The Data Quality Analysis View in Agilent Seahorse Wave Pro and Agilent Seahorse Analytics software

Part 1: Introduction

The Data Quality analysis view is an analysis feature available in Agilent Seahorse Wave Pro software and Agilent Seahorse Analytics Web-based software. This software feature automatically inspects data acquired during an XF assay for potential outliers or issues. There are more than 20 different data quality tests performed by this view; wells that contain data that exceed the warning and/or error test threshold values will be flagged for review and can be addressed directly from the Data Quality analysis view (e.g., unassign a well from a group, or flag for additional data review downstream). The Data Quality analysis view is intended for analysis of data files generated following standard XF assay workflow parameters – 37 °C operation, normoxia conditions, etc. The Data Quality analysis view is not intended for the analysis of data files generated at temperatures other than 37 °C, in hypoxic conditions, 3D biomaterial.

Data files generated by the Agilent Seahorse XF Pro Analyzer and the Agilent Seahorse XF HS Mini Analyzer are compatible with the Data Quality analysis view. Data files generated by Agilent Seahorse XFe, XFp, or XF Analyzers are not compatible with the Data Quality analysis view.

Part 2: How to use the Data Quality analysis view

The Data Quality analysis view is a feature embedded in every assay result file (XFLR) generated by an XF Pro Analyzer or an XF HS Mini Analyzer. To access the Data Quality analysis view, open an assay result file generated by these XF Analyzers in the Wave Pro software, or the Seahorse Analytics Web-based application.

Using Wave Pro software

1. Open an assay result file (XFLR) from your PC by double-clicking the file icon from File Explorer. If Wave Pro software is already open on your computer, you can use the Results view to open a result file.

2. When the file opens, you will find the Data Quality analysis view in the top-level functions ribbon (Figure 1). The button to open the Data Quality analysis view will appear yellow if there are warnings or errors detected in your result file (Figure 2). If the button has a gray/white color, like the other buttons in the function ribbon, this indicates that no warnings or errors were detected in your result file (Figure 3).
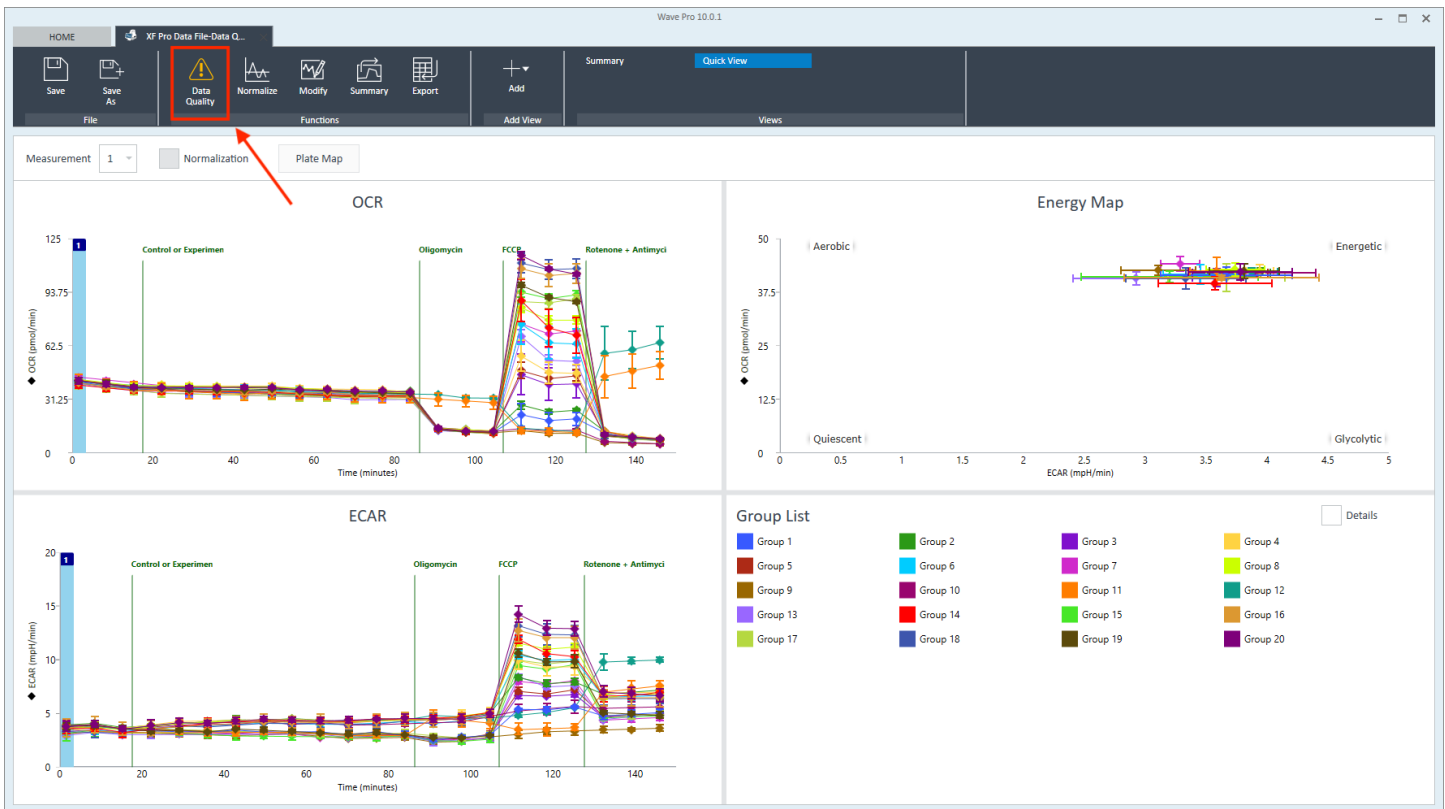

Figure 1. The Data Quality analysis view is enabled by clicking the indicated button.
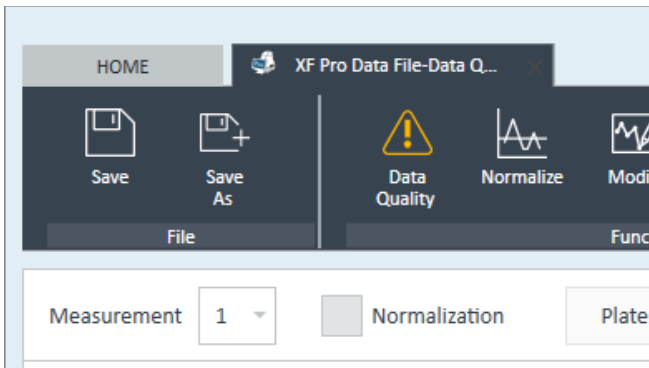
Figure 2. A yellow Data Quality button means there are warnings or errors detected in the result file.
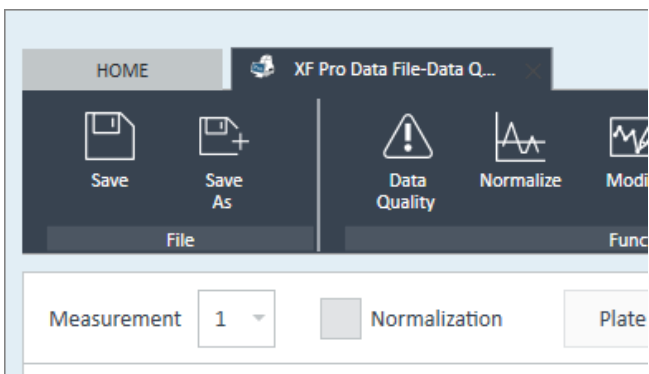

Figure 3. A normal or white Data Quality button means there are no warnings or errors detected in the result file.

