Quanterix™
The Science of Precision Health

Simoa® HD-X Analyzer Data Analysis Guide

USER-0073 01

simoa™

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Contents

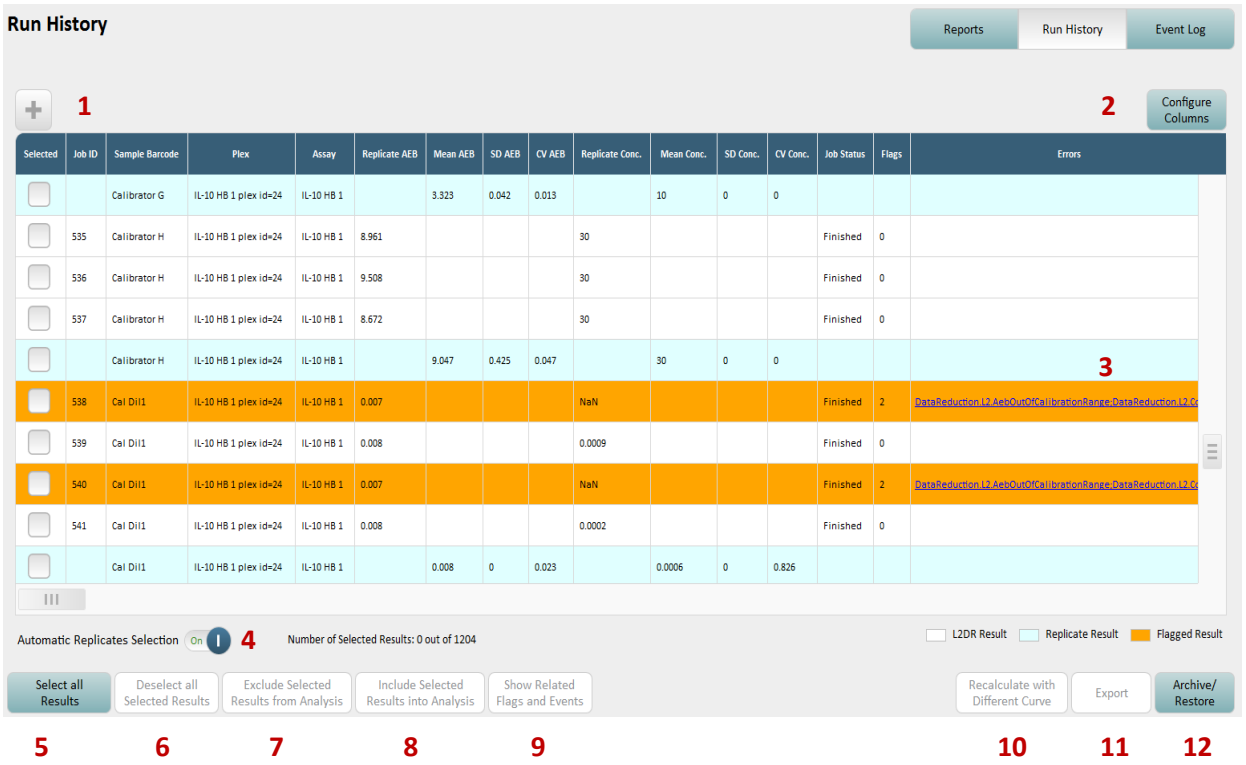
1 Overview

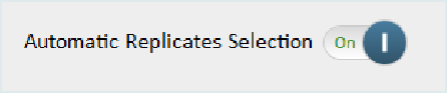
This document explains how to analyze data when an assay run is complete. You can analyze results with the Simoa® HD-X instrument software or with an external analysis package. See the *Simoa HD-X Analyzer User Guide* for more information on using the instrument.

Run History Screen Overview

The Run History screen is used to select the results you want to review. See the table on the next page for an explanation of the numbered items. See *Selecting Results on the Run History Screen* on page 8 for information on using this screen.

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

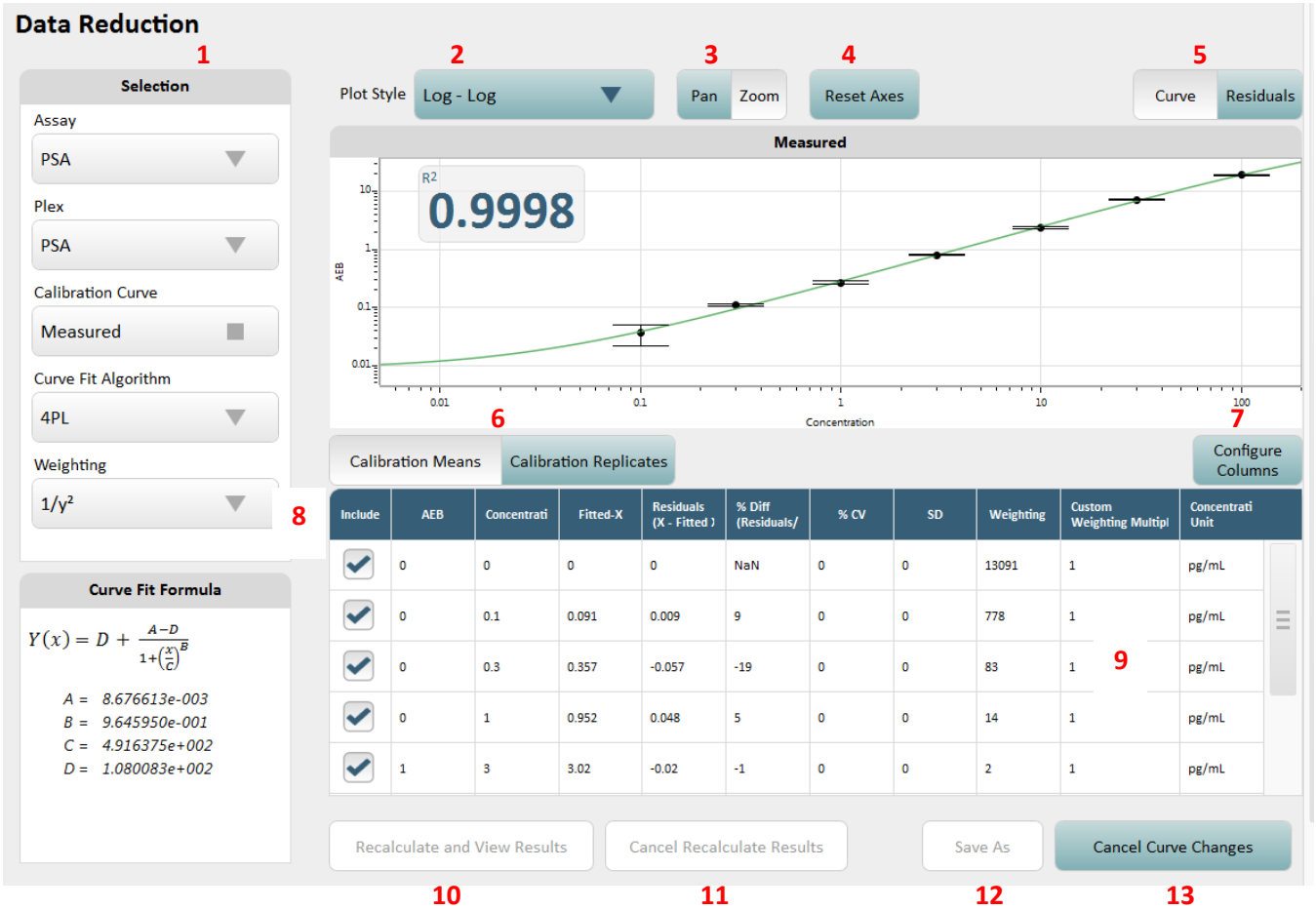


Column Name	Description
1. Add New Filter 	Adds filter criteria that determine what results appear in the table. Multiple filters are allowed. Touch the Add New Filter button (+) to add a filter. Touch the X within the filter button to remove the filter from the table. **TIP:** Loading a large amount 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 the user to configure column display and ordering. See *Column Definitions* on page 10 for a description of the columns.
3. Errors (link to complete error message)	Click on the error message link to see the full text of the error. Results with flags will be highlighted with orange.
4. Automatic Replicates Selection	When this option is turned on, the software automatically selects all replicates when one of the replicate results is selected. 
5. Select all Results	Selects all currently displayed results, as determined by data filters, if any are in place.
6. Deselect all Selected Results	Clears all selections made on results in the table.
7. Exclude Selected Results from Analysis	Marks selected results for exclusion in analysis. Only results that have been automatically included in analysis can be excluded manually. Isingle is not recalculated when excluding results in the Run History table. For more information on Isingle, see the listing in the table beginning on page 10. **TIP:** Ensure the Automatic Replicates Selection button is turned off > select the replicates to be excluded from analysis, then touch the **Exclude Selected**

Column Name	Description
	Results from Analysis button for the result.
8. Include Selected Results into Analysis	Marks selected results for inclusion in analysis. Only results that have been automatically excluded from analysis can be manually included.
9. Show Related Flags and Events	Displays all flags and event messages for selected results.
10. Recalculate with Different Curve	Allows the user to recalculate concentration results by manipulating the default calibration curve used or to select a new calibration curve. See *Recalculating Sample Results with a Different Calibration Curve* on page 19.
11. Export	Exports displayed results to a comma separated value (CSV) file. The software will export all columns displayed in the table. If you have not selected a column to display with Configure Columns (Item 2 in this table), it will not be exported. Additionally, if you applied data filters to the table, only results matching the filter criteria will be exported.
12. Archive/Restore	Provides the 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 Simoa HD-X software running on another computer.

Data Reduction Screen

This section provides an overview of the Data Reduction screen. The table on the next page describes each component of the screen. See *Reviewing Calibration Curves in the Data Reduction Tab* on page 18 for information on using this screen.



The following table describes the numbered items on the *Data Reduction Screen* on page 4.

Column Name	Description
1. Selection	Controls which calibration curve is displayed and the fitting algorithm and weighting used to generate the curve. Note the following minimum number of points needed for each curve formula.

