptying the containers. Reuse of fluids has been associated with a reduction in data quality.

- **DI water and wash buffer 1:** Lift the container up and then pull out.



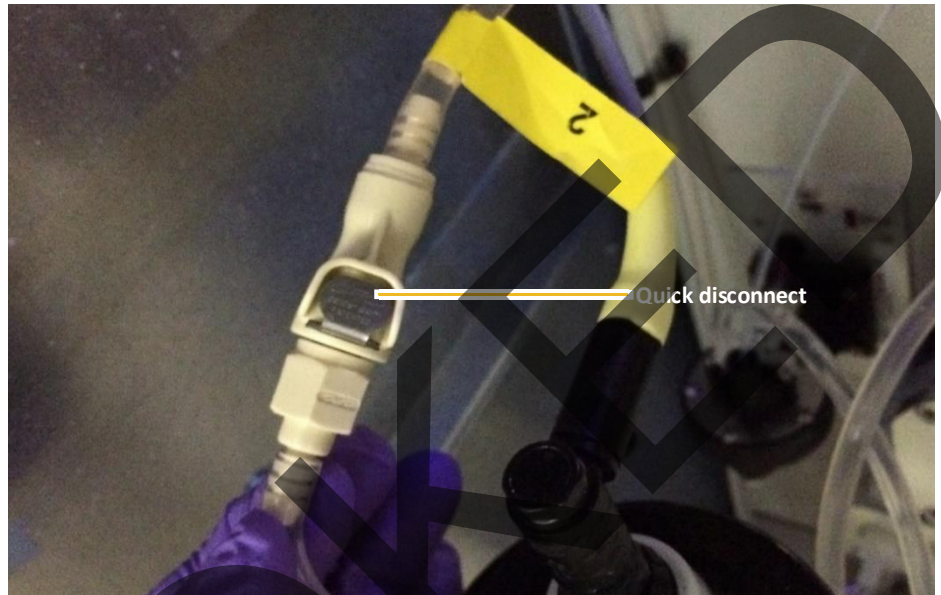
- **Wash buffer 2:** Disconnect the sensor and fluid port and then lift the container up.



- 3 Empty and dispose of all secondary and wash buffer 2 container fluids.

Note! Cross-contamination of the wash buffers may result in poor assay performance. Take care during this procedure to prevent cross-contamination. Do not reuse the fluids after emptying the containers. Reuse of wash buffers can result in a reduction of data quality.

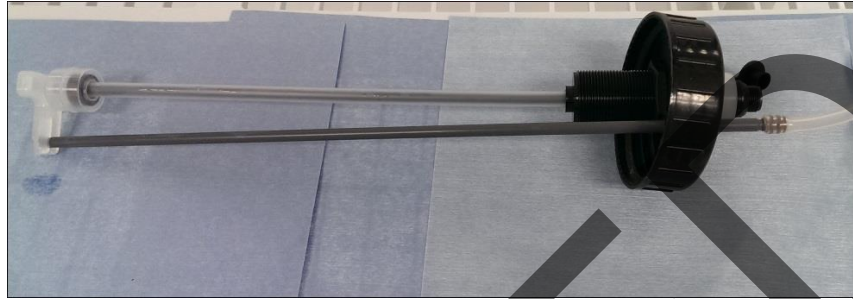
- 4 Remove all primary containers. The process is the same for each fluid type.
 - Disconnect the sensor by pulling the black connector up.



- Press the metal button on the quick disconnect fitting to detach the two ends of the liquid line.
- Remove the primary container by pulling forward and then up.



- Unscrew the black cap and place it on a clean lint-free paper towel.



- 5 Empty and dispose of all primary container fluids.
- 6 Wash all fluid containers (primary, secondary, and wash buffer 2) by triple-rinsing them with DI water. Fill the containers to approximately 1/3 of their total capacity with DI water and shake to rinse all surfaces.
- 7 Clean the liquid level sensors by running under DI water.

❖ To prime the system with DI water

- 1 Fill the containers with DI water as follows. Do not fill the secondary wash buffer 1 container.
 - **DI primary** – Full
 - **Wash buffer 1 primary** – Full
 - **Wash buffer 2** – Half full
 - **DI secondary** – Half full

- 2 Replace the sensor caps for the DI water and wash buffer 1 primary containers. If they have been separated from the containers, identify the caps by the physical differences in the tubing. DI water uses blue tubing, wash buffer 1 uses white and wash buffer 2 uses yellow.



- 3 Replace the primary containers (DI water, wash buffer 1), wash buffer 2 container, and secondary DI container into the instrument. Do not replace the secondary container for wash buffer 1.
- 4 Reconnect the sensor cable and screw the fluid tubing together. Refer to the color code listed previously to ensure proper container connections.
 - Connect the two ends of the quick disconnect fitting. You will hear an audible click sound.
 - Connect the electrical connector into the top of the primary container lid, making sure that the tab on the cable is well-aligned with the slot in the lid. You will hear an audible click sound.
- 5 Turn on the instrument and open the Simoa HD-X software.
- 6 After initialization completes, run the Monthly Fluid Prime task. This step takes approximately 30 minutes to complete.
 - a Touch the Maintenance Tab > touch the Monthly Fluid Prime task checkbox.
 - b Touch **Run Task**. The task takes approximately 30 minutes to complete. The task window has a timer that indicates when the task will complete.
- 7 Close the Simoa HD-X software and shut down the instrument.

❖ To prime the system with wash buffer

- 1 Disconnect and remove the wash buffer 1 primary container and the wash buffer 2 container. Do not remove the DI Water primary container.
- 2 Unscrew the black cap for the wash buffer 1 primary container and place it and the liquid level sensors on a clean lint-free paper towel.
- 3 Empty the remaining water out of the containers.
- 4 Replace the wash buffer 1 primary container while it is still empty. Screw the sensor cap back on and connect the tubing and sensor again. Refer to the earlier instructions for detail.
- 5 Fill the containers with the following volumes:
 - **DI Water Secondary** – at least 5 L
 - **Wash buffer 1 Secondary** – at least 5 L
 - **Wash buffer 2** – at least 2 L
- 6 Replace the secondary containers and wash buffer 2 container into the instrument.
- 7 Turn on the instrument and open the Simoa HD-X software.
- 8 After initialization completes, run the Monthly Fluid Prime task again. This will take another 30 minutes to complete.

Maintaining Idle Instruments

When an instrument has been idle for a period of time, air can be introduced into the fluid lines, potentially impacting data quality and precision. To ensure proper instrument operation, perform maintenance tasks on idle systems as described in the following table.

Idle Period	Procedure
Less than 4 weeks	Perform the following maintenance tasks twice per week for each week of inactivity. <ol style="list-style-type: none"> 1 Power on computer and instrument. 2 Run Start of Day maintenance. 3 Run End of Day maintenance. 4 Power off computer and instrument. If you cannot perform these tasks on schedule, run the Monthly Maintenance task (which performs an extended fluid prime) followed by a Simoa Qualification Test.

Idle Period	Procedure
Greater than 4 weeks	Schedule a visit with Quanterix Service to perform maintenance before and after the scheduled idle period.

REVOLOKED

11 Configuring Software Settings

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Software Settings

The Software Settings screen has the following tabs:

- SystemSettings
- User Settings
- Account Management (for users with administrator privileges only)
- ReportingSettings

❖ **To view the software settings**

Touch the Settings icon at the top right of the software to open the Software Settings screen.



Settings icon

Changing System Settings

In the System Settings screen, you can change the following settings:

- Sample loading mode
- Automatic lock out time
- Password policies
- Synchronization of loading screen and bay doors
- Default number of decimal places
- Customer-defined system name

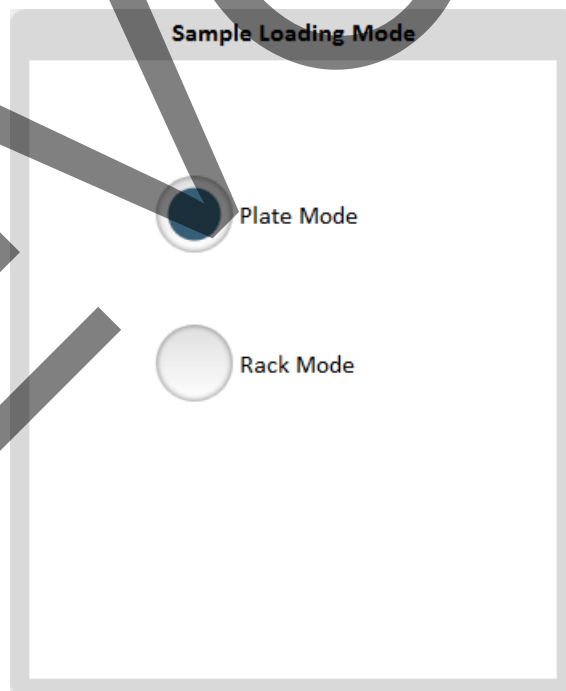
It is not necessary to restart the software after changes to the system settings.

Selecting the Sample Loading Mode

Plate mode is selected when you first open the software. To process samples in tubes, select Rack Mode.

❖ To change the sample loading mode

In the System Settings tab, touch **Plate Mode** or **Rack Mode** > touch **Done** in the upper right corner of the tab.