3. To display the Data Quality analysis view and interact with the results, click the button in the functions ribbon (Figure 4).
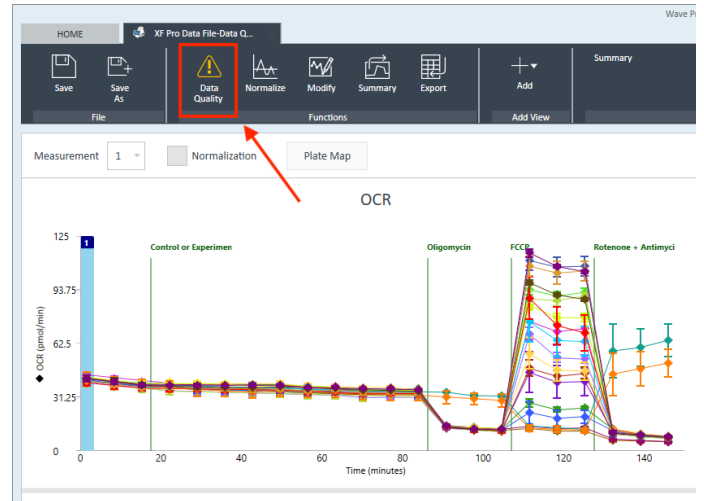

Figure 4. Data Quality button in the Functions ribbon.

4. When the view appears, if your result file contains warnings or errors, you will see warning icons on the plate map wells containing possible issues for your review (Figure 5).
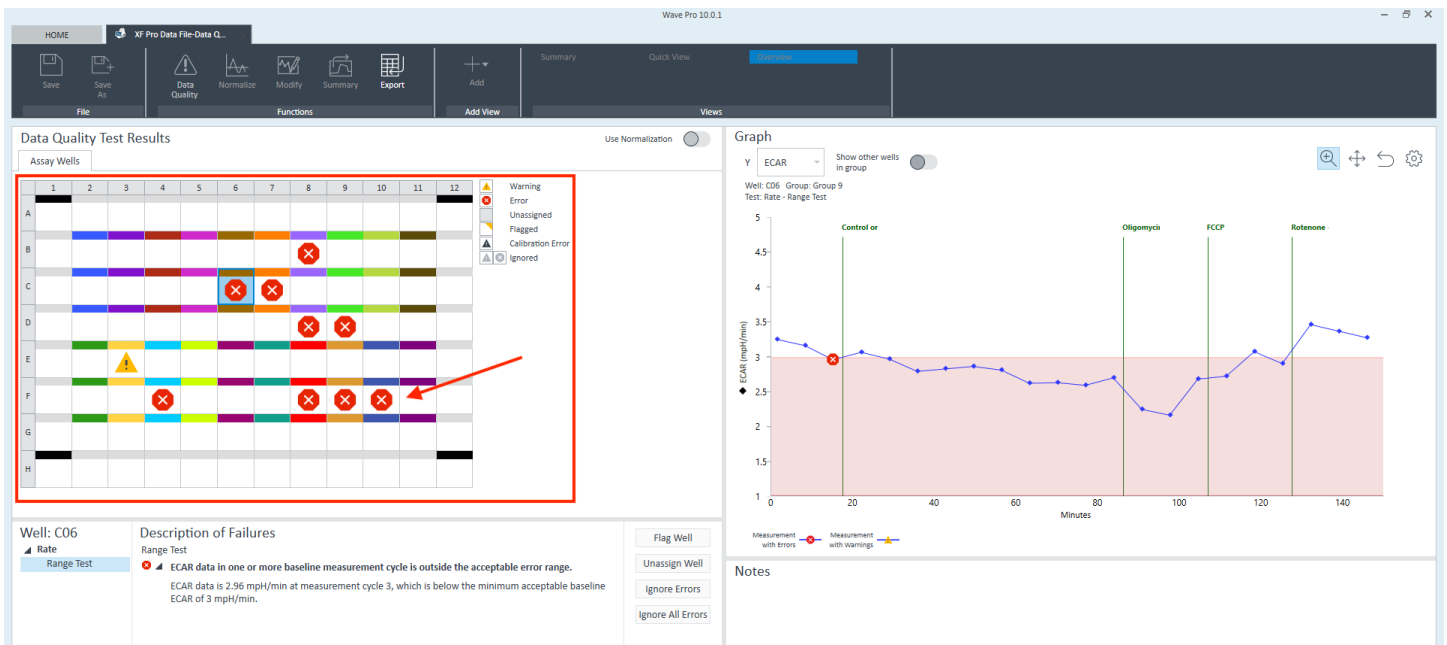

Figure 5. Warning and error icons appear on the plate map display.

5. If there are Temperature or Background warnings or errors, those will appear in a separate tab above the plate map (Figure 6), and must be addressed (e.g., ignore the error/warning or unassign the from the plate map) before you review assay well warnings/errors. For the complete list of data quality tests and descriptions of each test, please see section 3 of this document.
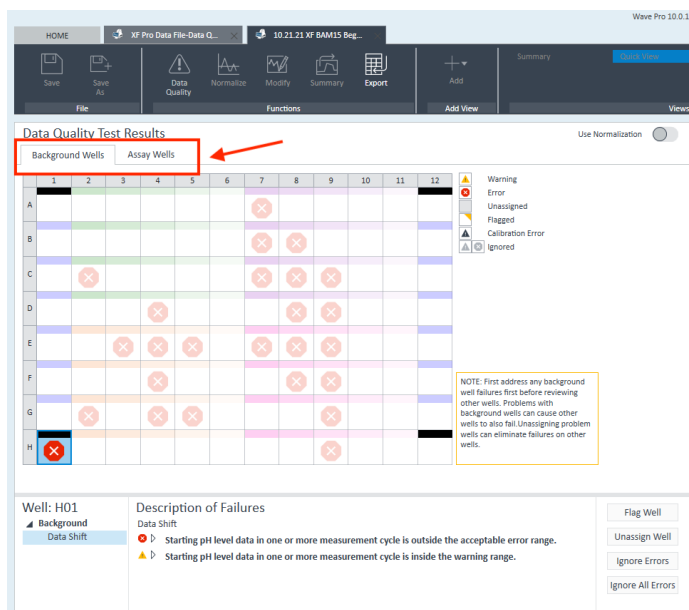

Figure 6. Temperature and Background warnings and errors tabs.

6. When a well contains a warning or an error, click the well to display the kinetic graph data and associated warnings or error test descriptions below the plate map (Figure 7).