Points Needed	Curve Formula
2	Piecewise Linear, Linear, Exponential, Log-Log
3	Quadratic
4	Cubic, 4PL
5	5PL

2. Plot Style	Controls how the data is plotted.
3. Pan/Zoom	When set to pan, changes focus as you drag across the plot. When set to zoom, zooms in on the area you specify by dragging.
4. Reset Axes	Reset the axes to the default values.
5. Curve/Residuals	**Curve** shows the curve itself. **Residuals** shows errors of each data point.
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 that you want to display in the calibrator data table.
8. Include	To exclude individual replicate results from analysis, uncheck the **Include** checkbox in the row you want to exclude.

9. Calibrator data table	Contains all calibration data points generated during a batch that can be used to create a curve. An automatically generated curve uses only valid data points. You can choose the calibrator levels or individual calibrator replicates used to create the curve. Manipulating an existing curve's data does not alter the curve; you must save it as a new curve. Calibration curves are batch based, meaning that calibrators can be placed across one or more plates. The curve generated during a batch run will be applied to all specimen samples that have the corresponding assay test assigned to them. **Note:** If results have been changed to **Hide Result** on the Assay Plexes tab of the Custom Assay screen, the results do not appear in the table. The table includes aggregated replicates data when **Calibration Means** is selected; toggling **Calibration Replicates** mode will show individual replicates. **Excluding Selected Results:** To exclude individual replicate results from analysis, see **Exclude Selected Results from Analysis** in the table on page 2.
10. Recalculate and View Results	After selecting a different calibration curve, recalculates the results and displays them in the Run History table.
11. Cancel Recalculate Results	Revert to previous calculation results.
12. 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.
13. Cancel Curve Changes	Resets any changes made to the existing calibration curve.

2 Creating New Calibration Curves

Once a calibration curve is generated, it cannot be modified. However, you can create a new curve based on an existing curve with changed concentrations and/or excluded replicates. This new curve can be used instead of the old one for obtaining the concentration of a specimen.

To generate a new curve

1. Select the curve in the data reduction screen you wish to use as the base curve.
2. Ensure the **Calibration Means** view of the table is active.
3. Change concentrations and/or exclude selected replicates:
   - Double-click on a concentration, and enter the requested value. You can modify any number of calibrator concentrations prior to saving the new curve.
   - Exclude replicates from the curve by changing to the **Calibration Replicates** view and deselecting the **Include** checkbox of specific replicates.
4. Touch **Save As** and name the new curve.

This procedure does not modify the original curve; instead, a new curve with a new name is created.

After generating a new curve, all specimens remain associated with the old curve.

To read concentrations from the new curve

1. Navigate to Run History screen on the History & Reports tab.
2. Ensure **Automatic Replicate Selection** is turned on.
3. Select the specimen you want to read off the new curve.
4. Touch **Recalculate with Different Curve** to change to the Data Reduction screen.
5. Choose your new curve on this screen and touch the **Recalculate and View Results** to return to the old screen.

The concentration of the specimen is now associated with the new curve.

Again, calibration curves cannot be modified and specimens do not automatically change over to a new curve when one is generated. You can only create new curves based on existing ones, you must save the new curve, and you must select the curve from which a sample is read.

3 Exporting Results for Analysis

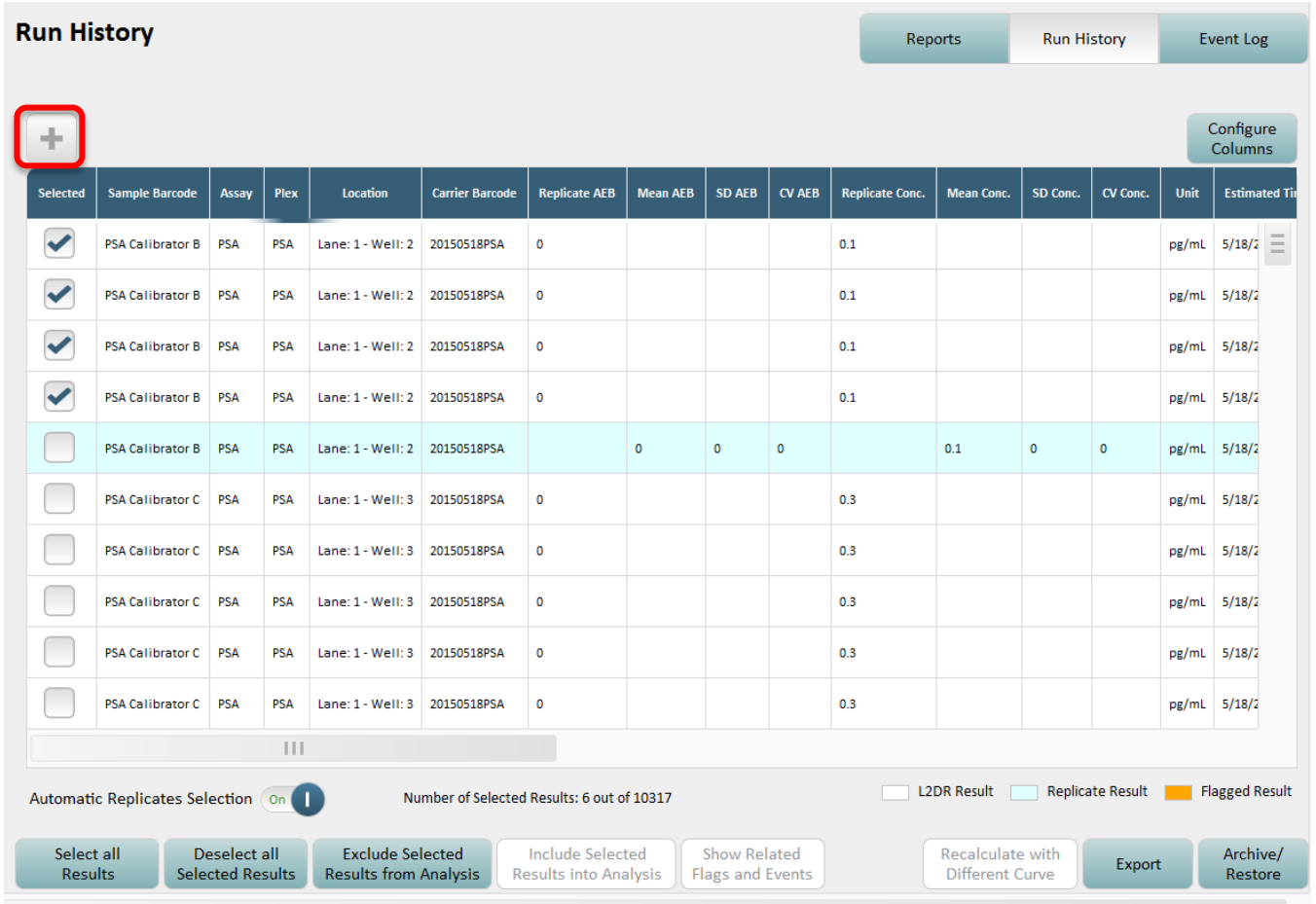
This section explains how to select the results you want to analyze (for singleplex and multiplex assays) and how to export them to a CSV file for external analysis. See *Run History Screen Overview* on page 1 for more information on selecting results.

Selecting Results on the Run History Screen

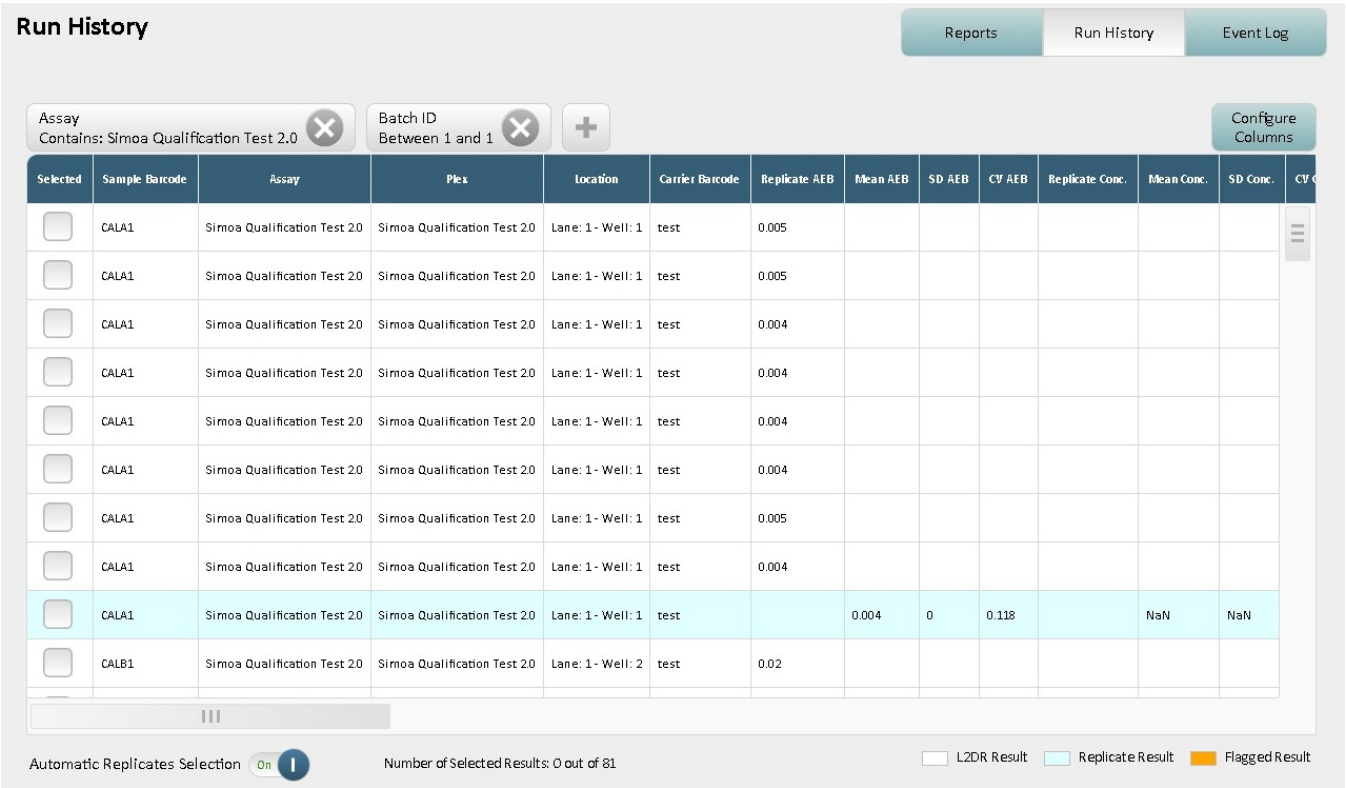
Selecting Results

Typically, you can filter on Batch ID and Plex Name to select results for singleplex assays.

1. Navigate to the Run History screen on the History & Reports tab.
2. Clear all filters in the results table.
3. Touch the **Add Filters** button (+).



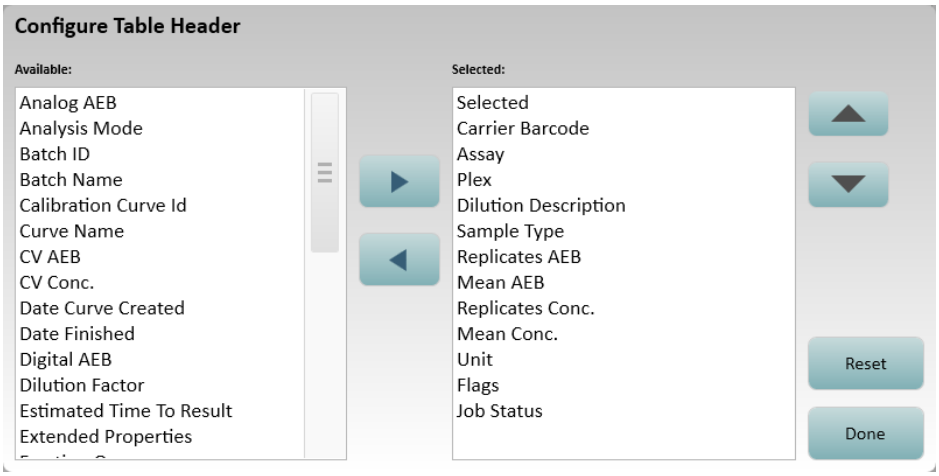
4 Select a filter from drop-down list > touch Next.
Add filters to narrow the results displayed in the table to the experiment data that you want to export or review in the Data Reduction screen (typically Batch ID and Assay Name). If you are exporting multiplex results, filter on Plex to export one plex at a time.



5 Touch **Configure Columns**.

6 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 Column Definitions on page 10 for an explanation of each column. Touch Done.

Note: You can rearrange columns directly in the results table by holding and dragging the column headers.



7 Touch **Select All**.

8 Export the results (see *Exporting Results* on page 16).

Column Definitions

The following table describes each column in the Configure Table Header screen.

Column Name	Description
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 (see *What is the majority rule?* on page 33).
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.
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.

Column Name	Description
Bead Concentration	Value that specifies bead concentration of Homebrew beads during a bead aggregation run.
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.
Completion Date	Date and time when processing of the sample result completed.
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.
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.
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 (see *What is the majority rule?* on page 33).
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

Column Name	Description
	concentration is reported. For example, for 4x dilution, the Dilution Factor will be 4.
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.
Extended Properties	A collection of image analysis diagnostic information. For system diagnostic use only.
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 a bead aggregation run.
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.
Isingle	Estimated average signal growth of beaded wells that contain one enzyme. Certain conditions are required to calculate this value; see *If Isingle is NaN* on page 22 for details.

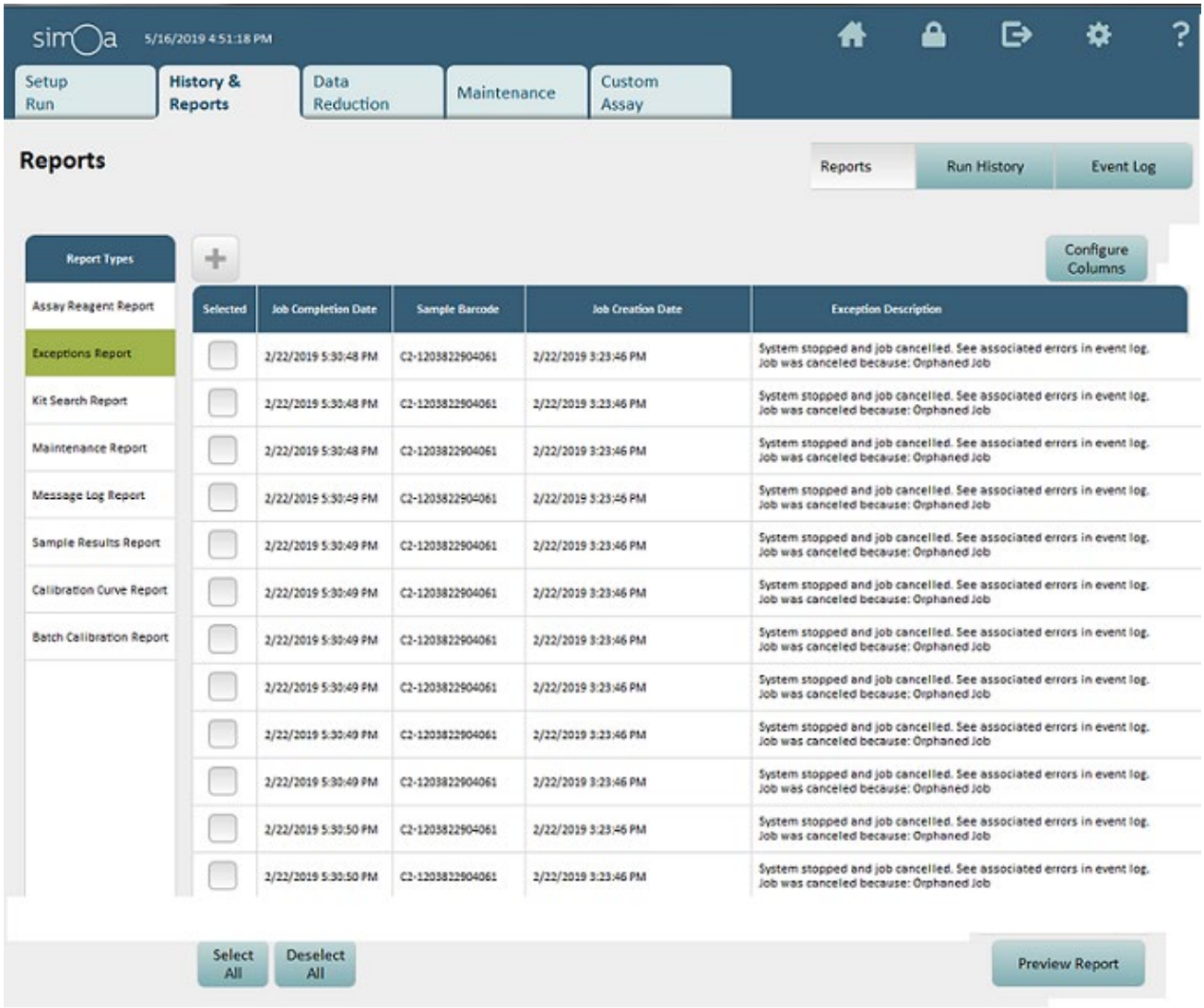
Column Name	Description
Job ID	Unique system identifier for a set of activities that lead to one result. For example, if a sample is scheduled to run an assay with one specimen in Neat and 4x dilutions in triplicate, the system will generate results for 6 unique jobs.
Job Start Cycle	The internal system clock used to coordinate sample processing activities. For system diagnostic use only.
Job Status	Status of each job.
Location	Location where the sample was loaded in the instrument.
Mean AEB	Average of sample replicate AEB values for a given plex.
Mean Conc.	Concentration calculated by averaging available individual replicate concentrations.
Number of Beads	Number of beads identified and used to produce the result for a given plex.
Plex	Name of the assay plex.
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.
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.

Column Name	Description
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.
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.
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.
Unit	Unit of sample concentration.
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.

Displaying Sample Batch Messages

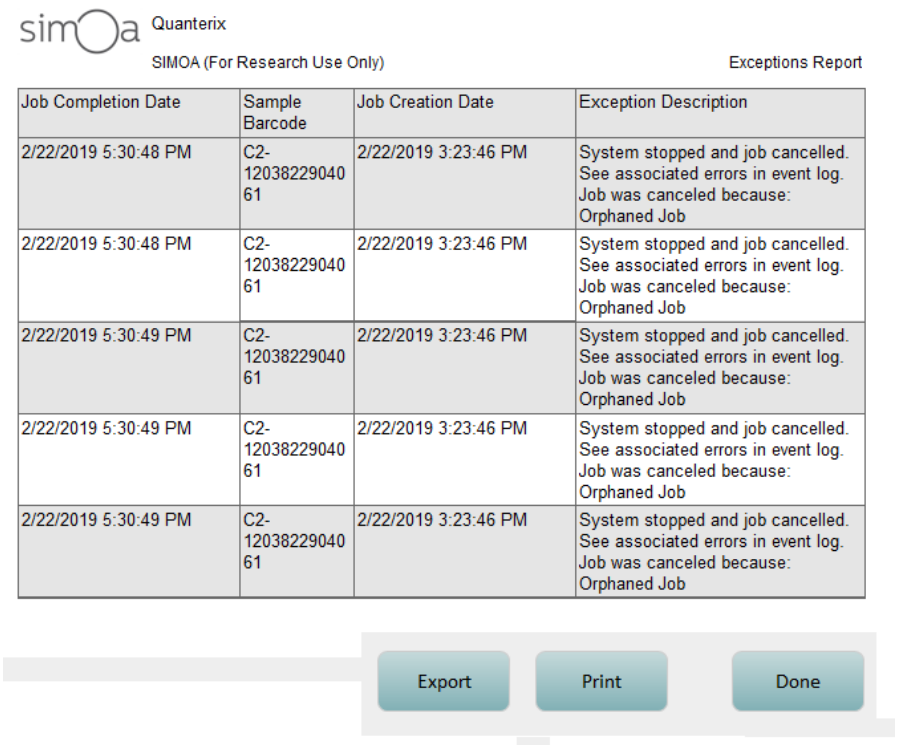
Follow these steps to display a log of events that occurred during the assay run. Events contain relevant information about events that may affect data precision.

1. Access your results on the Run History screen in the History & Reports tab and configure the table columns to include Batch ID and Test Order ID. Note the Batch ID of your results.
2. In the History & Reports tab, touch **Reports**.
3. On the Reports screen, touch **Exceptions Report** on the Report Types pane.



4 Select the option to filter by Batch ID and type the Batch ID of your results, as noted in step 1.

5 Touch **Report Preview** and wait for the preview screen to show.



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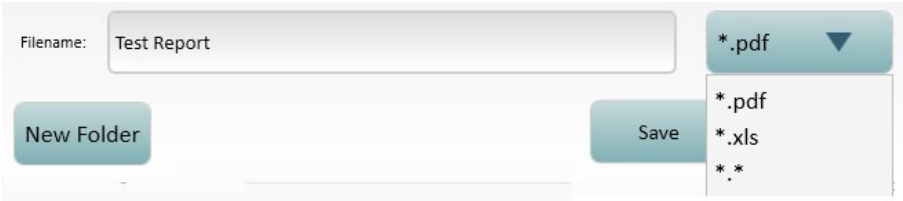
SIMOA (For Research Use Only) Exceptions Report

Job Completion Date	Sample Barcode	Job Creation Date	Exception Description
2/22/2019 5:30:48 PM	C2-1203822904061	2/22/2019 3:23:46 PM	System stopped and job cancelled. See associated errors in event log. Job was canceled because: Orphaned Job
2/22/2019 5:30:48 PM	C2-1203822904061	2/22/2019 3:23:46 PM	System stopped and job cancelled. See associated errors in event log. Job was canceled because: Orphaned Job
2/22/2019 5:30:49 PM	C2-1203822904061	2/22/2019 3:23:46 PM	System stopped and job cancelled. See associated errors in event log. Job was canceled because: Orphaned Job
2/22/2019 5:30:49 PM	C2-1203822904061	2/22/2019 3:23:46 PM	System stopped and job cancelled. See associated errors in event log. Job was canceled because: Orphaned Job
2/22/2019 5:30:49 PM	C2-1203822904061	2/22/2019 3:23:46 PM	System stopped and job cancelled. See associated errors in event log. Job was canceled because: Orphaned Job

Export Print Done

6 Click **Export**, select a folder, and save the report as a .pdf or .xls file.



7 Match the Test Order ID value from your results page to the Test ID in the Exceptions Report.

Exporting Results

Follow these steps to export assay results for analysis or troubleshooting.

1 Access your results in the Run History screen on the History & Reports tab (see *Selecting Results on the Run History Screen* on page 8).

2 Touch **Export**.

3 Name the export file, choose the directory, and touch **Save**.

4 Open the CSV file in Excel or your external analysis software.

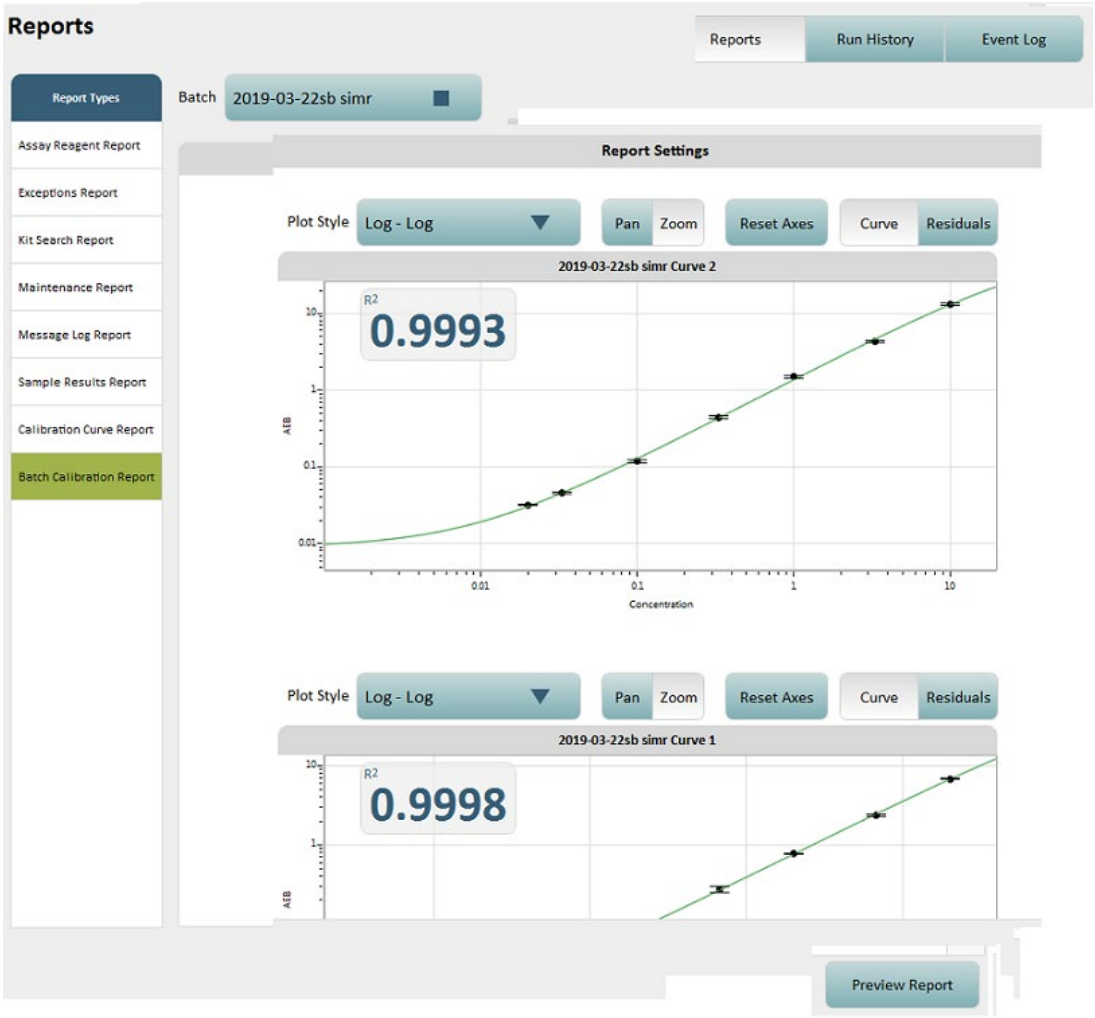
Exporting a Batch Report

The batch report displays a graph of your calibration curves and a table listing all reagents, samples, concentrations, and reported flags.

1 Navigate to the History & Reports tab.

2 Touch **Reports**.

3 Touch **Batch Calibration Report** in the Report Types pane.



4 Choose a batch to view from the Batch menu.

5 In the main pane, specify the curve display options (plot style, curve vs. residuals, for example) for the report.

6 Touch **Preview Report** to display the report.

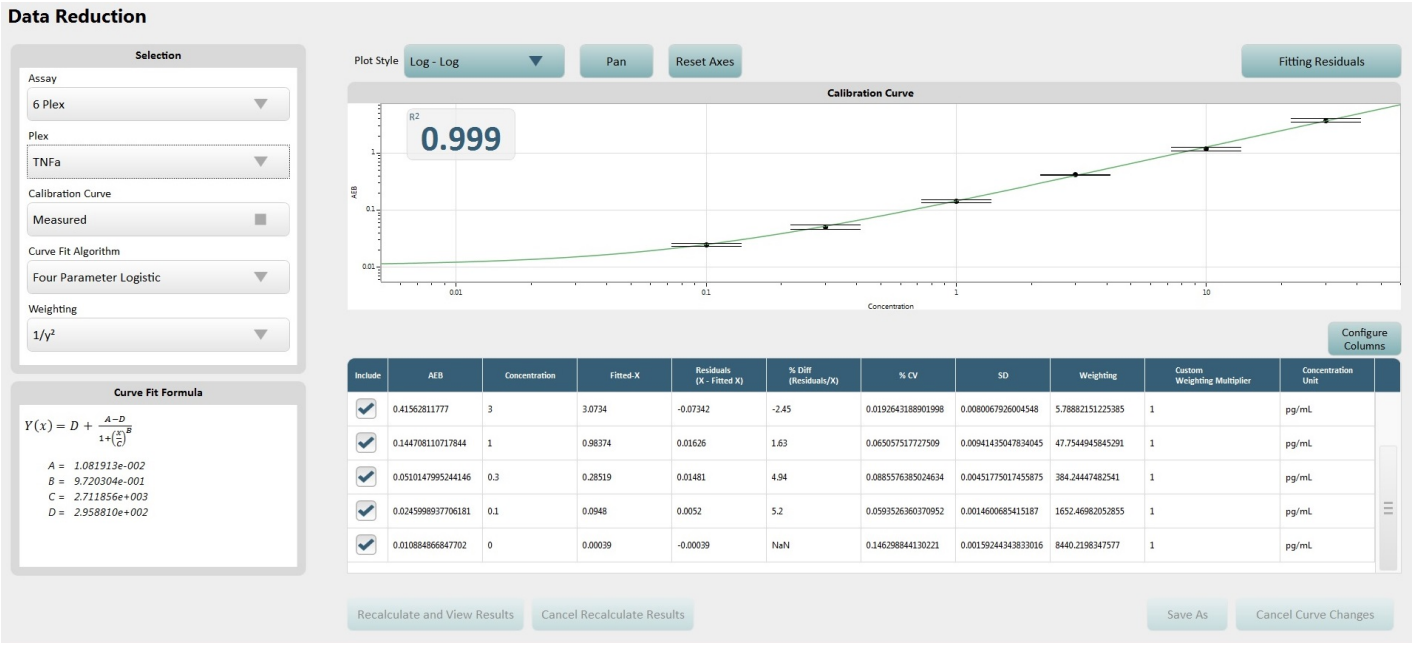
7 Optionally, touch **Export** to save this report to your computer or to a network drive in PDF or XLS format.

4 Reviewing Calibration Curves in the Data Reduction Tab

The following example shows one plex curve in a multiplex assay (6-plex). These instructions apply to results that contain calibrators. The software does not create a new curve for runs that do not contain a full set of assay calibrators.

The calibration curve provides an indication of how the assay plexes are performing. The R^2 value measures how well the data points fit the curve, with an R^2 value of 1 representing the best fit. You can view only one plex curve at a time.

1 From the Data Reduction tab, select the appropriate assay name from the Assay dropdown list.



2 Select a Plex.

3 Select a Calibration Curve, touch **Done** and review the data. If there are multiple curves with the same date, select the curve with the most recent timestamp.

Note: If your assay run contains samples only (without calibrators) you can select a previously run calibration curve (from an earlier run or a different date) to fit and quantify the sample concentrations.

4 Repeat steps 2 and 3 for each plex in the assay.

5 Recalculating Sample Results with a Different Calibration Curve

This section explains how to recalculate sample results with a different or modified calibration curve.

1. In the Run History screen on the History & Reports tab, add the following filters to narrow results to specific assay plex samples (do not include calibrators in the result):
   - Batch ID
   - Assay Name
   - Plex name (if it is a multiplex assay and you are exporting to an external analysis package)
   - Sample Type > Specimen
2. Make sure that the Calibration Curve ID is the same for all samples. Do not select samples that do not have values in the Calibration Curve ID column.
3. If you ran your samples as replicates, select all sample replicates. If **Automatic Replicates Selection** is turned on, the software automatically selects all replicates when one of the replicate results is selected.
4. Touch **Select all Results** or select individual results to recalculate.

Note: **Recalculate with Different Curve** is enabled only when the selection contains results from the same assay plex. It is disabled if your selection contains more than one assay plex, regardless of the associated assay.

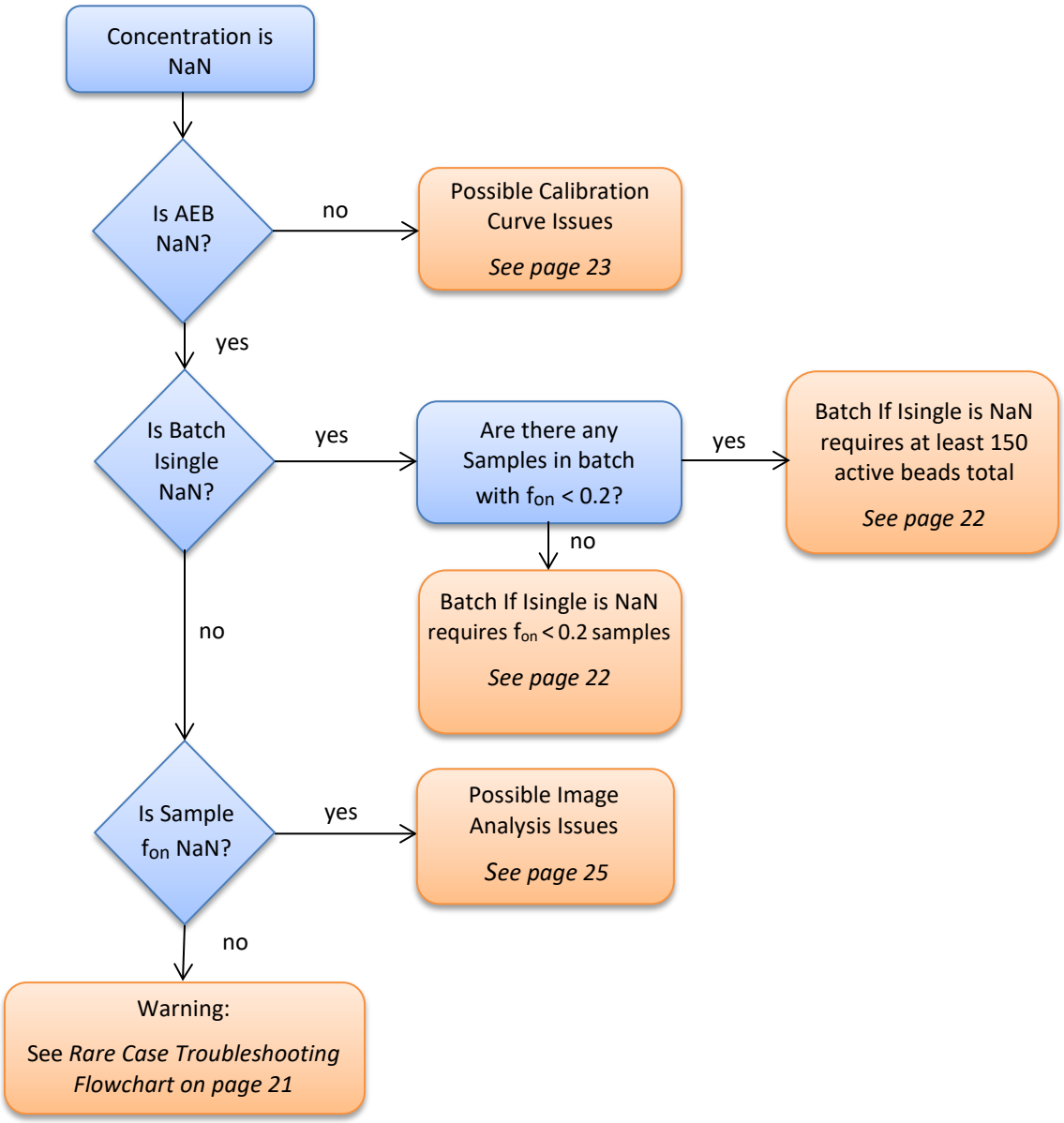
5. Touch **Recalculate with Different Curve**. The Data Reduction tab appears.
6. Modify the curve parameters until you are satisfied with the resulting curve and touch **Save As**. See page 7 for the different possibilities for modification.
7. Name your curve and touch **Save**.
8. Touch **Recalculate and View Results**. The software recalculates the sample results and displays them in the History & Reports tab when the calculations are complete.

6 Troubleshooting

The information in this chapter will help you investigate the root cause of missing result outputs from data analysis.

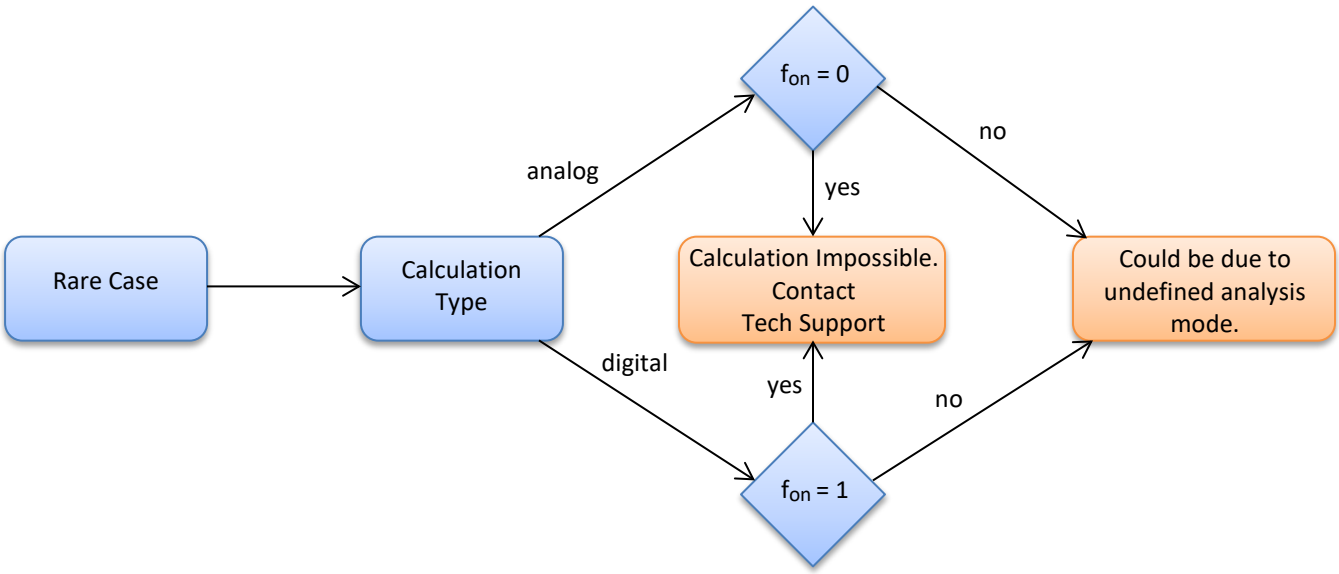
Troubleshooting Flowchart

Use this flowchart to identify and navigate to a troubleshooting topic. If you have a specific error message, see *Sample Error Messages* on page 29. If troubleshooting results in a rare case outcome, see the *Rare Case Troubleshooting Flowchart* on page 21.



Rare Case Troubleshooting Flowchart

Use the Rare Case Troubleshooting Flowchart to determine when you should contact Quanterix Technical Support.



Not a Number (NaN) and Where to Find It

Not A Number (NaN) is an undefined output that is not available. The Simoa HD-X Analyzer uses NaN to represent missing outputs due to (1) a value needed to calculate the output is missing, (2) the conditions that the output requires are not met, or (3) the calculation result is undefined. There are many instances where NaN replaces a result output, as listed below (the name in italics is the header name for each result output in the Run History Table).

- Concentration – *Replicates Conc.*
- AEB – Replicates AEB
- Isingle – *Isingle*
- f_{on} – Fraction On
- Ibead – *Ibead*

For more information on why a particular result output is NaN, see *What to Do When You Have NaN as a Value* on page 22, where each result output is described in more detail.

To Determine if a Result Output is NaN

1. Go to the History and Reports tab in the Simoa HD-X Analyzer software.
2. Touch **Run History**.
3. Find the column with the desired result output, for example, Replicates Conc. for concentration.
4. If the column is not in view, use the horizontal scroll bar to navigate through the columns.
5. If the column cannot be found, touch **Configure Columns** to add the result output to the columns.

For more information on how to add or edit displayed columns, see the *Simoa HD-X Analyzer User Guide.*

What to Do When You Have NaN as a Value

If Concentration is NaN

The Replicate concentration is calculated using the calibration curve and AEB. Since the calibration curve describes the relationship between concentration and AEB, concentration can be determined given an AEB value. Without an AEB value, the concentration cannot be obtained. There are instances in which an AEB value in range will return a NaN for concentration. In this case, there can be other problems with the calibration curve (see *Calibration Curve Issues* on page 23) such as the calibration curve not being calculated.

If AEB is NaN

- If f_{on} or Ibead is NaN, AEB is always NaN unless the majority rule is undefined. (see Q 7- What is the Majority Rule? in the Q & A section)
- If Isingle is NaN, AEB is NaN only for analog samples because the calculation of AEB requires the Isingle value (see *If Isingle is NaN*)

If Isingle is NaN

Isingle is NaN when the following calculation conditions are not met:

- At least one result with $f_{on} < 0.2$, and all other plexes in the same job all have $f_{on} < 0.7$
- Total number of active beads > 150 for results meeting the criteria above
- The number of active beads per result is the number of beads of that plex multipled by the f_{on}

The weighted batch Isingle value is plex-specific and assay-specific. For example, imagine a user runs a batch containing two assays because they want to compare the performance between the assays:

- Assay A: a 2-plex assay with plexes IL-6 and TNFa

- Assay B: a 3-plex assay with plexes IL-6, TNFa, and GM-CSF

In this batch, 5 different Isingles will be calculated:

- Assay A IL-6
- Assay A TNFa
- Assay B IL-6
- Assay B TNFa
- Assay B GM-CSF

In a different example, if a user runs a batch containing two homebrew assays of homebrew conditions C and D of the same analyte, then a unique Isingle is calculated for condition C and condition D. It is possible for one of the homebrew conditions to have a calculated Isingle, but the other condition to have too few active beads with a missing Isingle.

If f_{on} or Ibead is NaN

f_{on} gives the fraction of beads that have enzyme activity. Ibead gives the average intensity of beads with enzyme activity. A failure in image analysis is most likely the cause of NaN for either of these values. To learn more about image analysis errors, see *Image Analysis Issues* on page 25.

If Mean, SD, or CV for AEB or Concentration is NaN

Replicate statistics require at least two replicates to calculate the mean, standard deviation (SD), and coefficient of variation (CV). To calculate values for the replicate concentration, AEB cannot be NaN or undefined. When calculating AEB statistics, all NaN values for the batch are ignored; however, out of range Mean AEBs are included.

Determining Calibration Curve or Image Analysis Problems

Calibration Curve Issues

Calibrators are run with the instrument to produce data points. A method of curve fitting is selected and used to fit the data points and to generate the calibration curve. Using the calibration curve, concentration is determined given an AEB value. AEB and concentration values must be within a valid range of the calibration curve.

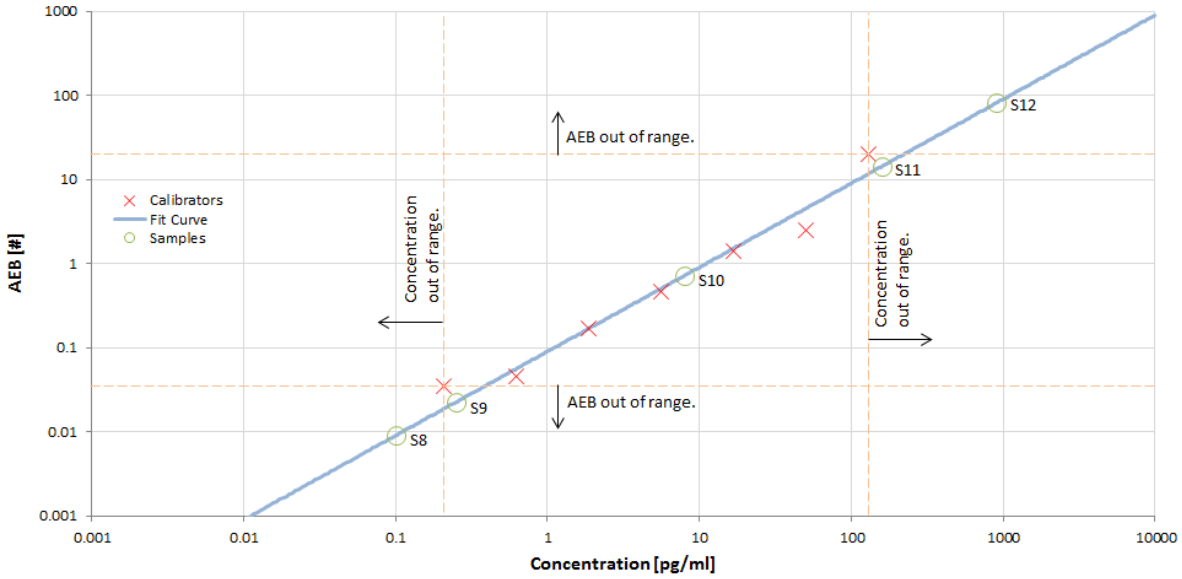
If a result's concentration is less than lowest calibrators or more than highest calibrators, then the software flags that result as having its concentration out of range. The rules for flagging AEB as out of range differ depending on the type of fit curve:

- If a polynomial curve is selected, the software flags AEB as out of range when an AEB is either less than all calibrators or greater than all calibrators.

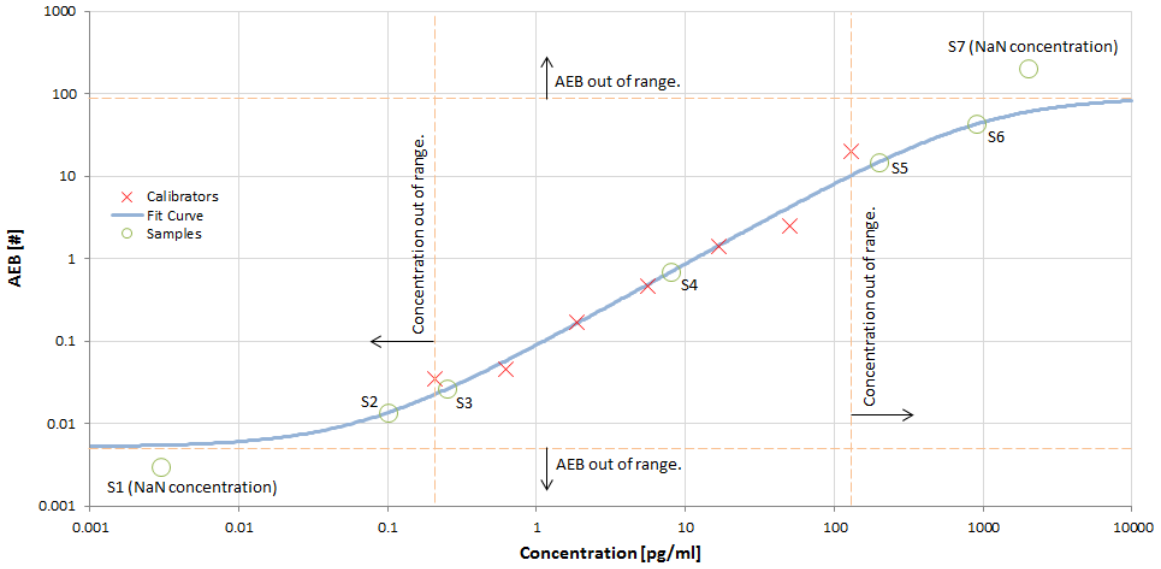
- If a 4PL or a 5PL curve is selected, an AEB out of range flag is applied when the AEB is less than the minimum parameter of the curve fit or more than the maximum of the curve fit.

In the Simoa software, these two parameters are parameter A and parameter D, respectively. The two figures below show the flagging rules for the different curve types. The table on the next page applies to both flagging examples.

Polynomial Flagging Example



4PL Flagging Example



	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12
AEB flagged as out of range?	X						X	X	X			X
Concentration flagged as out of range?	X	X			X	X	X	X			X	X

To generate a calibration curve, all calibrator levels defined for the assay must be present and the number of points must meet the minimum requirement of the selected fitting algorithm (table below). If a calibration curve fails to fit, for example because too many calibrators failed to return results, then relevant samples will be marked with a flag related to the fit failure.

Element	Description
Fit Algorithm	Select the fit equation to be used with your calibrators. Note that each fit algorithm needs a minimum number of calibrator levels.

Levels Needed	Fit Algorithm
2	Piecewise Linear, Linear
3	Quadratic
4	Cubic, 4PL
5	5PL

Flags related to determining concentration and curve fit are specific to each plex in each job. Samples run in replicate may have flags on none, some, or all of the replicates. Multiplex jobs may have flags on none, some, or all of the plexes for a specific job. For example, running a zero-concentration sample in duplicate in a 4-plex assay format might have an AEB out of range flag on only one plex of one job with no other flags.

Image Analysis Issues

Image analysis issues can prevent the software from calculating analyte concentrations. These problems often result in a f_{on} of NaN or no active beads. To view the error messages related to image analysis failure, review the Event Logs (see *Using the Event Log to Investigate Error Messages* on page 26).

A common problem is too much fluorescence for image analysis. One of the following error messages will appear:

- "Too much fluorescence in resorufin channels for analysis. Check the detector and RGP reagents; if there is no problem, please dilute the source and rerun."
- "Too much fluorescence in the resorufin channels for analysis."

This error signals that the concentration is limited by saturation of system components, such as the imaging system or substrate amount in the wells. If you are running a homebrew assay with failing calibrators, check that the detector concentration is reasonable. When running a Quanterix kit assay or a homebrew assay with no issues analyzing calibrators, this error signals a specimen has a concentration that is too high for analysis. In this case, dilute and rerun the sample.

In a multiplex format, any of the plexes being too concentrated for the assay format will cause this failure mode. That is, if running a sample as part of a 2-plex assay and one plex has zero concentration but the other plex is too concentrated to read, this error message is returned. The rationale for failing all plexes is due to cross-reactivity constraints that cannot be captured and corrected. As with a singleplex assay, dilute and rerun the sample when receiving this message.

If you receive the "Too much fluorescence" error message for a calibrator or control in a Quanterix kit assay, or if you receive any other error message for one of these samples, contact Quanterix Customer Support for troubleshooting help.

Another common error message you may see when running specimens or homebrew assays is too few beads. This error message will appear as:

- "Too few beads loaded for analysis to proceed."
- "Unable to locate all plexes in this assay."

Two of the most common causes are poor bead resuspension and sample matrix effects. Make sure your beads are properly resuspended prior to running any assay. If you are uncertain about how to resuspend beads, please see the *Simoa HD-X Analyzer User Guide* for more information. If you repeatedly see too few beads messages, especially with a particular specimen, try diluting the specimen further to reduce any matrix effects. Do not hesitate to contact your field application scientist or Quanterix support if you have any questions.

When using helper beads or multiplex assays in a homebrew context, using too few beads or a lopsided bead ratio will cause the "Unable to locate all plexes" error message. Check that your bead ratio between any two plexes does not exceed 1:4, and that you are using the recommended number of beads in your assay.

If you see an error message besides "Too Much Fluorescence" or "Too Few Beads," please contact Quanterix Support using the information provided at the start of this guide.

How to Find Error Messages for a Batch or Job ID

Using the Event Log to Investigate Error Messages

The Event Log is useful when investigating unusual result outputs or errors from a run. You can also use it to determine why NaN was assigned for a result output.

To View the Event Log of One Job ID

1. Navigate to the Run History screen on the History and Reports tab.
2. Ensure that Automatic Replicate Selection is off.

3 Select the job of interest.

Note: When viewing Event Log entries of a single job, select only one item at a time. To view the Event Log entries of multiple jobs, see *To View the Event Log of Multiple Job IDs or Batches* below.

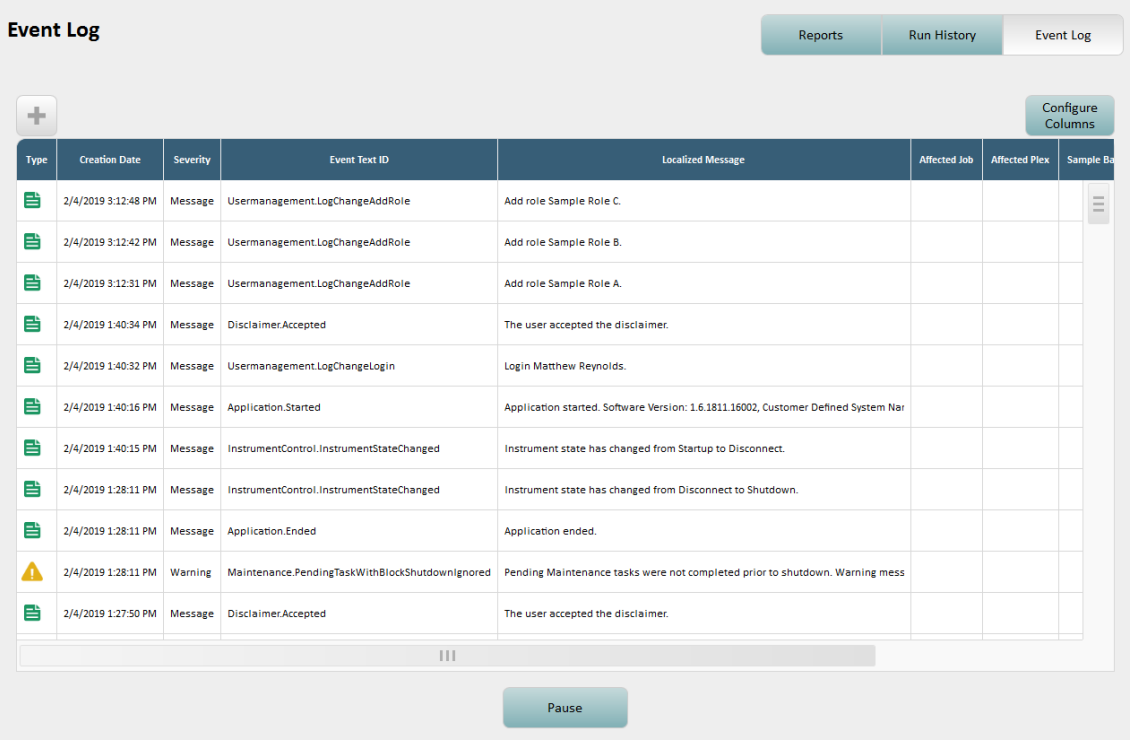
4 Touch **Show Related Flags and Events**.

An example of an Event Log output with error messages is shown on the next page. This particular example shows that the calculated concentration and the given AEB were out of the calibration range.

To View the Event Log of Multiple Job IDs or Batches

1 From the Run History tab, note the Job ID, Batch ID, or Creation Date of the batch that you want to investigate (other fields will work as well).

2 Touch **Event Log**.



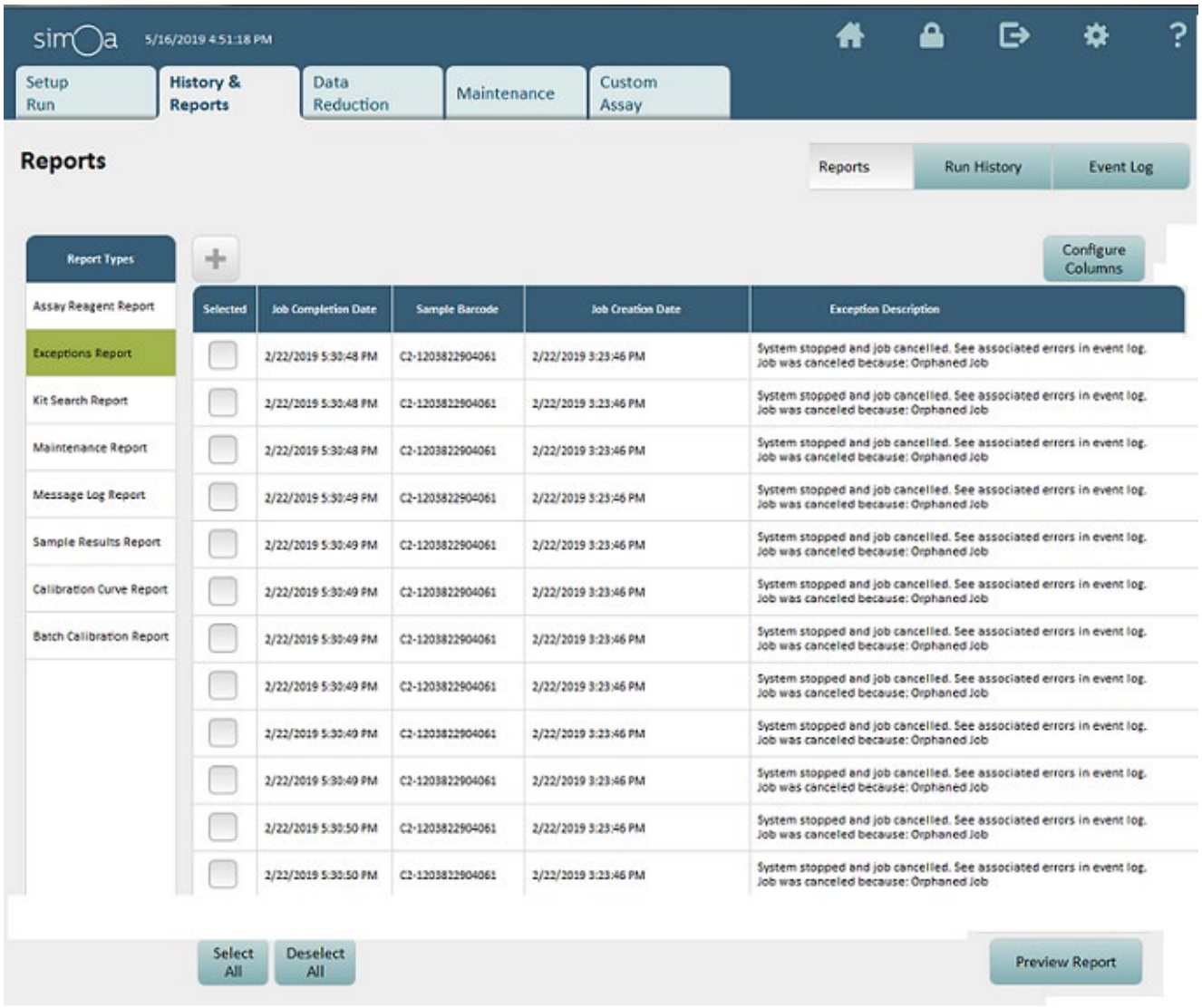
3 Touch the Add Filters button (**+**) to add a filter to the displayed error messages.

4 Use the Job ID, Batch ID, or Creation Date for the filter. See the *Simoa HD-X Analyzer User Guide* for more details on how to filter results.

To Export Error Messages

1 Note Test ID, Sample Barcode, Creation Date, or Completion Date.

2 In the History & Reports tab, touch **Reports.**



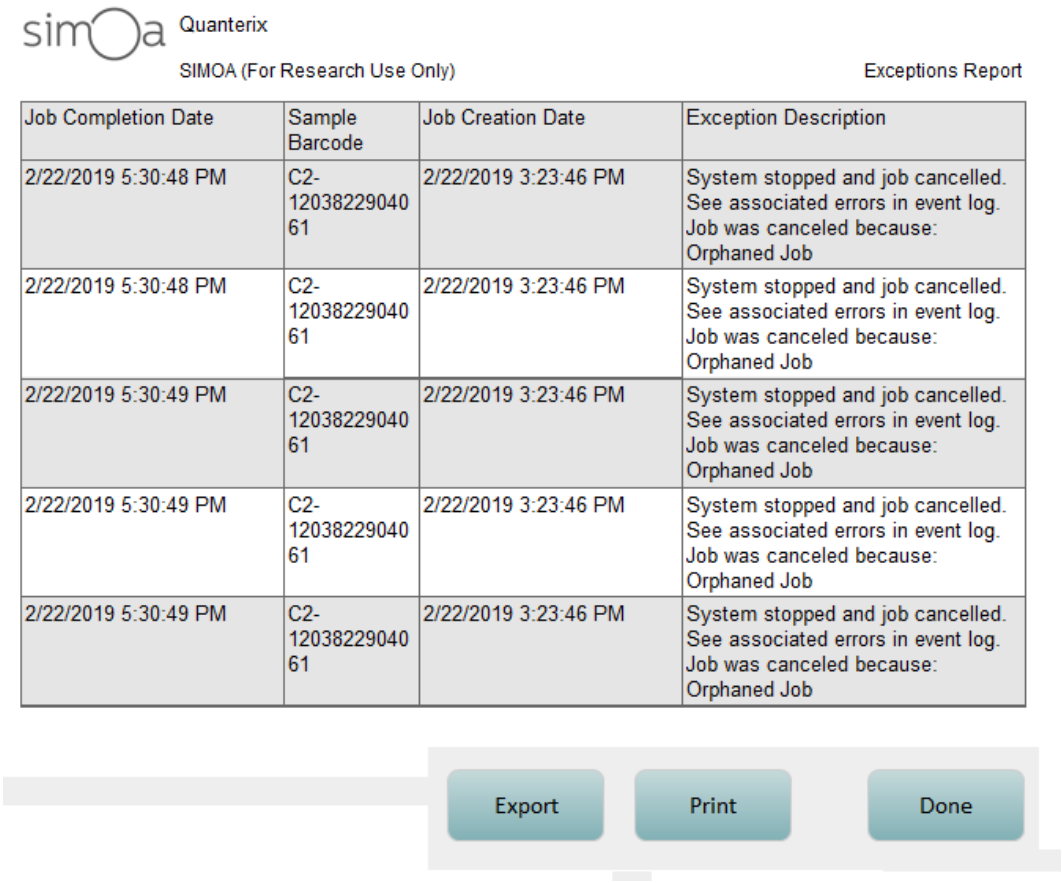
3 Select **Exceptions Report** from Report Types.

4 Touch the Add Filters button (**+**) to add a filter to the displayed error messages.

5 Select the check boxes of the messages to export.

6 Touch **Preview Report** and wait for the report to show.

7 Touch **Export** and specify the destination of the file.



Quanterix

SIMOA (For Research Use Only)

Exceptions Report

Job Completion Date	Sample Barcode	Job Creation Date	Exception Description
2/22/2019 5:30:48 PM	C2-1203822904061	2/22/2019 3:23:46 PM	System stopped and job cancelled. See associated errors in event log. Job was canceled because: Orphaned Job
2/22/2019 5:30:48 PM	C2-1203822904061	2/22/2019 3:23:46 PM	System stopped and job cancelled. See associated errors in event log. Job was canceled because: Orphaned Job
2/22/2019 5:30:49 PM	C2-1203822904061	2/22/2019 3:23:46 PM	System stopped and job cancelled. See associated errors in event log. Job was canceled because: Orphaned Job
2/22/2019 5:30:49 PM	C2-1203822904061	2/22/2019 3:23:46 PM	System stopped and job cancelled. See associated errors in event log. Job was canceled because: Orphaned Job
2/22/2019 5:30:49 PM	C2-1203822904061	2/22/2019 3:23:46 PM	System stopped and job cancelled. See associated errors in event log. Job was canceled because: Orphaned Job

Export Print Done

Sample Error Messages

The Sample Error Message table lists error messages that can appear in the Event Log.

Message	Description
Could not calculate AEB across replicates. Check the event log for possible individual replicate error messages.	The analysis mode is undefined for all of the samples. Check the event log of the individual replicates to troubleshoot.
Unable to correct for optical signal interference.	There was a problem with image analysis which did not allow analyte concentrations to be calculated. Contact Quanterix Customer Support for more information.
Could not calculate the required ISingle value.	The Isingle value was not calculated or is undefined. Check individual replicates for failure. In a Homebrew assay, ensure you followed

Message	Description
	guidelines for Isingle calculation.
Too few beads loaded for analysis to proceed.	There were not enough active beads in the batch or plex. In a Homebrew assay, check bead concentrations. Otherwise, contact Quanterix Customer Support for more information.
Too few valid wells found for analysis.	Contact Quanterix Customer Support.
Too much debris found in the image to proceed with image analysis.	Excessive debris prevented image analysis from completing. Contact Quanterix Customer Support.
Too much fluorescence in resorufin channels for analysis. If no problem is found, dilute the source and rerun.	Excessive fluorescence prevented image analysis from completing. See *Image Analysis Issues* on page 25 for more details. **TIP:** Diluting and rerunning the samples can decrease resorufin activity and fluorescence.
Image analysis failure due to subimage misalignment.	Image analysis could not complete because the sample images could not be aligned. Contact Quanterix Customer Support.
Unable to match input bead types to image.	An exception was raised because image analysis could not determine which wells were beaded and which wells were not. Contact Quanterix Customer Support.
Invalid AEB numeric value found for a calibration data point.	A calibrator level value was reported as NaN. To create a calibration curve, all calibrator levels must have values. Manually excluding calibrator levels with NaN values will allow for curve creation. Check the individual replicates for failure reasons.
Calculated Concentration *X* is out of calibration range for the current curve.	The calculated concentration is either above the highest calibrator concentration or below the lowest calibrator concentration.

Message	Description
Could not calculate calibration curve, because the given number of data points is insufficient.	The calibration curve could not be fit into the data points because there were not enough points. The required number of data points depends on the algorithm used to fit a curve.
Sample AEB value, *X,* is outside of the range established by the calibration curve.	A NaN value is displayed for the calculated concentration. No replicate results are calculated. Mean, SD, CV, AEB, and concentration values are not available.
A valid calibration curve fit does not exist for current plex.	The calibration curve for a given plex was not found or could not be created. In a Homebrew assay, ensure your curves are set up correctly. Otherwise, check the individual failures.
Analog AEB unavailable for plex X.	Analog AEB is unavailable because Isingle is NaN. See *If Isingle is NaN* on page 22 for information on troubleshooting Isingle issues.
Insufficient volume detected by probe.	The software calculates how much volume is left based on the geometry of the container and compares it against how much is to be aspirated. If the volume available is less than the volume to aspirate, this error appears.
Job canceled. No cuvette available for job.	A scheduler issue prevents the system from providing the needed cuvettes.
Job canceled. No liquid level detected.	To prevent physical damage, each pipettor has a maximum Z position at which the pipette stops moving. If no liquid is detected when the pipette moves down to aspirate for a job at the maximum position, the job is canceled.
Need at least two valid results for this plex in order to calculate a replicate result.	Mean, SD, or CV cannot be calculated because there are fewer than two replicate results.
No cuvette available for job.	See Job canceled. No cuvettes available for job in

Message	Description
	described earlier in this table.
No liquid found by probe.	See Job canceled. No liquid level detected described earlier in this table.
System stopped and job canceled.	System halts (for any reason) and all unfinished jobs are canceled.

Frequently Asked Questions

Questions and Answers

1 **When is the Replicate AEB out of range?**

Replicate AEB is out of range when it is above the highest calibration level or below the lowest calibration level. The AEB concentration figure shows the highest and lowest calibration level (see *Calibration Curve Issues* on page 23). The concentration will still be calculated.

2 **When is the Concentration out of range?**

Concentration is out of range when it is above the concentration of the highest calibrator or below the lowest calibrator.

3 **AEB was out of range but I got a concentration. What is happening?**

If AEB is out of range, the software will calculate the concentration but will flag the result.

4 **What are the conditions for the calibration curve to be created?**

For the calibration curve to be generated, all calibrator levels defined for the assay must be present and the number of points must meet the minimum requirement of the selected fitting algorithm.

5 **What are the conditions for the Mean AEB to be calculated?**

To calculate the Mean AEB, you need at least two replicates for samples that are run in replicate.

6 **If the replicate AEB is out of range, is it still included in the calculation of Mean AEB?**

No, the calculation of Mean, SD, and CV of AEB does not include AEB values that are out of range.

7 **What is the majority rule?**

For replicate samples, the majority rule is a method to determine whether samples are treated as analog or digital. It counts the number of analog or digital samples in a batch and uses the method with the majority. The rule increases the system robustness to several physical effects.

8 **When is the analysis mode undefined?**

The analysis mode is undefined when the Fraction On or Digital AEB is NaN. It can also be undefined if the sample is run in replicate and all but one of the replicates has a job that is canceled.

9 **Are samples with AEB out of range included in the majority rule?**

Yes, to calculate sample AEB, the majority rule must be applied first. Determination of whether AEB is out of range takes place after AEB is calculated, thus after the majority rule is applied.

10 **Are the Mean, SD, and CV for AEB and concentration calculated for specimens that have one or more NaN values?**

Yes, Mean, SD, and CV are calculated from replicates that do not have NaN values.

11 **I have all the calibrator values but the software did not return a curve.**

This could be a problem with a severe outlier that causes the curve fit to fail. In such cases, exclude the outlier and perform the curve fit again.

12 **What happens when the number of analog and digital replicates is the same?**

When the number of analog and digital replicates is the same, the majority rule goes into a tie breaker and defaults to the analog method.

13 **Majority rule could not be extracted. What does this mean?**

When the majority rule cannot determine if an analog or digital analysis method is used, an error message appears indicating that the majority rule could not be extracted. This is because all of the replicates have undefined analysis methods.

14 **How is the majority rule evaluated when there is a manual exclusion/inclusion of replicate samples?**

Manual exclusion/inclusion of replicate samples forces the majority rule to be re-evaluated.

15 **What is the difference between the analog and digital analysis methods?**

The digital analysis method uses f_{on} to calculate the value of AEB. The analog analysis method requires f_{on}, Ibead, and Isingle to calculate the AEB value. The digital method yields better precision when the AEB is lower than 1.2, corresponding to an f_{on} of 0.7, whereas the analog method is better when the AEB is higher than 1.2.

16 **How is a sample determined to be analog or digital?**

A sample's majority rule vote is digital when it's f_{on}, the fraction of beads with active enzymes, is less than 0.7, otherwise it is analog. Based on the votes from all sample replicates, a sample is either analog or digital.

17 **What determines if the sample is treated as analog or digital?**

The majority rule is used to determine if a sample is analog or digital.

18 **Why would a batch require at least one sample with $f_{on} < 0.2$?**

If the batch contains analog samples, an Isingle value is required to calculate AEB values for these samples. Isingle can only be calculated if at least one sample (including calibrators and controls) with $f_{on} < 0.2$ is present in the batch.