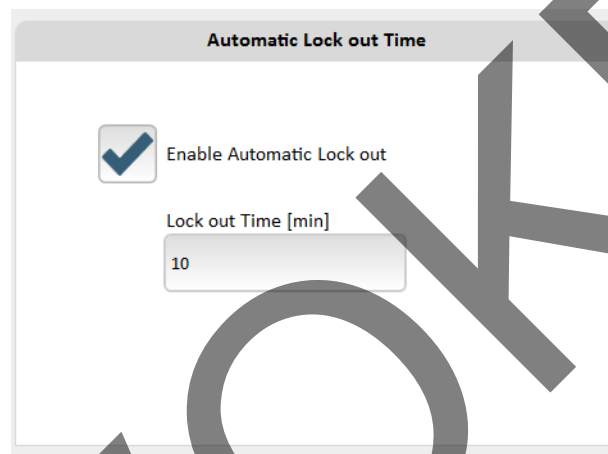
Setting an Automatic Lock Out Time

You can set the software to require users to log in again after a specified time period.

❖ To set a lock out time

In the System Settings tab, touch the **Enable Automatic Lock out** checkbox > type a Lock Out Time > touch **Done** in the upper right corner of the tab.

The Lock out Time value must be greater than 0 and less than or equal to 999.



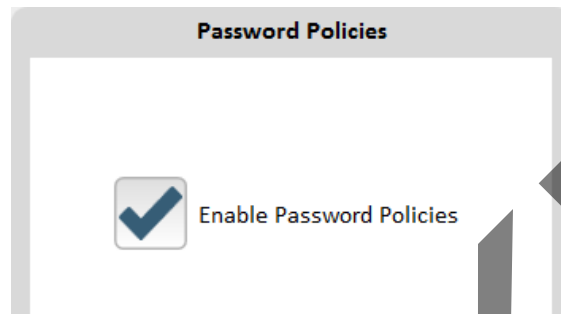
Enabling Password Policies

You can enable or disable the use of password policies. Enabling this setting places additional security requirements on new password values. The security requirements for new passwords are as follows:

- Usage of at least seven characters
- Usage of at least one upper case character
- Usage of at least one lower case character
- Usage of at least one non-alphanumeric character
- Usage of at least one numeric character

❖ To enable password policies

In the System Settings screen, scroll down to the General section > touch the **Enable Password Policies** checkbox > touch **Done** in the upper right corner of the tab.



Controlling Tab Switching when Bay Doors are Opened

By default, the software switches to the Load Reagents tab when you open the reagent bay door, and it switches to the Setup Run tab when you open the sample bay door. You can turn on/off tab switching with the **Synchronize Loading Screen and Bay Doors** setting.

❖ To set tab switching behavior

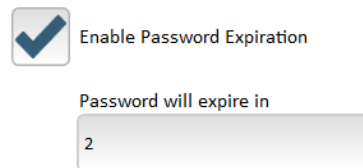
- 1 In the System Settings screen, scroll down to the General section.
- 2 Enable the **Synchronize Loading Screen and Bay Doors** checkbox to switch tabs when you open a bay door, or disable it to keep the current tab open.
- 3 Touch **Done** in the upper right corner of the tab.

Setting a Password Expiration Time

You can enable password expiration and set the amount of time in which you want user passwords to expire.

❖ To set a password expiration time

In the Systems Settings tab, touch the **Enable Password Expiration** checkbox > type the number of days in which you want user passwords to expire > touch Done in the upper right corner of the tab.



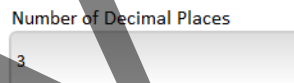
Enable Password Expiration
 Password will expire in
 2

Setting Default Decimal Places

You can specify the number of decimal places that appear for all numbers (except concentrations, which are controlled at the Plex level). This setting controls the display of numbers throughout the application (in result tables, reports, etc.).

❖ To set the default digits to the right of the decimal place

In the System Settings screen, scroll down to the General section > enter an integer from 0 to 15 > touch **Done** in the upper right corner of the tab.



Number of Decimal Places
 3

Specifying the Customer Defined System Name

To assist with troubleshooting, you can specify a name that describes the system configuration. For example:

- Version 1.2.3.4
- Simoa 1234
- Lab Machine 12

This name is captured in the Event Log screen and the shellDebug log file when the software is started.

❖ To set the customer defined system name

In the System Settings screen, scroll down to the General section > type a string that describes the system configuration (up to 20 characters) > touch **Done** in the upper right corner of the tab.

Customer Defined System Name

Locking an Account due to Incorrect Login Attempts

You can set the software to lock an account after a specified number of incorrect login attempts.

❖ To lock an account after a configurable number of incorrect login attempts

In the System Settings tab, scroll down to **General** > touch the **Lock Account after Configurable Number of Incorrect Login Attempts** checkbox > type the allowed number of incorrect login attempts > touch **Done** in the upper right corner of the tab.

Lock Account after Configurable Number of Incorrect Login Attempts

Allowed number of incorrect login attempts

Specifying User Settings

All users can change their own system passwords. If you are a user with administrative privileges, you can:

- change another user's password
- show assays belonging to all users, even if the **Visible only to me** setting (in the Overview screen of the Custom Assay tab) is enabled for the account.

❖ To change a password

In the User Settings tab, type the user name, existing password, and new password > touch **OK**.

Change Password

User Name:

Password:

New Password:

Confirm Password:

OK Cancel

❖ To display/hide assays from other users



Note! You must be a user with administrative privileges to override the **Visible only to me setting** (in the Overview screen of the Custom Assay tab).

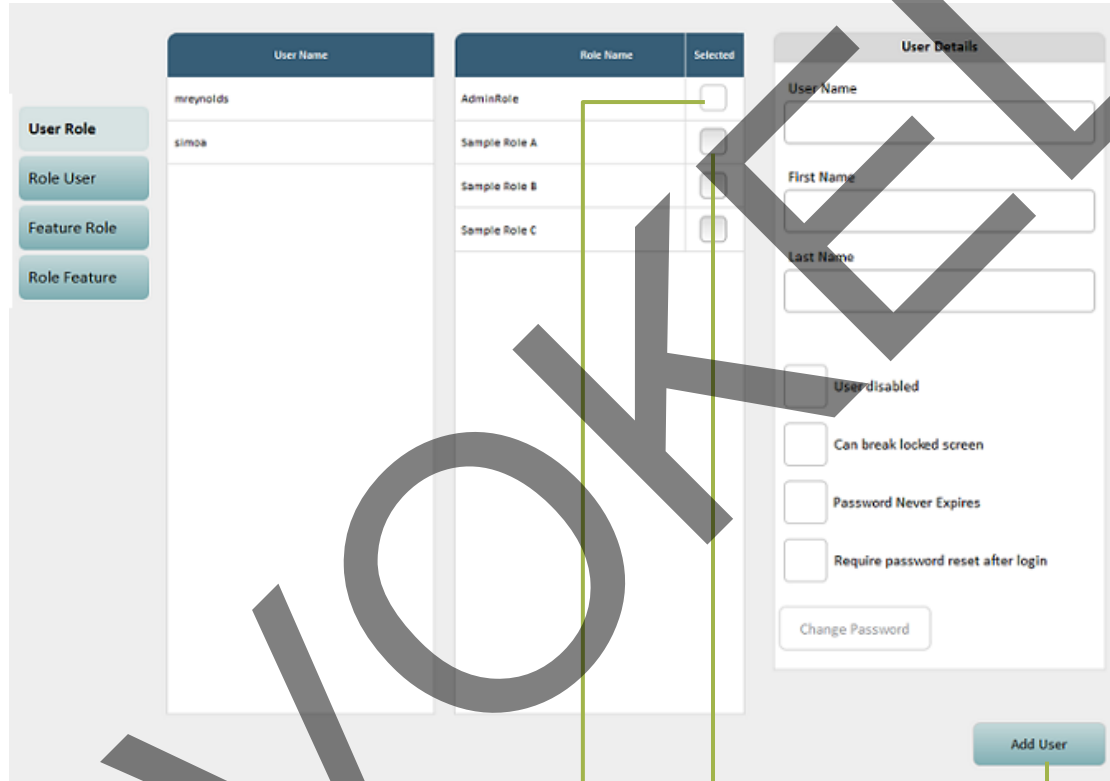
- 1 In the User Settings tab, enable **Show Assays from other Users** to display all assays, or disable it to show only your assays.
- 2 Touch **Done** in the upper right corner of the screen.

Assay Visibility

Show Assays from other Users

Specifying Account Management Settings

Use the Account Management screen to create and manage users and customize roles. The user that you define when you sign into the instrument software for the first time is assigned the AdminRole and appears as a user on this screen.



Touch to assign administrator-level privileges

Touch to assign user-level privileges

Add a new user

Managing Users

In the Account Management screen, you can add new users and define their access privileges.

❖ To add a new user

- 1 Touch **User Role**.
- 2 Touch **Add User**. Specify the User Name, First Name, Last Name, Password and Confirm Password.
- 3 To require the new user to reset their password after logging in, touch the **Require password reset after login** checkbox.
- 4 Touch **OK**.
- 5 Touch the **Selected** checkbox for the role you want to assign in the Role Name list.
- 6 If you have completed Account Management tasks, touch **Done**.

Create new user

User Name:

First Name:

Last Name:

Password:

Confirm Password:

Require password reset after login

OK Cancel

❖ To change a user's role

- 1 Touch **User Role**.
- 2 Select the user in the User Name list.
- 3 Touch the **Selected** checkbox for the role you want to assign in the Role Name list.
- 4 If you have completed Account Management tasks, touch **Done**.

❖ To change a user's password

- 1 Touch **User Role**.
- 2 Select the user in the User Name list.
- 3 Touch User Settings tab.
- 4 Touch **Change Password**.
- 5 Touch **Done** in the upper right corner of the screen.
- 6 Type the existing password and the new password.
- 7 Touch **OK**.
- 8 If you have completed Account Management tasks, touch **Done**.

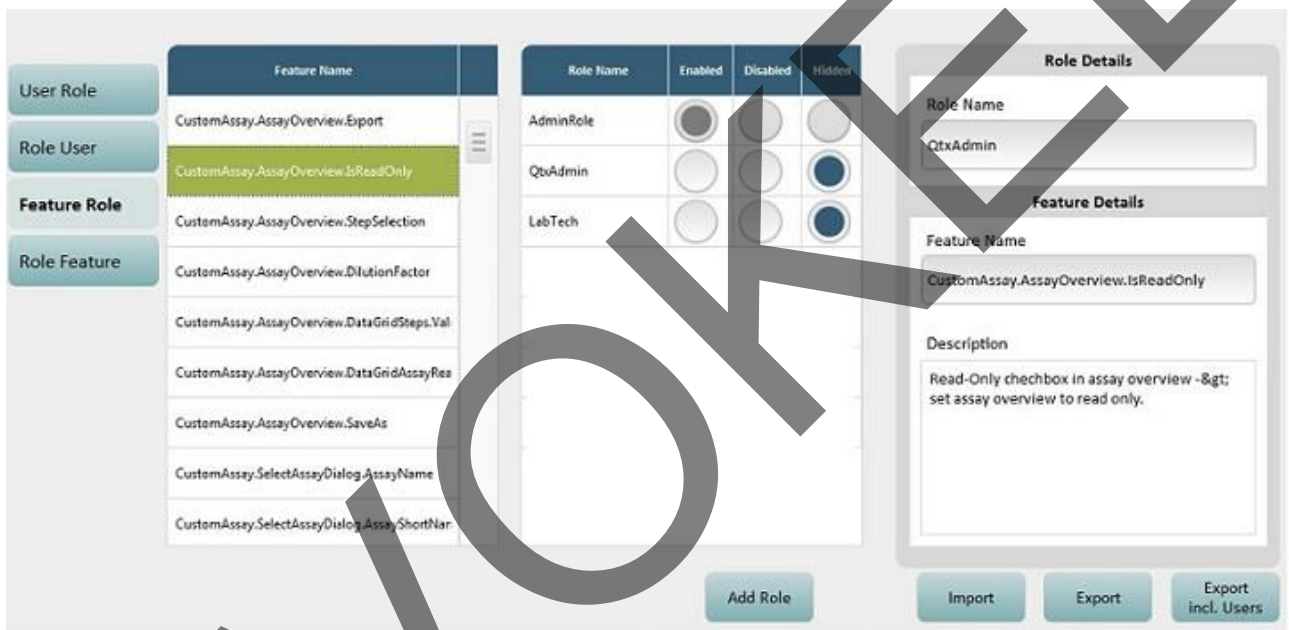
❖ To assign multiple users to a single role

- 1 Touch **Role User**.
- 2 Select the role you are assigning in the Role Name list.
- 3 Touch the **Selected** checkbox for each user you want to assign.
- 4 If you have completed Account Management tasks, touch **Done**.

Managing Roles and Features

In the Account Management screen, you can create new roles and define the product features that each role can access. For example, you might want to limit the ability to modify imported assay definitions to administrators only. To do this, you can enable the Hidden option for the Read/Write button on the Custom Assay screen for nonadministrator roles.

The default role, AdminRole, can access all features. You cannot change the status of any feature to Disabled or Hidden for AdminRole.



❖ To define features for a role

- 1 Touch **Feature Role**.
- 2 Select the feature you are assigning in the Feature Name list or select multiple features with the Shift key or Control key.
- 3 Touch **Enabled** (role can use the feature), **Disabled** (role cannot use the feature), or **Hidden** (feature does not appear in the software) for each role.
- 4 If you have completed Account Management tasks, touch **Done**.



Note! You can use the **Import**, **Export**, and **Export incl. Users** options to share settings among multiple instruments.

❖ To define multiple features for a role

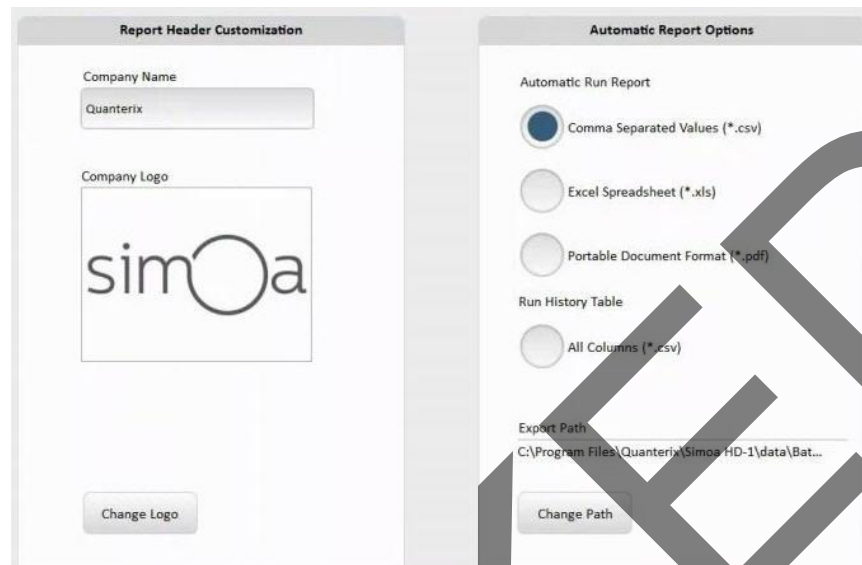
- 1 Touch **Role Feature**.
- 2 Select the role name you are configuring in the Role Name list or select multiple roles with the Shift key or Control key.
- 3 Touch **Enabled** (role can use the feature), **Disabled** (role cannot use the feature), or **Hidden** (feature does not appear in the software) for each feature.
- 4 If you have completed Account Management tasks, touch **Done**.

❖ To add a new role

- 1 Touch **Feature Role**.
- 2 Touch **Add Role**.
- 3 Enter a **Role Name**.
- 4 Optionally, select a role that is similar to the one you are creating from the dropdown list.
- 5 Touch **OK**.

Managing Report Settings

In the Report Settings tab, you can customize the company name and logo (.jpg, .jpeg, .jpe, .png, or .bmp format) that appear on reports. You can also specify the default output format and path for automatic batch reports and run history table. There is the option to select All Columns and export the run history report in .csv format.



❖ To customize report headers

- 1 In the Report Settings screen, enter the Company Name.
- 2 Touch **Change Logo**.
- 3 Browse to the logo file > touch **Open**.
- 4 Touch **Done** in the upper right corner of the tab.

❖ To customize report export defaults

- 1 In the Report Settings screen, select the Format.
- 2 Touch **Change Path**.
- 3 Browse to the folder you want to use for report output, or touch **New Folder**, specify a new folder name for reports.
- 4 Touch **Select**.
- 5 Touch **Done** in the upper right corner of the tab.

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Appendix A – Configure Columns Definitions

The following table describes each column in the Configure Table Header screens. The Configure Column function is accessed on the History & Reports tab for the Assay Reagent, Exceptions, Kit Search, Maintenance, Message Log, Sample Results, Run History and Event Log reports. The Configure Column function for the Maintenance report is located on the Maintenance tab.

Column Name	Comments
Affected Assay	Contains the name of the assay of the related job that is affected by the event (this does not relate to changes made to assays).
Affected Job	Displays which jobs are affected by reported messages/events.
Affected Plex	Contains the name of the plex of the related job or test order result that is affected by the event.
Affected Test Order	Contains the unique ID to identify the test order that is affected by the event.
Analog AEB	AEB value calculated using the analog mode. See Analysis Mode in this table to find out whether digital or analog mode was used to calculate the result AEB value. If the sample was run in replicate, the majority rule is used to determine the mode.
AEB	Average enzymes per bead.
Analysis Mode	Indicates whether the AEB calculation was performed using the digital or analog mode.
Assay	Name of the assay scheduled for the sample.
Assay Revision	The revision of the assay definition used.

Column Name	Comments
Batch ID	Unique system identifier of the sample batch.
Batch Name	Name of the batch specified on the Setup Run screen. If you do not specify a batch name, it defaults to batch creation date and time.
Blocks Sample Processing	Prevents user from starting a run when the task is not complete.
Blocks Shutdown when Due	Enable/disable. When enabled, a prompt appears when you shut down the software to either perform maintenance tasks or proceed with shutdown.
Calibration Curve ID	Unique system identifier of the calibration curve used in sample concentration calculations. This value is blank for calibrators as more than one calibration curve can be generated against calibrator AEB values.
Carrier Barcode	Barcode value of a microtiter plate or tube rack specified in the Setup Run screen.
Category	Categories for the given event. Possible values are: General, Maintenance, Job, L1DR, and L2DR.
Completion Date	Date and time when processing of the sample result completed.
Concentration	-
Creation Date	-
Curve Name	Name of the calibration curve used in sample concentration calculations. This value defaults to Measured. The calibration curve name can be edited in the Data Analysis tab.
CV AEB	Coefficient of variation of sample replicate AEB values for a given plex.

Column Name	Comments
CV Conc.	Coefficient of variation of sample replicate concentration values for a given plex.
Date Curve Created	Date and time when the calibration curve used in the sample concentration calculations was created. This value can be used to distinguish between curves if they all have the default name of Measured.
Dates Reagents Were Used	-
Description	Description of maintenance task performed.
Digital AEB	AEB value calculated using the digital mode. See Analysis Mode in this table to determine if digital or analog mode was used to calculate the result AEB value. If the sample was run in replicate, the majority rule is used to determine the mode.
Dilution Description	Name of the dilution applied to the sample test. The dilution description normally appears as a ratio of sample to diluent, for example, 4x, but it can carry any name, for example, One to Four, etc.
Dilution Factor	Multiplication factor applied to sample results when the concentration is reported. For example, for 4x dilution, the Dilution Factor will be 4.
Entry ID	Numeric value that shows the unique event ID from the database.
Errors	IDs of error flags associated with a sample test. To view additional information in the Event Log, click the error hyperlink.
Estimated Time to Result	Date and time when the system expects to deliver the result value.
Event ID	Represents a unique ID to identify a specific event.

Column Name	Comments
Event ID Hex	Represents a unique ID to identify a specific event - built of hex numbers and dots ".".
Event Text ID	Brief description of event.
Exception Description	Description of error message for a particular run.
Extended Properties	A collection of image analysis diagnostic information. For system diagnostic use only.
First Loading Time	-
Flags	The number of warning or error flags associated with processing of a sample test.
Fraction Monomeric Beads	Fraction of bead content that is monomeric – used to calculate actual bead concentration during bead aggregation assays.
Fraction On	Fraction of beaded wells with enzyme activity for a given plex. Both the digital and analog AEB calculation methods use this value.
Ibead	Average signal growth of beaded wells with enzyme activity. This value is used to calculate the result AEB value in analog mode. In digital mode, this value is not used. Isingle is calculated with Ibead values of jobs meeting the appropriate constraints.
Image Quality Score	Metric that helps the software determine whether a result should be included or excluded from analysis, as determined by image-processing algorithms. Currently, the value defaults to 1 for all sample results. For system diagnostic use only.
Instrument SN	Unique instrument identifier.

Column Name	Comments
Isingle	Estimated average signal growth of beaded wells that contain one enzyme. Certain conditions are required to calculate this value.
Interval	Identifies frequency of the task (e.g., daily, monthly, database size limit reached, etc.).
Job Completion Date	-
Job Creation Date	-
Job ID	Unique system identifier for a set of activities that lead to one result. For example, if a sample is scheduled to run an assay with one specimen in Neat and 4x dilutions in triplicate, the system will generate results for 6 unique jobs.
Job Start Cycle	The internal system clock used to coordinate sample processing activities. For system diagnostic use only.
Job Status	Status of each job.
Last Run Date	Date and time of last maintenance task run.
Localized/English Message	Detailed description of event.
Location	Location where the sample was loaded in the instrument.
Lot Numbers	Components/kits have a lot number that identifies the specific manufactured lot.
Lot Expiration Date	-
Mean AEB	Average of sample replicate AEB values for a given plex.

Column Name	Comments
Mean Conc.	Concentration calculated by averaging available individual replicate concentrations.
Message	Shows the full description of the error.
Name	Name of maintenance task performed (see Page 124).
Next Run	-
Number of Beads	Number of beads identified and used to produce the result for a given plex.
Plex	Name of the assay plex.
Reagent Barcode	Barcode located on reagent bottle identifies the reagent. Instrument uses barcoded information during processing.
Reagent Lots	-
Reagent Name	-
Remaining On-Board Time	Recommended time on-board until reagent expiration.
Replicate AEB	Sample AEB value for a given plex of a given job.
Replicate Conc.	Sample concentration value for a given plex of a given job.
Replicate Result ID	Unique system identifier for a replicate summary result. For example, if a sample was processed in triplicate, the replicate summary result will contain the mean, standard deviation, and coefficient of variation of the replicates (see Mean AEB, Mean Conc., SD AEB, SD Conc., CV AEB, CV Conc. in this table). For system diagnostic use only.
Result ID	Unique system identifier for a single result generated by the system. For system diagnostic use only.

Column Name	Comments
Result Status	Indicates whether the result was included or excluded from result calculations. The value can be one of: Automatically Included, Automatically Excluded, Manually Included, and Manually Excluded.
Sample Barcode	Sample identifier assigned by the user in the Sample Assignment screen. The value is entered manually or by scanning a barcode.
Sample Type	Specimen or calibrator sample type.
Selected	A column of check boxes for selecting records.
Severity	Defined as either a message, warning, problem, processing error, system halted or software failure.
SD AEB	Standard deviation of sample replicate AEB values for a given plex.
SD Conc.	Standard deviation of sample replicate concentration values for a given plex.
Source	Identifies location from which event originates such as PC, firmware, etc.
Status of Last Run	Status will be displayed as pending or finished. If status is pending, it may need to be executed prior to run.
SW Version	Software version. Provide to Quanterix Customer Support during troubleshooting.
Test Order ID	Unique system identifier for a grouping of sample jobs. For system diagnostic use only.
Type	An icon that specifies the severity of the event. The number in center of icon signifies how many messages of that type are listed in the log. See Severity.

Column Name	Comments
Unit	Unit of sample concentration.
Used Disc Lot Numbers	-
Used Reagents	A list of reagent barcodes used to produce the result.
User Name	The user who initiated the sample test that produced this result.

REVOKED

Appendix B – Instrument Specifications

The instrument must be installed by qualified service personnel only. Please do not move the instrument once it is installed.

Physical Specifications

Weight (without fluids)	294 kg (648 lb)
Dimensions	Width: 141.4 cm (55.66 in) Depth: 78.7 cm (31 in) Depth with monitor: 89.7 cm (35.3 in) Height: 161.2 cm (63.5 in)

Installation & Service Dimension Requirements

Doorway width for installation	80.0 cm (31.5 in)
Service clearance at instrument rear	60.0 cm (23.6 in) If clearance at the rear is not sufficient, enough front clearance must be available to move the instrument away from the wall for service.
Service clearance at instrument sides	60.0 cm (23.6 in)
Operator clearance at instrument front	100.0 cm (39.38 in)
Ventilation clearance at instrument rear	Minimum of 20.5 cm (8 in)
Earthquake safety	For sites that require the system to be secured from tipping due to an earthquake, adhesive straps must be affixed to the <u>rear panel</u> of the instrument only.

Power Requirements Instrument

Instrument	Electrical receptacles (2 each): <ul style="list-style-type: none"> • Europe: DIN49441 • North America: 5 – 15R
Voltage	100 V – 240 V \pm 10%
Frequency	50 – 60 Hz
Input current	7.5 A @100 VAC, 3.2 A @240 VAC
Fuse	Thermal overload protection

Computer and UPS

Computer	Input: 100 – 240 VAC ~50 – 60 Hz, 300W Ethernet (RJ45)
Recommended UPS	APC Smart-UPS SRT 2200VA 120V recommended

Environmental Operating Conditions

Operating temperature	18°C to 25°C (64.4°F to 77°F)
Operating humidity	30% to 80%, noncondensing
BTU output during operating conditions (non-standby)	3288 BTU/Hr

Noise Emission

Noise emission	72 dB(A), distance 1 m (39.4 in)
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Lasers

The Simoa HD-X Analyzer is a class 2 laser product. It incorporates the following laser sources.

Laser Barcode Scanner

Maximal output radiation	1.3 mW
Maximal pulse duration	110 μ s
Wavelength	650 nm
Reading distance	3.0 – 30.0 cm

Laser Light Barrier

Maximal output radiation	1.0 mW
Wavelength	650 nm

Packaging

See delivery note enclosed with the instrument for the contents, weight, and dimensions.

Number of crates	3
Crate 1	
Contents	Instrument
Dimensions (WxDxH)	160.0 cm x 92.0 cm x 178.0 cm (63 in x 36.2 in x 70.1 in)
Weight	378 kg (833 lb)

Crate 2	
Contents	Instrument covers
Dimensions (WxDxH)	157.0 cm x 80.0 cm x 97.0 cm (61.8 in x 31.5 in x 38.2 in) (Box 3 on top of crate 2 bundled)
Weight	130 kg (287 lb)
Crate 3	
Contents	Accessories
Dimensions (WxDxH)	120.0 cm x 80.0 cm x 50.0 cm (47.2 in x 31.5 in x 19.7 in) (packaged in card board box and can be handled separately)
Weight	39 kg (86 lb)

Environmental Conditions

The following table shows the range of conditions needed to run the system safely.

Environmental conditions	The system is made for indoor use.
Temperature	Operating: 18°C to 25°C (64.4°F to 77°F) Storage: 1°C to 45°C (33.8°F to 113°F) Transport: -20°C to 60°C (-4°F to 140°F)
Humidity	Operating: 30% – 80% noncondensing Storage: 5% – 80% noncondensing Transport: 20% – 80% noncondensing
Pollution degree	Degree 2
Overvoltage category	Class 2

Limit class	Class A (For industrial use. Domestic use restricted.)
Sunlight	No direct sunlight. May mislead optical sensors and affect performance.
Altitude	Up to 2000 m (1.24 mi) above mean sea level.
Dust	No excessive dust

Instrument Installation Dimensions

The following diagram shows a top-down view of the instrument.