7. There are several options to resolve assay wells that contain an error or warning:
   – Do nothing. If desired, you can review the warning(s)/error(s), simply ignore the errors, and return to your analysis using Wave Pro and Seahorse Analytics. Doing so will cause the Data Quality analysis button to remain yellow at all times.
   – You can flag the well, adding a small triangular notch in the upper-right corner. This flag will persist with this well within the data file so anyone who views a plate map for this data file will see the flag (unless the well is subsequently unflagged from an analysis view) (Figure 8A).
   – You can unassign the well from the group. This will cause the data quality analysis tests to be rerun (Figure 8B).
   – You can ignore the error, graying out the warning/error symbol in the well. If you ignore an error within a well, you can reset this by simply clicking the **Reset Errors** button (Figure 8C).
   – You can ignore all errors, graying out all warning/error symbols in all wells on the plate. If you ignore all errors for the file, you can reset this by clicking the **Show All Errors** button (Figure 8D).
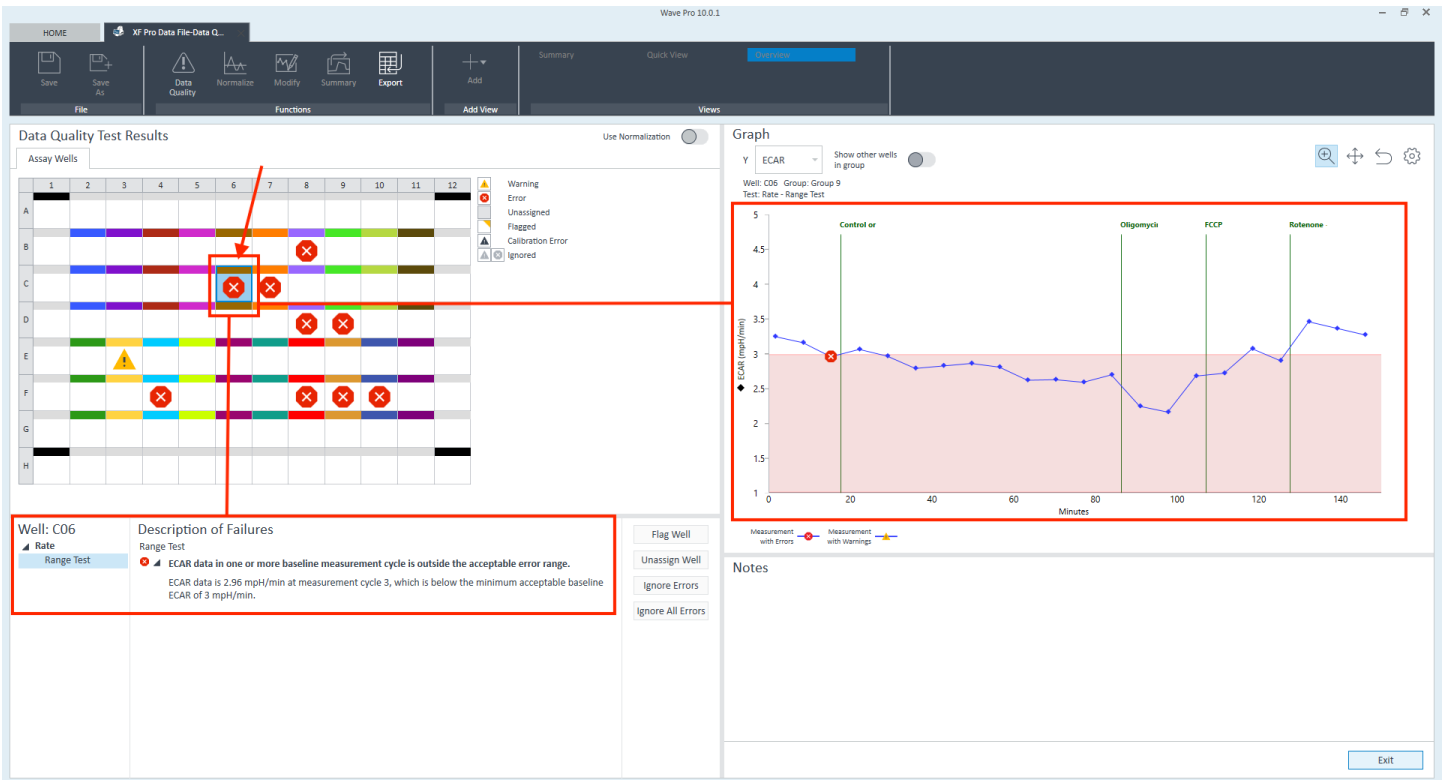
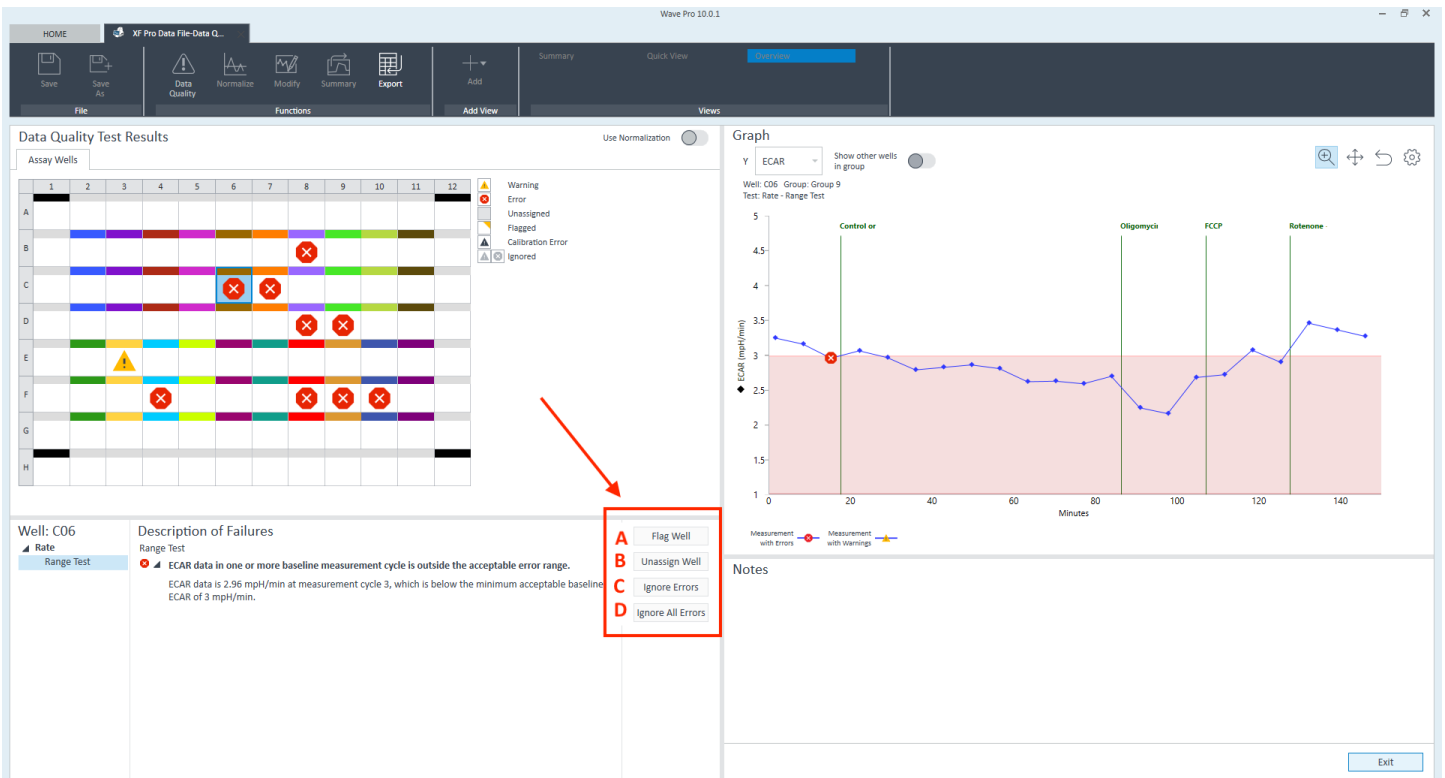**Figure 7.** Kinetic graph data and a description of the problem.

Figure 8. Warning/error user actions.

8. When you have selected a well containing a warning/error and data for the selected well is displayed on the kinetic graph, you can:

   – Toggle the Y-axis setting between O_2, pH, OCR, and ECAR. You will see the corresponding warning/error icon next to the Y-axis option containing the warning(s)/error(s) (Figure 9A).

   – Show other wells in the group, which can be useful to compare the response in the problematic well with others in the same group to understand why it is flagged (e.g., injection failure or problem) (Figure 9B).

   – Use the zoom, pan, reset, and graph options buttons to customize how the kinetic graph appears for improved interaction with the graphed data (Figure 9C).

9. Each action performed in the Data Quality analysis view is logged in the Notes area. You can enter your own custom notes into the Notes field, and you can edit the automatically logged notes and any custom notes, if they exist in the file (Figure 10).

10. When you exit the Data Quality analysis view, you will be prompted to save your changes. Choose the appropriate option and you will be returned to the previous analysis view (Figure 11).

11. You can open the Data Quality analysis view again by clicking the Data Quality button in the functions ribbon.
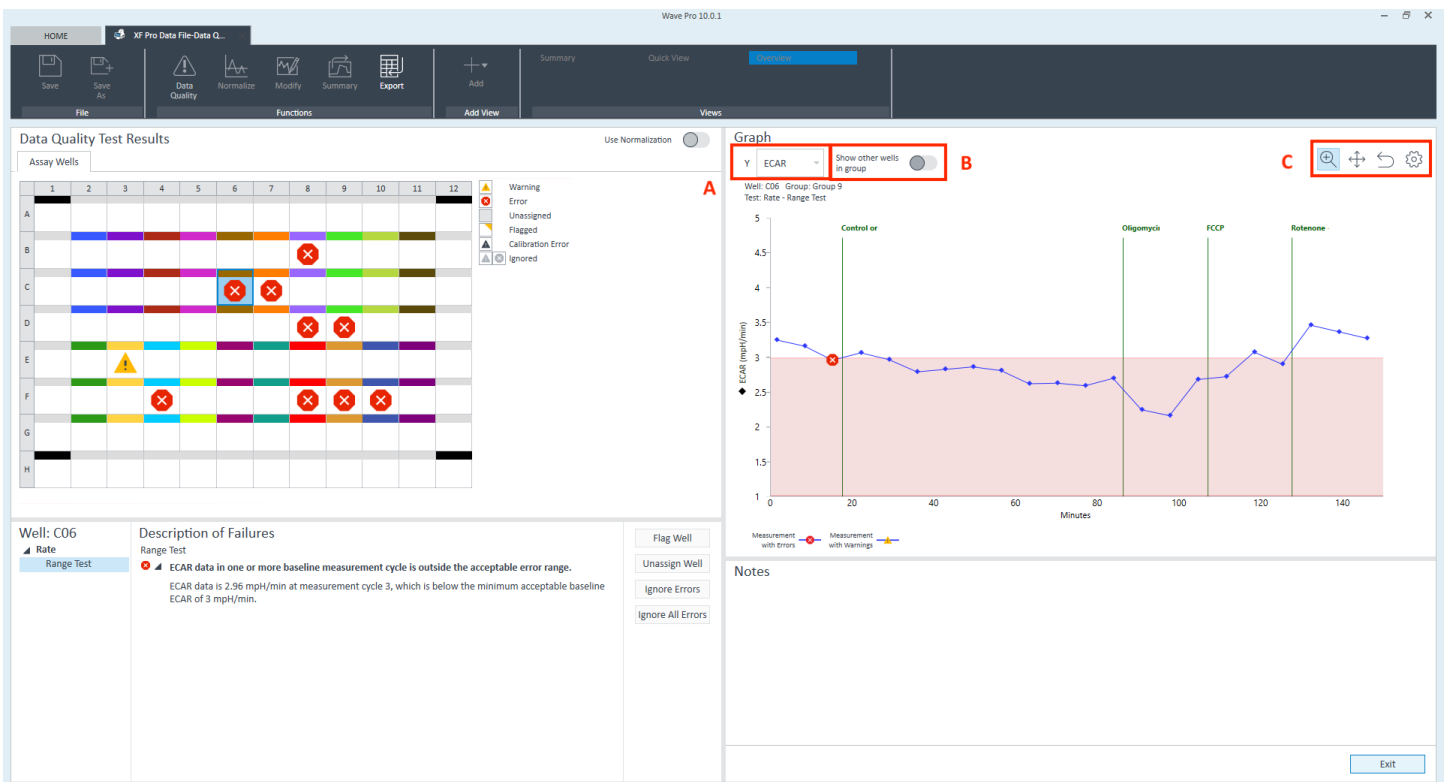

Figure 9. Kinetic data display options. (A) Select Y-axis options from the drop-down. (B) Toggle to show other wells in the same group as the selected well. (C) Zoom, pan, reset, and graph options buttons.
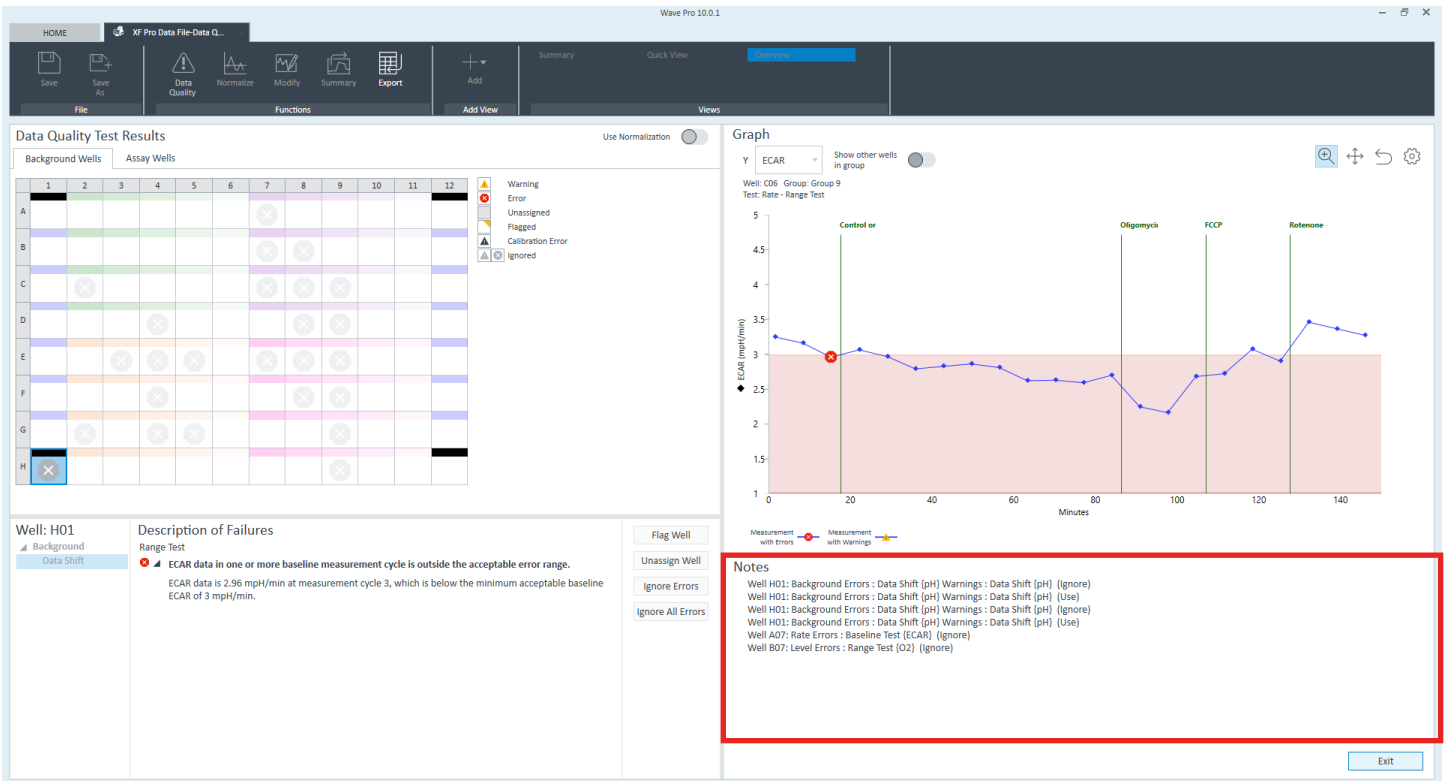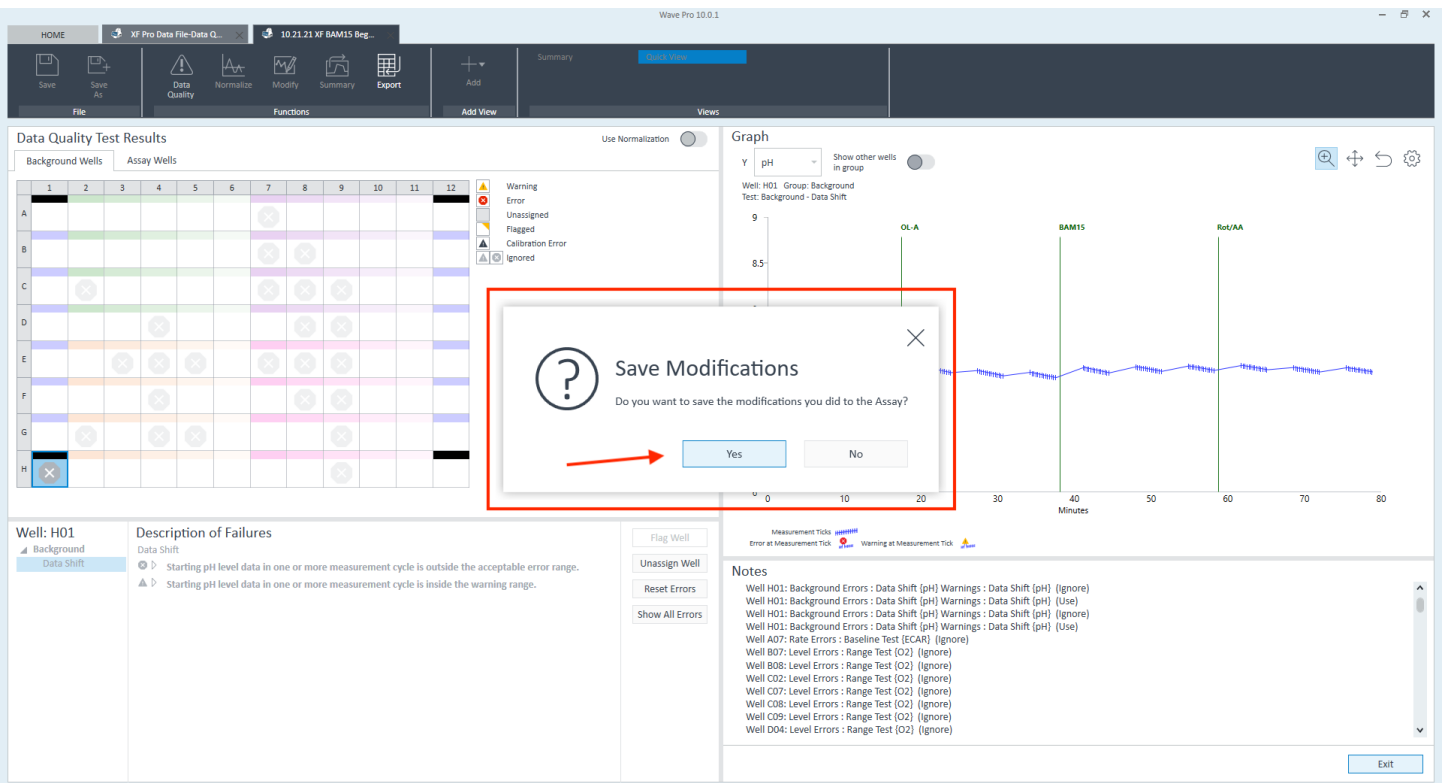
Figure 10. Notes area.

Figure 11. Save modifications exit notice.

Using Seahorse Analytics

1. Create an account, or log in to your Seahorse Analytics account: https://seahorseanalytics.agilent.com

2. Upload the desired assay result file (XFLR) if necessary.

3. Click the assay result file (XFLR) in your file list to open the file.

   – If this is the first time you have analyzed the data file, you will see the **Add View** dialog. The Add View window allows users to select widgets for creating an analysis workspace. Create an analysis view for this file by selecting at least one widget (Figure 12).

   – If this file was analyzed using Wave Pro, the file will automatically open to an analysis view called Wave Pro Analysis. Please see the Wave Pro software user manual for more information on this feature.
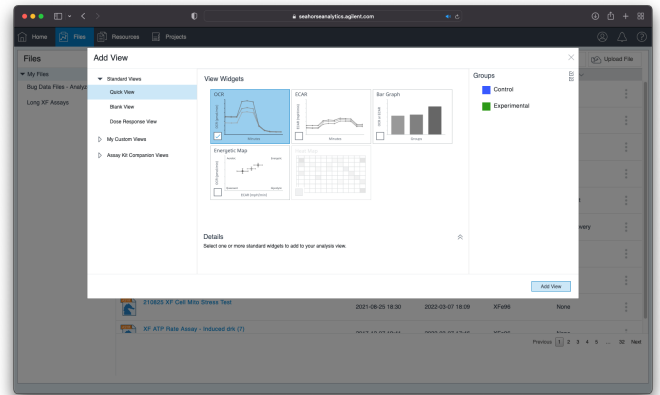
Figure 12. The **Add View** window.

4. Once the analysis view is generated, you will be able to access the Data Quality view. Look on the right side of the top-level dark blue ribbon for a triangle with an exclamation point. If this triangle has a yellow fill, then there are warnings or errors detected in your result file. If this triangle does NOT have a yellow fill, then no warnings or errors are detected in your file at that time (Figure 13).

Figure 13. Data Quality warnings and errors icon.

5. Open the Data Quality analysis view by clicking the triangle. When the view appears, if your result file contains warnings or errors, you will see icons on the plate map wells indicating possible issues for your review (Figure 14).
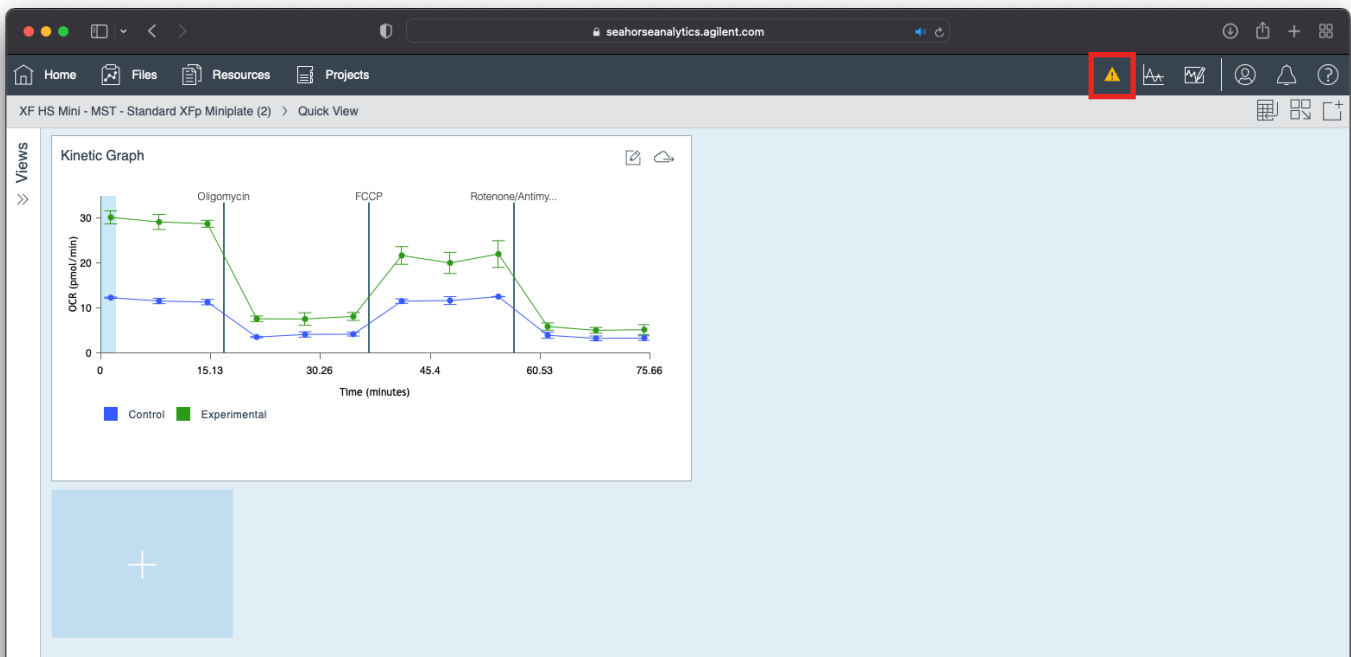
6. If there are Temperature or Background warnings or errors, those will appear in a separate tab above the plate map and must be addressed (e.g., ignore the error/warning or unassign the well from the plate map) before you review assay well warnings/errors. For the complete list of data quality tests and descriptions of each test, please see section 3 of this document.
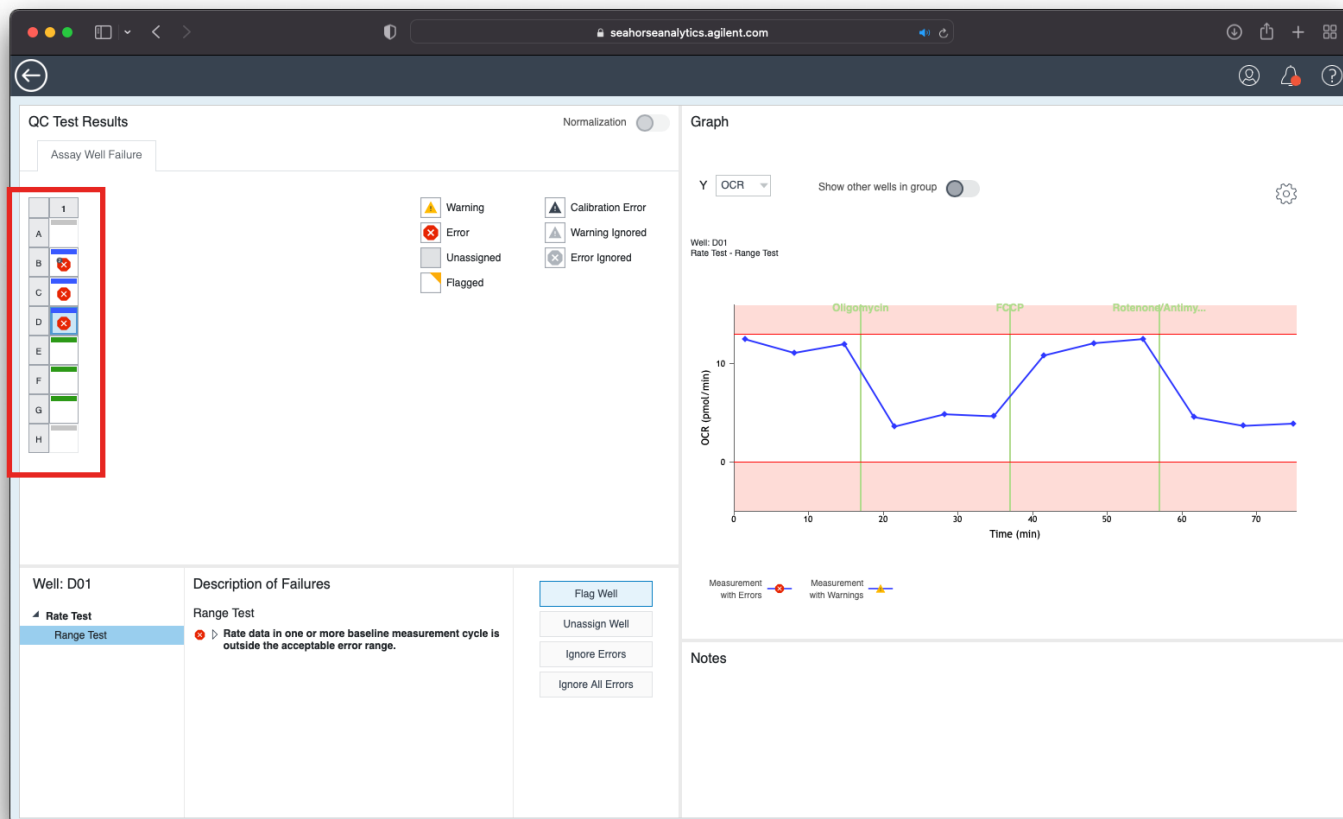

Figure 14. The Data Quality analysis view, showing error icons on the plate map.

7. When a well contains a warning or error, click the well to display the kinetic graph data and associated warnings or error test descriptions below the plate map (Figure 15).

8. You have several options to resolve assay wells that contain an error or warning:

   – Do nothing. If desired, you can review the warning(s)/ error(s), simply ignore the errors, and return to your analysis using Wave Pro and Seahorse Analytics. Doing so will cause the Data Quality analysis button to remain yellow at all times.

   – You can flag the well, adding a small triangular notch in the upper-right corner. This flag will persist with this well within the data file so anyone who views a plate map for this data file will see the flag (unless the well is subsequently unflagged from an analysis view) (Figure 16A).

   – You can unassign the well from the group. This will cause the Data Quality analysis tests to be rerun (Figure 16B).

   – You can ignore the error, graying out the warning/error symbol in the well. If you ignore an error within a well, you can reset this by clicking the **Reset Errors** button (Figure 16C).

   – You can ignore all errors, graying out all warning/error symbols in all wells on the plate. If you ignore all errors for the file, you can reset this by clicking the **Show All Errors** button (Figure 16D).
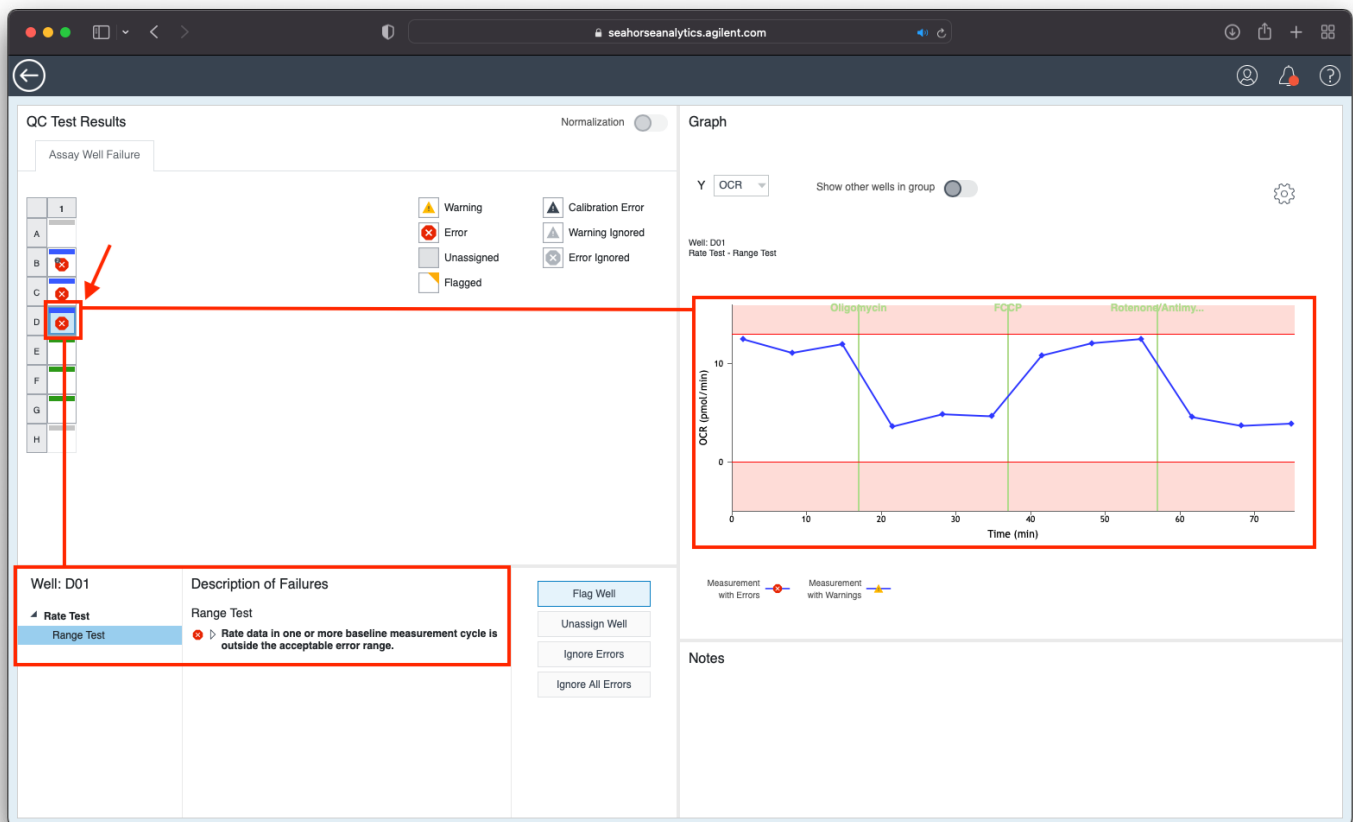

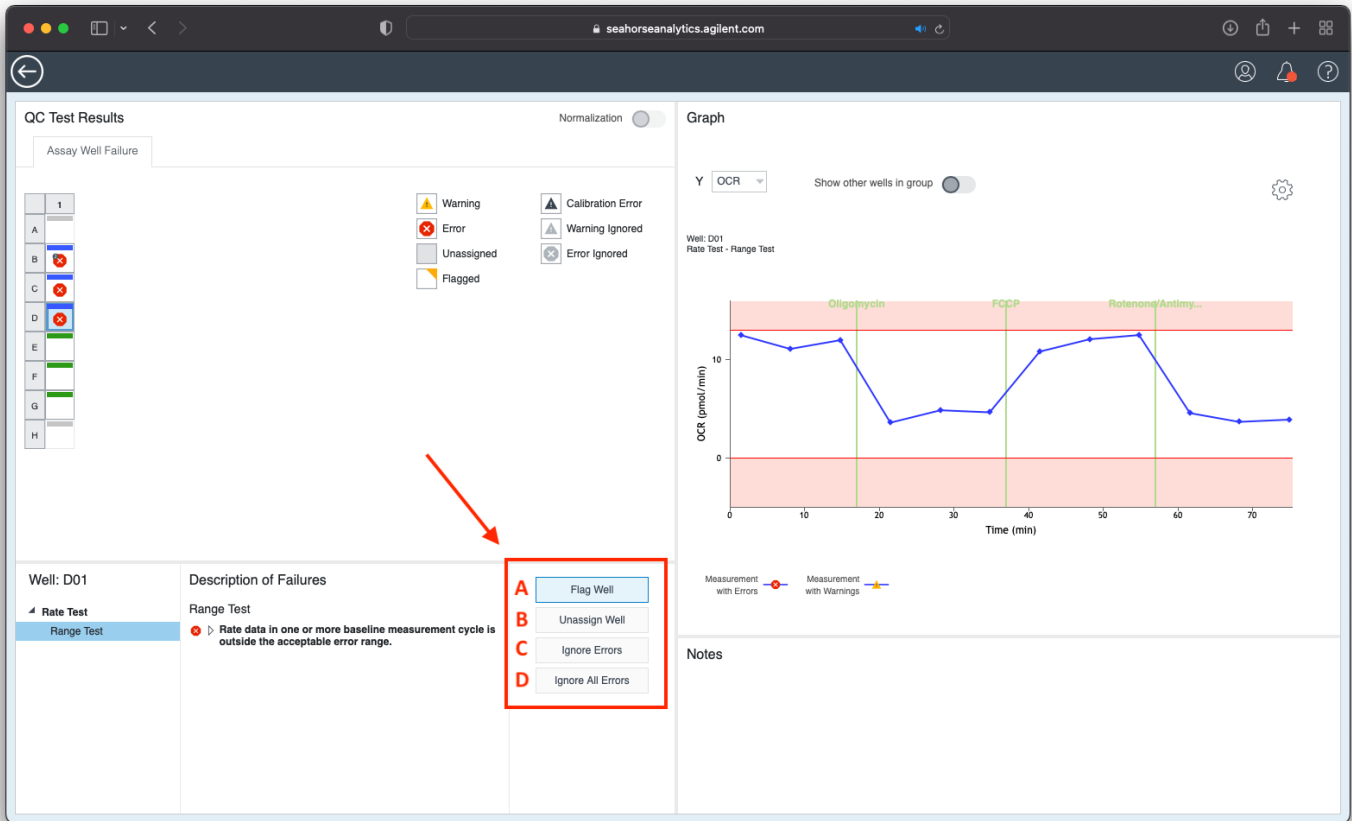**Figure 15.** Kinetic graph data and a description of the problem

Figure 16. Warning/error user actions.

9. When you have selected a well containing a warning/error and data for the selected well is displayed on the kinetic graph, you can:

– Toggle the Y-axis setting between O_2, pH, OCR, and ECAR. You will see the corresponding warning/error icon next to the Y-axis option containing the warning(s)/error(s) (Figure 17A).

– Show other wells in the group, which can be useful to compare the response in the problematic well with others in the same group to understand why it is flagged (e.g., injection failure or problem) (Figure 17B).

– Use the graph options button to customize how the kinetic graph appears for improved interaction with the graphed data (Figure 17C).

– Use your mouse to move around the kinetic graph.

10. Each action performed on the Data Quality analysis view is logged in the Notes area. You can enter your own custom notes into the Notes field, and you can edit the automatically logged notes and any custom notes, if they exist in the file (Figure 18).

11. Exit the Data Quality analysis view by clicking the back arrow in the upper-left corner (Figure 19).

12. You can open the Data Quality analysis view again by clicking the triangle in the top-level dark blue ribbon.
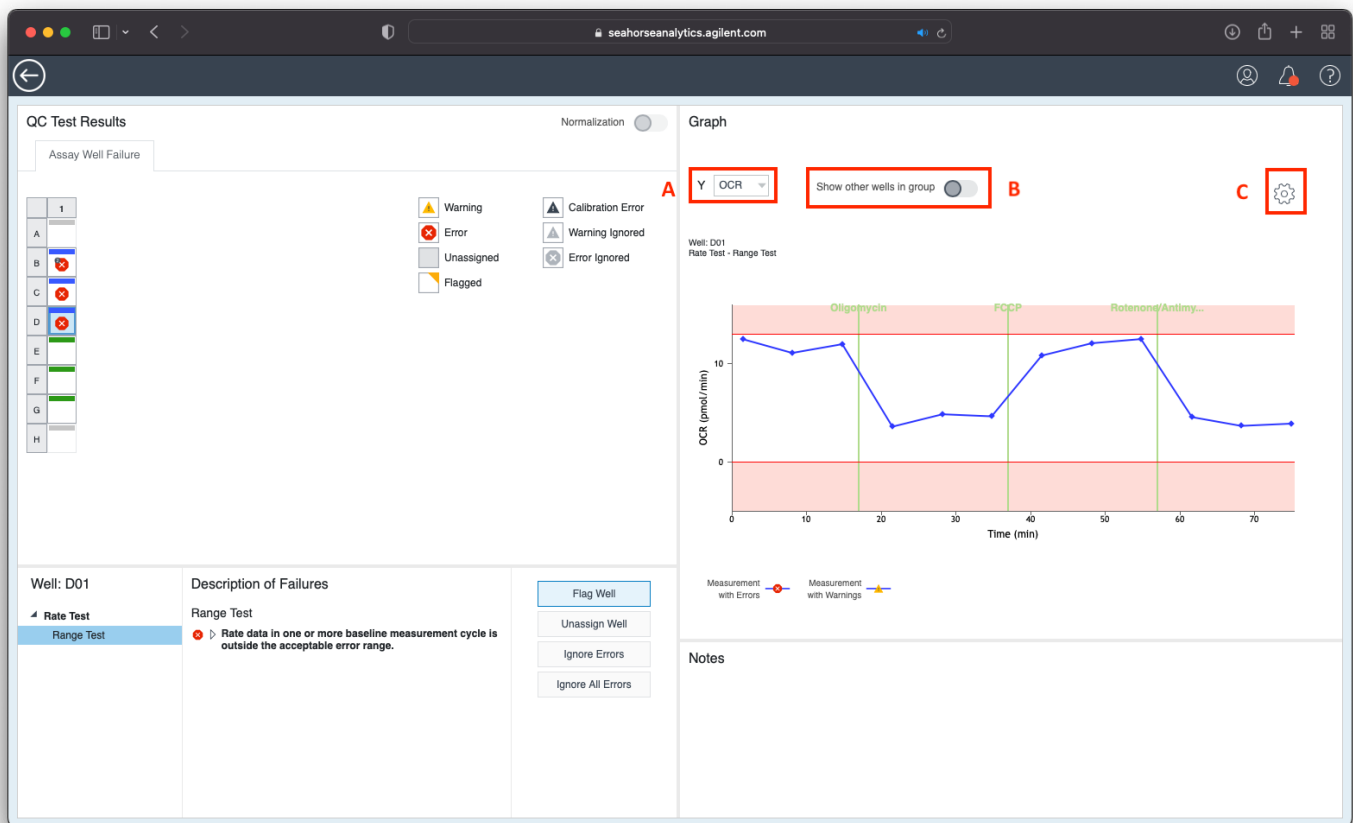

Figure 17. Kinetic data display options. (A) Select Y-axis options from the drop-down. (B) Toggle to show other wells in the same group as the selected well. (C) Graph options button for customizing the data display.
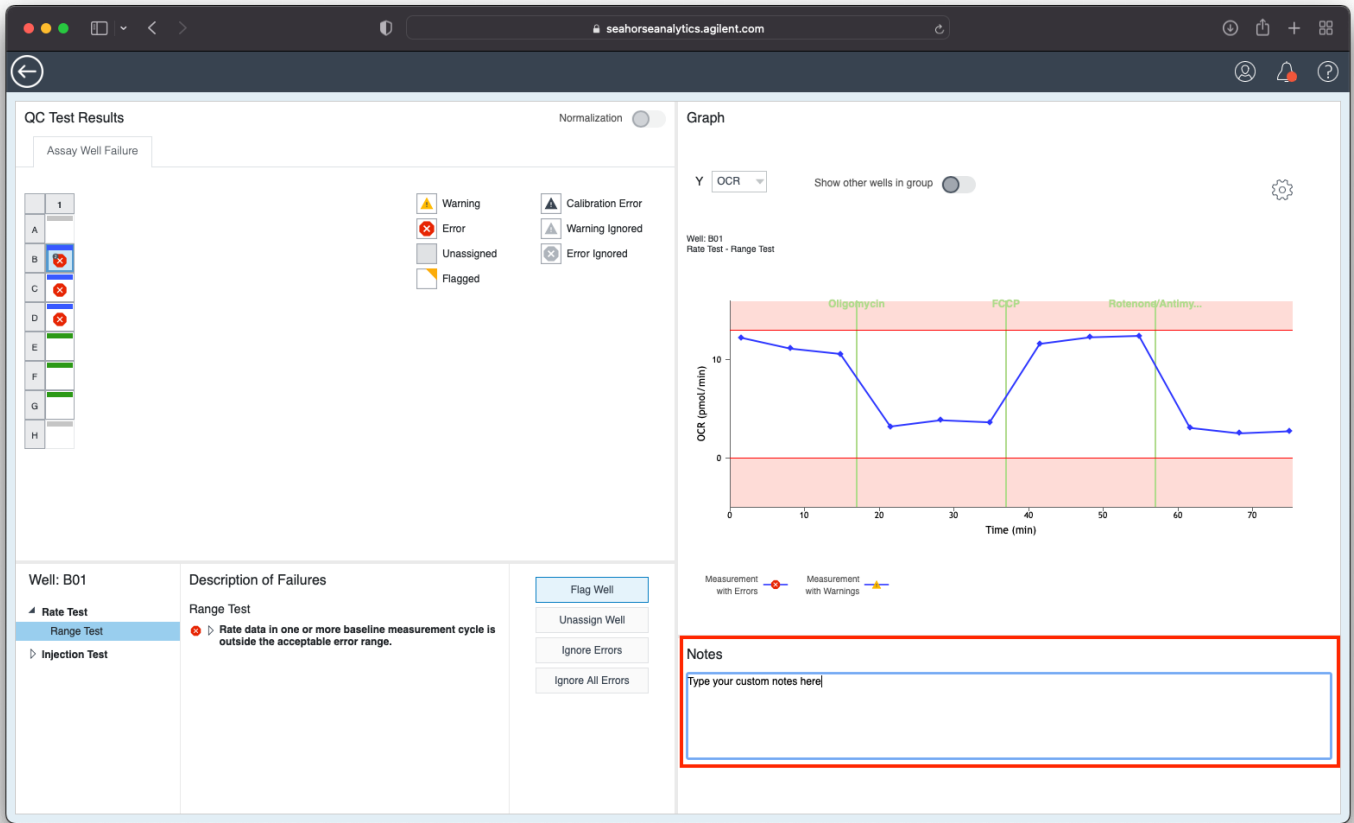
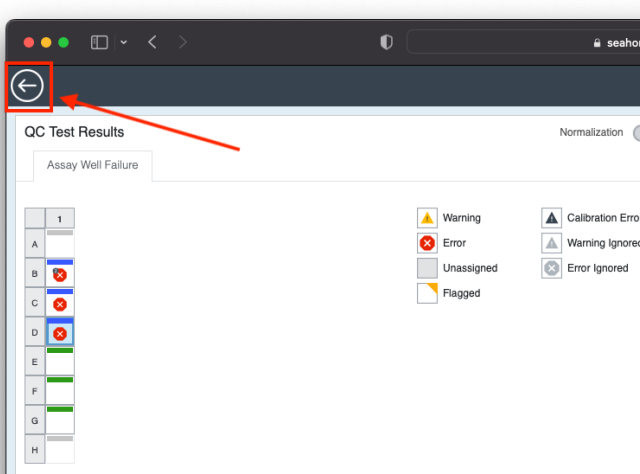**Figure 18.** Notes area.

Figure 19. The Data Quality Exit icon.

Part 3: Data Quality test explanations and other important information

There are currently 25 data quality tests performed by the Data Quality analysis view. These tests are grouped into six categories: temperature, calibration, background, level, rate, and injection.

Temperature
Assay result files are excluded from the temperature quality tests if: - It is a hypoxia assay result file. - The XF Pro or XF HS Mini set temperature is not 37 °C. - The atmospheric pressure is not 760 mm Hg. - No background wells are assigned to the plate map.

Temperature
- **Sub-Test − Low Temperature:** This sub-test sends an error if temperature of the plate for each measurement period is lower than the minimum allowed temperature.
  - Low Temperature = (instrument set temperature) − (temperature tolerance saved in the file)
  - **Note:** If the temperature tolerance is set to 0, then a default value of 0.4 will be used.
- **Sub-Test − High Temperature:** This sub-test sends an error if the temperature of the plate for each measurement period is higher than the maximum allowed temperature.
  - High Temperature = (instrument set temperature) + (temperature tolerance saved in the file)
  - **Note:** If the temperature tolerance is set to 0, then a default value of 0.4 will be used.

Calibration
Assay result files are excluded from the calibration quality tests if: - It is a hypoxia assay result file. - The XF Pro or XF HS Mini set temperature is not 37 °C. - The atmospheric pressure is not 760 mm Hg. - No background wells are assigned to the plate map.
Calibration - **Sub-Test − O_2 Calibration:** This sub-test displays an error for any well that fails O_2 calibration. The results of this test are just informative. Background wells that fail calibration are automatically excluded from background correction, and assay wells that fail calibration are automatically excluded from group calculations. - **Sub-Test − pH Calibration:** This sub-test displays an error for any well that fails pH calibration. The results of this test are just informative. Background wells that fail calibration are automatically excluded from background correction, and assay wells that fail calibration are automatically excluded from group calculations.

Background
Assay result files are excluded from the background quality tests if: - It is a hypoxia assay result file. - The XF Pro or XF HS Mini set temperature is not 37 °C. - The atmospheric pressure is not 760 mm Hg. - There are less than two background wells assigned to the plate map. **Note:** Background wells with errors should be unassigned to remove them from background correction of assay wells. Bad background wells affect all calculations when background correction is ON.
Calibration
- **Sub-Test − O_2 Calibration:** This sub-test displays an error for any background well that fails O_2 calibration. If this sub-test fails, no more calibration sub-tests are performed on the failed analyte (O_2 of pH). - **Sub-Test − pH Calibration:** This sub-test displays an error for any well that fails pH calibration. If this sub-test fails, no more calibration sub-tests are performed on the failed analyte (O_2 of pH).
Data Shift

This sub-test is only performed on the first level tick of each measurement period.
- **Sub-Test − O_2 Data Shift:** This sub-test sends an error if the O_2 level of a well on the first tick of a rate is more than 20 mm Hg away from 152 mm Hg. If it is between 10 and 20 mm Hg, a warning will be displayed.
  - Parameters: Lower limit = 10 mm Hg; Upper limit = 20 mm Hg
- **Sub-Test − pH Data Shift:** This sub-test sends an error if the pH level of a well on the first tick of a rate is more than 0.2 pH different from the averaged level of the other background wells. If it is between 0.1 and 0.2 pH, a warning will be displayed.
  - Parameters: Lower limit = 0.1 pH; Upper limit = 0.2 pH

Data Stability

This sub-test is performed on all measurement periods. A data pop error can be displayed for one or more measurement periods.
- **Sub-Test − O_2 Data Stability:** This sub-test sends an error if the O_2 level of a well varies by more than 5 mm Hg within a measurement period for each consecutive tick. If the difference is between 2 and 5 mm Hg, a warning will be displayed.
  - Parameters: Lower limit = 2 mm Hg; