

Quanterix

Powering a Revolution in Healthcare

```
struct group_info Init_groups = { .usage = ATOMIC_INIT(2) }  
struct group_info Init_groups = { .usage = ATOMIC_INIT(2) }  
struct group_info *groups_alloc(int groupsize)  
struct group_info *groups_alloc(int groupsize)
```

Simoa HD-X Best Practices

Simoa Best Practices—Day 2

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2019

Simoa Best Practices



- Instrument Maintenances
- Overview of software Data Review
- Troubleshooting Review and Resources

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INSTRUMENT MAINTENANCE



Maintenance Checklist



Monthly Tasks	Performed by Enter initials and date completed below											
	Jan	Feb	Mar	Apr	May	June	July	Aug	Sep	Oct	Nov	Dec
Database Maintenance*												
Cleaned Surfaces												
Bulk Fluid Containers Rinsed												
Check for Leaks												
Check for Oil Leaks												
Temporary Files Cleanup												
Send Quanterix Reporting Tool data (QuaRT) if you report manually												

If you use your instrument four days per week or more, Quanterix recommends that you run the Database Maintenance task at **least once per week instead of once per month.*

Maintenance Tasks- Best Practices



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 156.
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 159.
Computer Memory Management	Monthly	Removes temporary files and performs disk utility that improves data access speeds.

Monthly Maintenance



- System fluid container cleaning and tubing rinse
- Cleaning the touchscreen with wet paper towel sprayed with glass cleaner (non ammonia)
- Clean external surfaces with wet cloth sprayed with 10% ethanol or water
- Clean other surfaces with 70% ethanol
 - System resource drawers
 - Sample and Reagent Bays
 - Bottom cabinet

Maintenance at Start of Day and End of Day



- If the instrument has completed an End of Day shutdown
 - Power cycle the instrument
 - Run Start of Day maintenance
- If the instrument has not completed an End of Day shutdown
 - Perform the End of day shutdown
 - Power cycle the instrument
 - Run Start of Day maintenance

Instrument Idle Best Practice



- If the instrument will not be used for **less than 4 weeks**, do the following twice per week of inactivity
 - Power on the computer and instrument
 - Do a Start of Day and End of Day
 - Power off the computer and instrument
- If the instrument will not be used for **greater than 4 weeks**,
 - Schedule a visit with Quanterix Service to perform maintenance before and after the scheduled idle period.



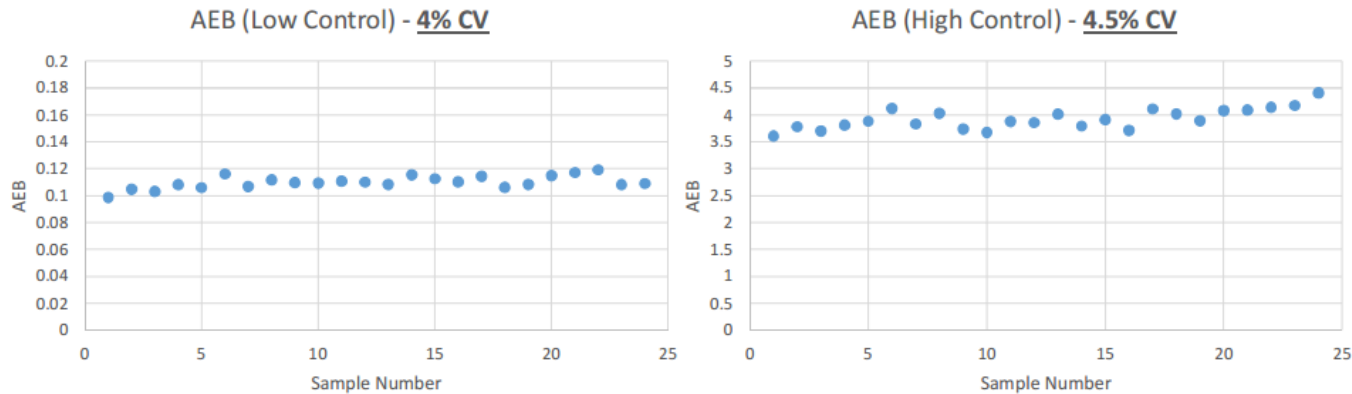
Customer Portal - Documentation

- Simoa HD-X Analyzer TM User Guide
 - Chapter 11: Maintaining the Simoa HD-X Analyze
- Simoa HD-X Analyzer Quick Start Guide
- Simoa HD-X Analyzer Monthly Maintenance Guide

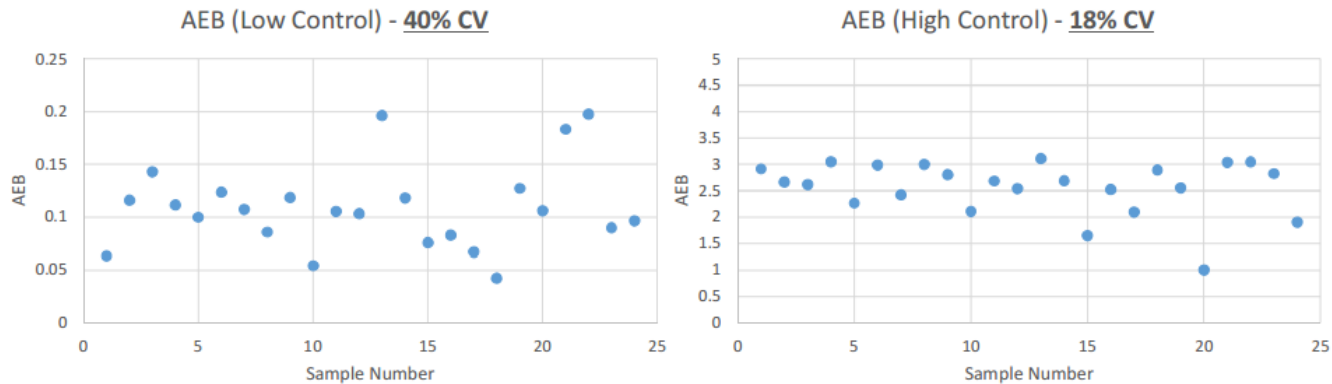
Instrument Maintenance



- Typical performance with required maintenance procedures being performed



- Performance when required maintenance tasks are ignored



Tech Bulletin: *Impact of Monthly Maintenance on Data Quality*
Simoa Instrument Maintenance for Version 1.5 Software

Assay Considerations: Sample Volume



Minimal Volume Calculation:

(Recommended sample volume (neat or divided by dilution factor) x Number of Replicates) + 30 uL dead volume for plates or 50 uL dead volume for Nalgene 5 mL tubes

Example for a recommended total volume of 100 uL – ASSAY DEPENDENT – check your kit instructions/package insert for correct volumes and dilutions

Calibrators (neat) in triplicates:

$(100 \text{ uL} \times 3 \text{ replicates}) + 30 \text{ uL dead volume} = 330 \text{ uL/well}$

Samples (4X dilution) in duplicates

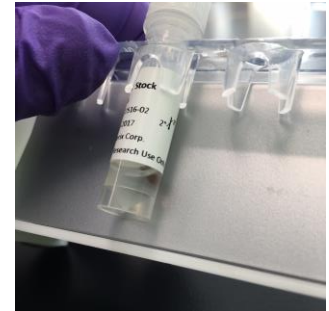
$((100/4\text{X dilution factor}) \text{ uL} \times 2 \text{ replicates}) + 30 \text{ uL dead volume} = (25 \times 2) + 30 = 80 \text{ uL/well}$

REMEMBER NOT TO LOAD MORE THAN 350 uL / well – Separate replicates in different wells if necessary

Discovery Kits



- Contains enough reagents for 192 tests
- Follow setup according to assay kit instructions/package insert.
 - Calibrator curve made by diluting calibrator concentrate. Dilute per kit instructions.
 - Reagents (Beads, Detector, SBG) are concentrated. Dilute to working concentration prior to running on instrument. Dilute SBG to final volume as soon as kit arrives
 - A magnet is required to wash beads before diluting to working concentration.



Advantage Kits with Cal Concentrate or Ref Cals



- Kits that come with a calibrator concentrate – check concentration on CoA (Certificate of Analysis) and prepare curve according to kit instructions/package insert
- Some of our kits come with pre-made reference calibrators. Check concentration of each calibration point on CoA (Certificate of Analysis), go to Assay Definition, Save As new definition, untick Read-Only and change concentrations under Plexes or change directly at the play layout

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OVERVIEW OF SOFTWARE DATA REVIEW

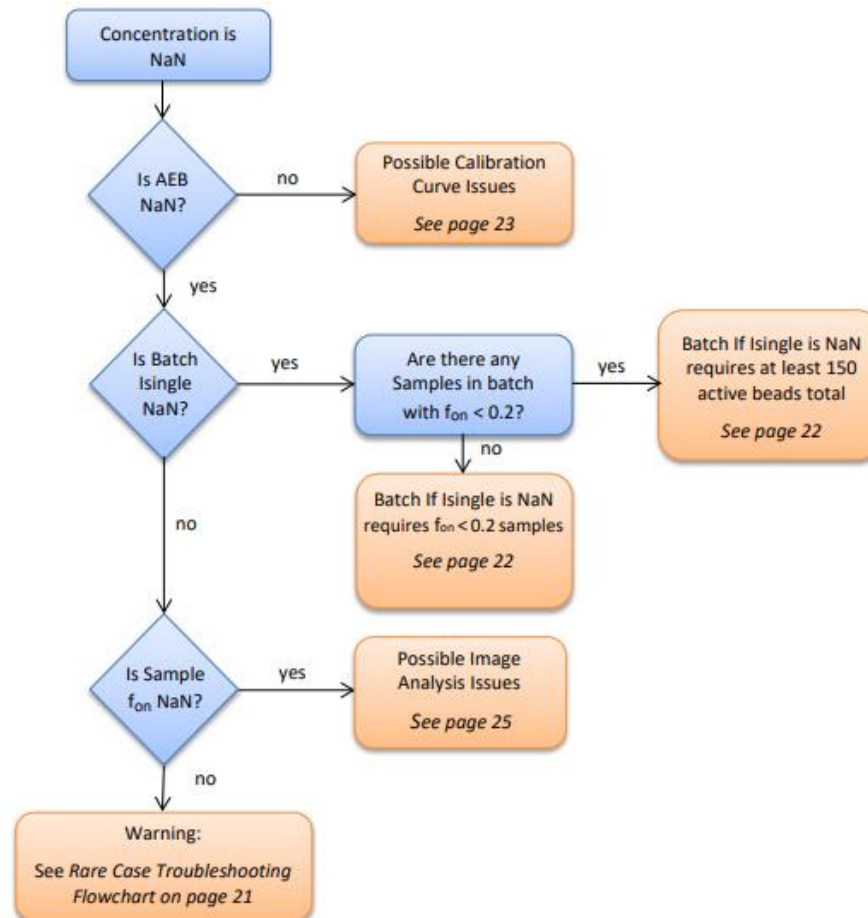




Customer Portal – Documentation

- Simoa HD-X Analyzer User Guide Software (Chapter 9) Analyzing Run Data
- Simoa HD-X Analyzer Data Analysis Guide
 - Chapter 5-Recalculating Sample Results with a Different Calibration Curve
 - Chapter 6-Troubleshooting

Troubleshooting Flowchart



Data Review

- Exporting Data – History & Reports Tab, Filter for Batch, Select all Results and Export CSV file of results
 - Add/Remove Columns and change column order using Configure Columns
- Exporting Batch Calibration Report – History & Reports Tab
- Calibration Curve – Data Reduction Tab

Run History

Selected	Batch ID	Sample Type	Calibration Curve ID	Curve Name	Replicate AEB	Replicate Conc.	Sample Barcode	Assay	Location	Mean AEB	SD AEB	CV AEB	Mean Conc.	SD Conc.
<input type="checkbox"/>	4	Calibrator			0.01	0	IL-6 2.0 Calibrator A	IL-6 2.0	Plate 1 - Well A5					
<input type="checkbox"/>	4	Calibrator			0.01	0	IL-6 2.0 Calibrator A	IL-6 2.0	Plate 1 - Well A5					
<input checked="" type="checkbox"/>	4	Calibrator			0.146	0.37	IL-6 2.0 Calibrator D	IL-6 2.0	Plate 1 - Well D5	0.051	0.001	0.011	0.123	0
<input checked="" type="checkbox"/>	4	Calibrator			0.146	0.37	IL-6 2.0 Calibrator D	IL-6 2.0	Plate 1 - Well D5	0.051	0.001	0.011	0.123	0

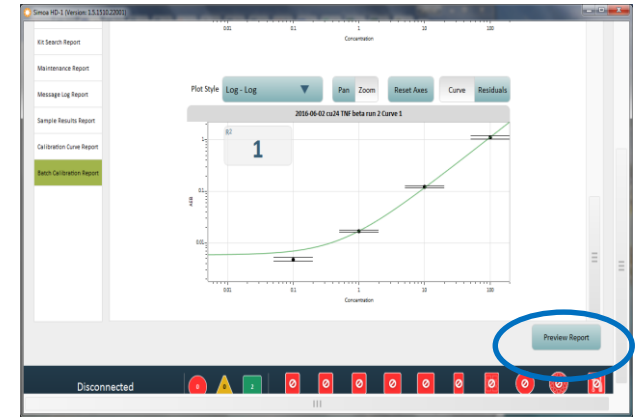
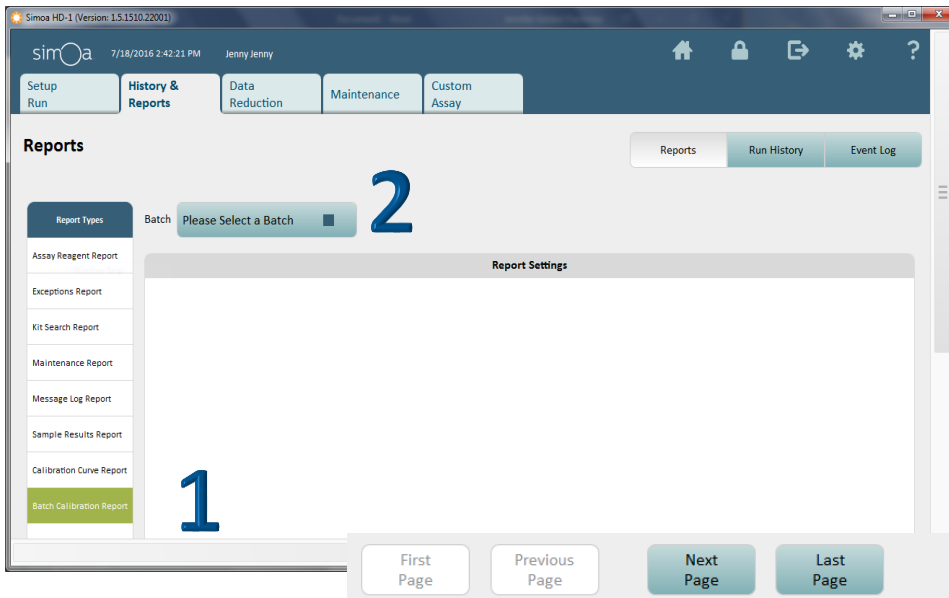
Automatic Replicates Selection On | Number of Selected Results: 33 out of 33

L2DR Result
 Replicate Result
 Flagged Result

Sample Type	Replicate	Replicate	Sample Barcode	Assay	Location	Mean AEB	SD AEB	CV AEB	Mean Conc	SD Conc.	CV Conc.	Unit
1	Sample Type											
2	Calibrator	0.010239	0	IL-6 2.0 Calibrator A	IL-6 2.0							pg/mL
3	Calibrator	0.010468	0	IL-6 2.0 Calibrator A	IL-6 2.0							pg/mL
4	Calibrator			IL-6 2.0 Calibrator A	IL-6 2.0	Plate 1 - V	0.010353	0.000162	0.015615	0	0	NaN
5	Calibrator	0.025331	0.0412	IL-6 2.0 Calibrator B	IL-6 2.0	Plate 1 - Well B5						pg/mL
6	Calibrator	0.026832	0.0412	IL-6 2.0 Calibrator B	IL-6 2.0	Plate 1 - Well B5						pg/mL
7	Calibrator			IL-6 2.0 Calibrator B	IL-6 2.0	Plate 1 - V	0.026082	0.001062	0.040718	0.0412	0	0
8	Calibrator	0.051829	0.123	IL-6 2.0 Calibrator C	IL-6 2.0	Plate 1 - Well C5						pg/mL
9	Calibrator	0.051008	0.123	IL-6 2.0 Calibrator C	IL-6 2.0	Plate 1 - Well C5						pg/mL
10	Calibrator			IL-6 2.0 Calibrator C	IL-6 2.0	Plate 1 - V	0.051419	0.00058	0.011286	0.123	0	0
11	Calibrator	0.146264	0.37	IL-6 2.0 Calibrator D	IL-6 2.0	Plate 1 - Well D5						pg/mL

History & Reports Tab – Reports Section Calibration Batch Report

- Select “Batch Calibration Report” from the left menu
- Select the Batch (pop up menu will appear) and press done

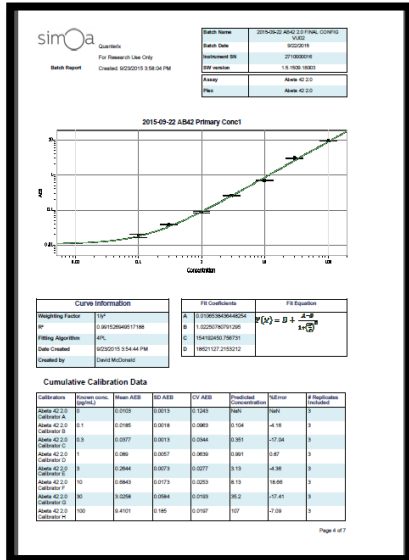


↑ A calibration curve preview will appear. Curve appearance can be adjusted – Press Preview Report

↓ Report Preview screen - press “Export” to save as a PDF or XLS.

History & Reports Tab – Reports Section

Calibration Batch Report



- Graph of Curve
- Curve Info.
- Fit Coefficients
- Fit Equation

Cumulative Sample Data

Sample	Location	Mean ABS	SD ABS	CV ABS	Dilution Factor	Mean	SD	CV	Concentration	Recovery
A	Rep#2-1	1045	1045	1045	1	1045	1045	1045	1	1
B	Rep#2-2	0.0968	0.0072	0.3629	1	0.109	0.0185	0.1434	1	1
C	Rep#2-3	0.0962	0.0062	0.1202	1	0.101	0.0052	0.0196	1	1
D	Rep#2-4	0.1124	0.002	0.0179	1	1.28	0.0049	0.019	1	1
E	Rep#2-5	0.0686	0.0061	0.0201	1	0.641	0.0077	0.0203	1	1
F	Rep#2-6	0.0662	0.0108	0.1914	1	10.8	0.162	0.0162	1	1
G	Rep#2-7	0.1208	0.0091	0.0476	1	0.15	0.0021	0.0046	1	1
H	Rep#2-8	0.1277	0.0064	0.0168	1	1.17	0.0196	0.019	1	1
I	Rep#2-9	0.1024	0.0107	0.2544	1	0.28	0.0213	0.0362	1	1
J	Rep#2-10	0.1228	0.0028	0.0214	1	0.13	0.006	0.028	1	1
K	Rep#2-11	0.0954	0.0095	0.0202	1	0.34	0.0207	0.0363	1	1
L	Rep#2-12	0.0854	0.0164	0.2642	1	0.2	0.0268	0.0364	1	1
M	Rep#2-13	0.0907	0.0042	0.0881	1	0.28	0.0118	0.028	1	1
N	Rep#2-14	0.0948	0.0025	0.0191	1	0.054	0.0041	0.0166	1	1
O	Rep#2-15	0.0206	0.0001	0.0019	1	0.004	0.0001	0.004	1	1

Calibration Replicate Data

Calibrator	Location	Mean	SD	CV	Concn	Recovery
A	Rep#1-1	0.0086	0	0	1	1
A	Rep#1-2	0.0105	0	0	1	1
A	Rep#1-3	0.0114	0	0	1	1
A	Rep#1-4	0.0137	0	0	1	1
A	Rep#1-5	0.0022	0.1	1	1	1
A	Rep#1-6	0.0112	0.009	0.1	1	1
A	Rep#1-7	0.0106	0.1	1	1	1
A	Rep#1-8	0.0061	0.3	1	1	1
A	Rep#1-9	0.0081	0.3	1	1	1
A	Rep#1-10	0.0043	1	1	1	1
A	Rep#1-11	0.003	1	1	1	1
A	Rep#1-12	0.0079	0	0	1	1
A	Rep#1-13	0.0035	0.1	1	1	1
A	Rep#1-14	0.0035	0.1	1	1	1
A	Rep#1-15	0.0035	0	0	1	1
A	Rep#1-16	0.0035	0	0	1	1
A	Rep#1-17	0.0035	0	0	1	1
A	Rep#1-18	0.0035	0	0	1	1
A	Rep#1-19	0.0035	0	0	1	1
A	Rep#1-20	0.0035	0	0	1	1
A	Rep#1-21	0.0035	0	0	1	1
A	Rep#1-22	0.0035	0	0	1	1
A	Rep#1-23	0.0035	0	0	1	1
A	Rep#1-24	0.0035	0	0	1	1
A	Rep#1-25	0.0035	0	0	1	1
A	Rep#1-26	0.0035	0	0	1	1
A	Rep#1-27	0.0035	0	0	1	1
A	Rep#1-28	0.0035	0	0	1	1
A	Rep#1-29	0.0035	0	0	1	1
A	Rep#1-30	0.0035	0	0	1	1

- Cumulative
- Calibration Data
- Sample Data

Sample Replicate Data

Calibrator	Location	Mean	SD	CV	Concn	Recovery
A	Rep#1-1	0.0072	0.001	0.0141	1	1
A	Rep#1-2	0.0081	0.001	0.0124	1	1
A	Rep#1-3	0.0077	0.001	0.013	1	1
A	Rep#1-4	0.0096	0.001	0.0104	1	1
A	Rep#1-5	0.0096	0.001	0.0104	1	1
A	Rep#1-6	0.0101	0.001	0.0099	1	1
A	Rep#1-7	0.0102	0.001	0.0098	1	1
A	Rep#1-8	0.0102	0.001	0.0098	1	1
A	Rep#1-9	0.0102	0.001	0.0098	1	1
A	Rep#1-10	0.0102	0.001	0.0098	1	1
A	Rep#1-11	0.0102	0.001	0.0098	1	1
A	Rep#1-12	0.0102	0.001	0.0098	1	1
A	Rep#1-13	0.0102	0.001	0.0098	1	1
A	Rep#1-14	0.0102	0.001	0.0098	1	1
A	Rep#1-15	0.0102	0.001	0.0098	1	1
A	Rep#1-16	0.0102	0.001	0.0098	1	1
A	Rep#1-17	0.0102	0.001	0.0098	1	1
A	Rep#1-18	0.0102	0.001	0.0098	1	1
A	Rep#1-19	0.0102	0.001	0.0098	1	1
A	Rep#1-20	0.0102	0.001	0.0098	1	1
A	Rep#1-21	0.0102	0.001	0.0098	1	1
A	Rep#1-22	0.0102	0.001	0.0098	1	1
A	Rep#1-23	0.0102	0.001	0.0098	1	1
A	Rep#1-24	0.0102	0.001	0.0098	1	1
A	Rep#1-25	0.0102	0.001	0.0098	1	1
A	Rep#1-26	0.0102	0.001	0.0098	1	1
A	Rep#1-27	0.0102	0.001	0.0098	1	1
A	Rep#1-28	0.0102	0.001	0.0098	1	1
A	Rep#1-29	0.0102	0.001	0.0098	1	1
A	Rep#1-30	0.0102	0.001	0.0098	1	1

- Replicate
- Calibration Data
- Sample Data
- Error Msg.

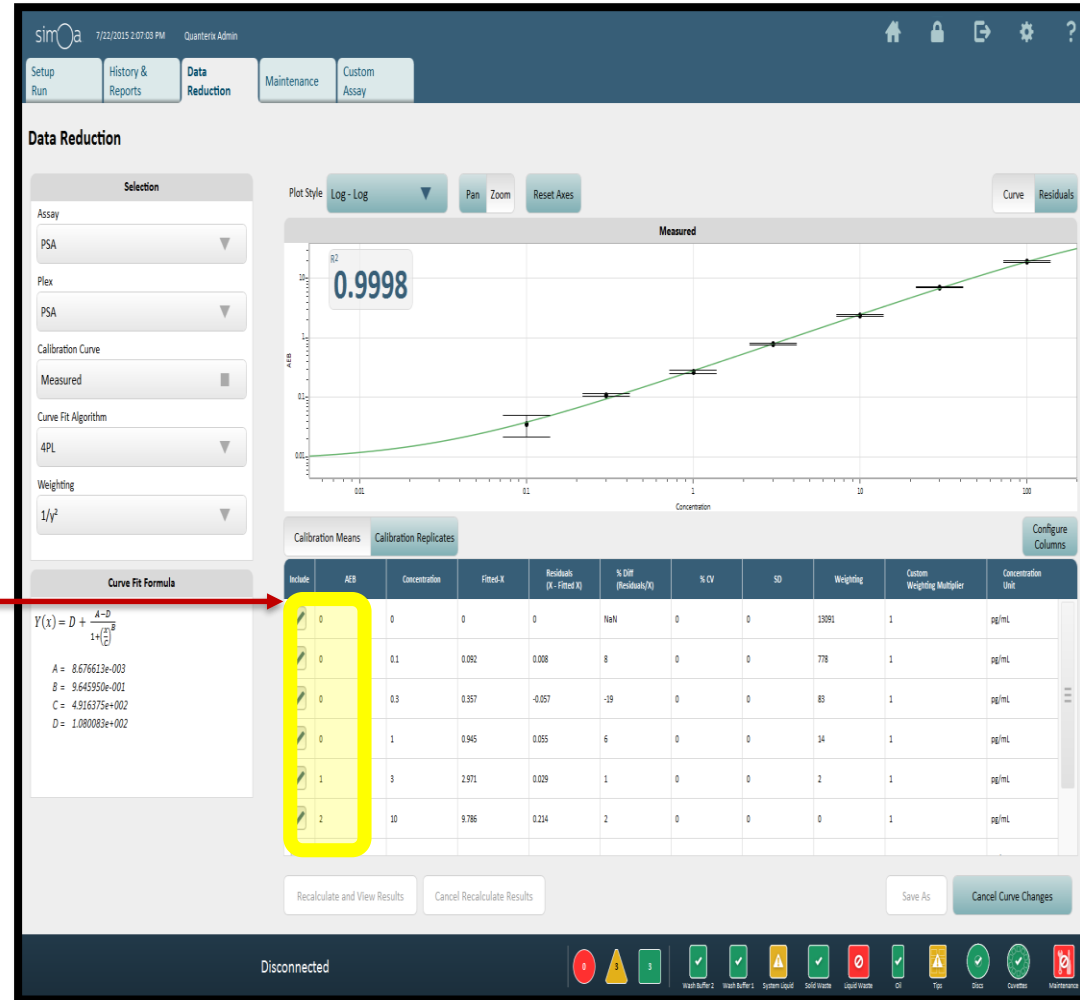
Assay Reagent Report

Reagent Name	Reagent Barcode	Lot Number	Lot Expiration Date
Alexa 422 Alexa	11211204800002	12048	10/15/2016
Alexa 422 DAPI	11211204800007	12048	10/15/2016
Alexa 422 DAPI	11211204800007	12048	10/15/2016
Alexa 422 DAPI	11211204800002	12048	10/15/2016
DAPI	110005127100020	11271	11/07/2016

- Assay Reagents
- Used Summary
- Lot Number
- Expiration

Data Reduction – Calibration Curve

- View Curve
 - Curve fit formula
- Adjust Curve
 - Remove single outliers
 - Remove calibrator level
 - Edit concentration values
- Press “Save As” when done
- Use New Curve to reprocess sample data
(History & Reports tab)



Simoa HD-X Data Analysis for Software Version 1.5

Re-analyzing Data – Changing Calibration Curve Values

- History and Reports Tab

1. Filter for your batch

2. Then add an additional filter for Sample type = Specimen

- Note: If Multiplex assay, add an additional filter for the plex

Selected	Batch ID	Sample Type	Replicate AEB	Replicate Conc.	Calibration Curve ID
<input type="checkbox"/>	2	Specimen	0.009	1.78882886419	3
<input type="checkbox"/>	2	Specimen	0.009	1.70965935251	3

3. Select all the samples you want to reprocess with the new curve

4. Press “Recalculate with Different Curve”. This will take you to the Data Reduction Tab

Automatic Replicates Selection 1 Number of Selected Results: 16 out of 16

2DR Result Replicate Result Flagged Result

Select all Results Deselect all Selected Results Exclude Selected Results from Analysis Include Selected Results into Analysis Show Related Flags and Events Recalculate with Different Curve Export Archive/Restore

Disconnected 0 3 Wash Buffer 2 Wash Buffer 1 System Liquid Solid Waste Liquid Waste Oil Tips Discs Cuvettes Maintenance

Re-analyzing Data – Changing Calibration Curve Values

- Choose the new calibration curve:
- Data Reduction tab
 - On left side of the screen, selection box:
Assay & Plex are filled in automatically, cannot be changed.
 - Select the Calibration curve you would like to use to recalculate your data
- Press “Recalculate and View Results”


The screenshot displays the 'Data Reduction' tab in a software interface. On the left, the 'Selection' panel includes dropdown menus for Assay (HB1 Singleplex 1), Plex (HB Plex 1), Calibration Curve (2016-07-17 Curve), Curve Fit Algorithm (Linear), and Weighting (None). Below this is the 'Curve Fit Formula' section with the equation $Y(x) = A + Bx$. On the right, a 'Log - Log' plot shows a linear trend with a data point highlighted at a concentration of 0.244. Below the plot is a table of calibration replicates.

Calibration Means		Calibration Replicates		
Include	AEB	Concentration	Fitted-X	Residuals (X - Fitted X)
<input checked="" type="checkbox"/>	0	0	-2.289406672	2.2894066726
<input checked="" type="checkbox"/>	0.002	0.244	-2.0105508964	2.2545508964
<input checked="" type="checkbox"/>	0.005	0.98	-1.5048675148	2.4848675148

At the bottom of the interface, the 'Recalculate and View Results' button is circled in blue.

Re-analyzing Data – Changing Calibration Curve Values

- This will take you back to the History & Reports tab
- It takes a few seconds for the re-calculation to complete. If you check the Calibration curve ID or name it will have the new value



Selected	Batch ID	Sample Type	Replicate AEB	Replicate Conc.	Calibration Curve ID
<input type="checkbox"/>	2	Specimen	0.009	1.78882886419	3
<input type="checkbox"/>	2	Specimen	0.009	1.70965935251	3
<input type="checkbox"/>	2	Specimen	0.011	2.08801956067	3

Selected	Batch ID	Sample Type	Replicate AEB	Replicate Conc.	Calibration Curve ID
<input checked="" type="checkbox"/>	2	Specimen	0.009	1.6296795205	10
<input checked="" type="checkbox"/>	2	Specimen	0.009	1.55646943631	10
<input checked="" type="checkbox"/>	2	Specimen	0.011	1.9069799101	10

Quanterix™

CUSTOMER SUPPORT TOOL



Customer Support Tool / Team Viewer



Customer Portal – Documentation

- Simoa HD-X Analyzer Customer Support Tool User Guide
 - Generate SQT Report
 - Export IPL images
 - Generate QuaRT Report
 - Database BackUp
- Simoa HD-X Analyzer Reporting Tool Technical Information (QuaRT)



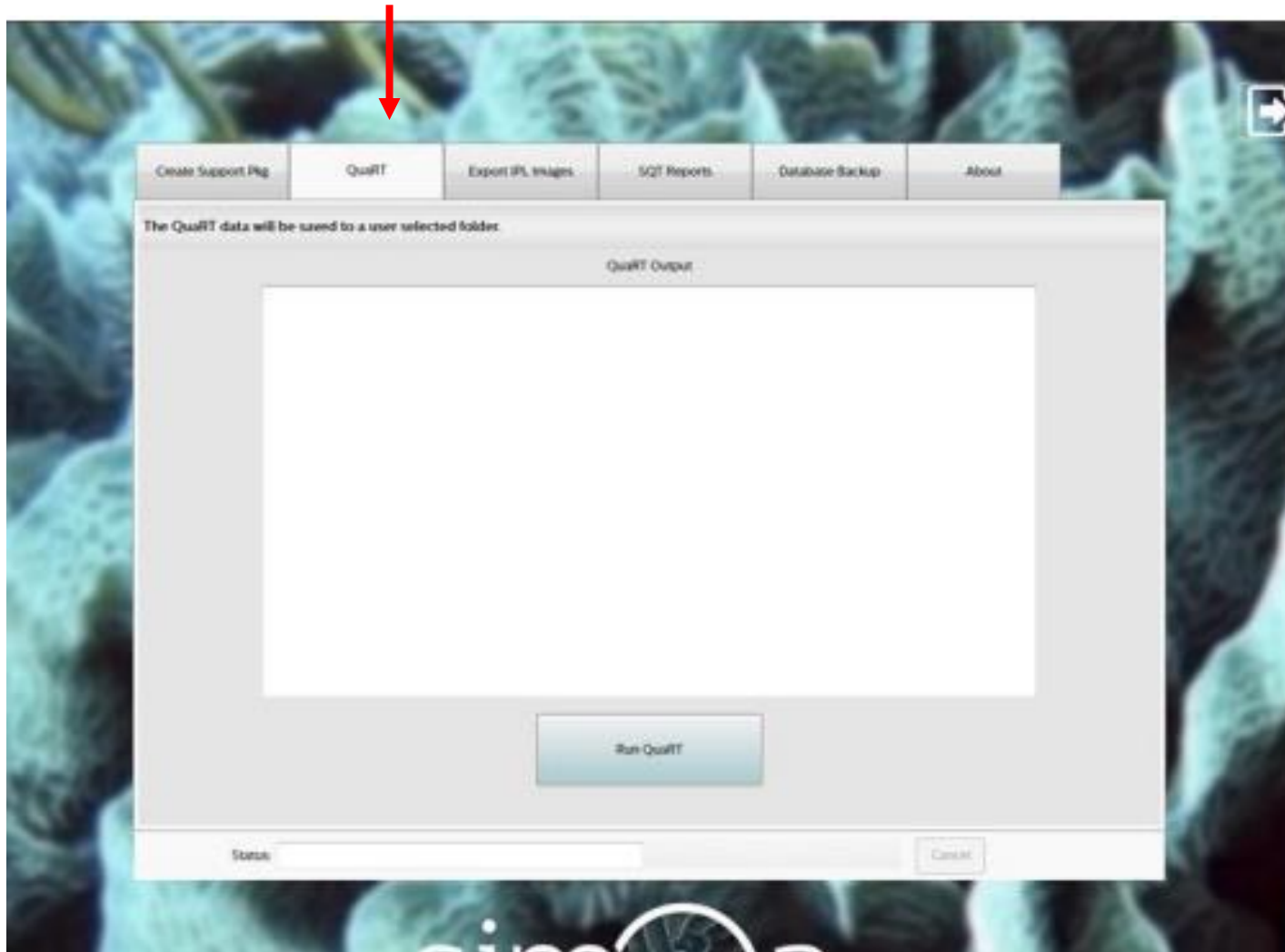
Can be configured to be uploaded to Quanterix automatically

If internet is not available for your instrument, another file share system (i.e. dropbox) can be used

Team Viewer

- Remote access if need assistance

QuaRT monitors reliability and performance of the HD-X

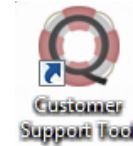




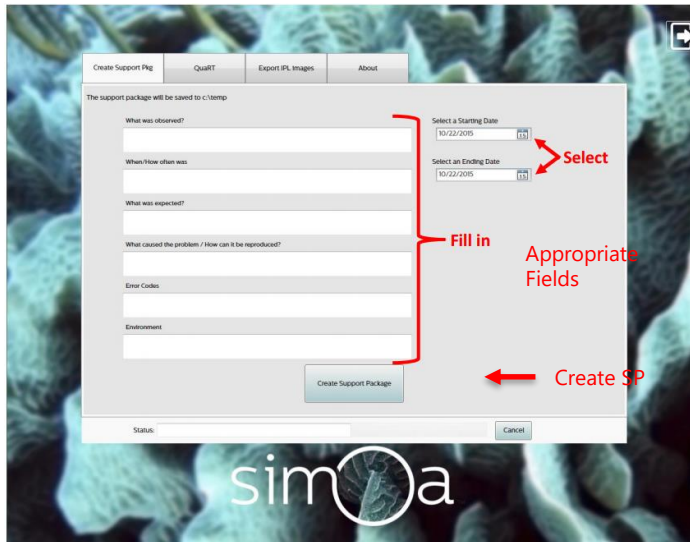
- Tracks performance on collected data
- Provide the customer feedback on instrument performance
- QuaRT obfuscates data from the database for some parameters that are considered customer-specific, sensitive, or proprietary
- As an example, a customer assay with the name “Homebrew Assay 1” is reported by QuaRT with a value of “EF0CD653EA048DA0994E8DBE91D6EE98” where the exact value is unique to each assay on each HD-X Analyzer

Customer Support Tool

- Generate Support Package
 - Fill in info about error
 - Collects log files for selected date(s)



- Generate IPL image files for selected batch
 - All image files (no boxes checked)
 - Failed images only (left box)
 - White Light images



Instrument / Assay Issues



- Upload **support package** for the day the issue was observed
- Upload **White Light Images** for the specific run
- Send the **csv file** for specific run and explain the problem and assay setup to techsupport@quanterix.com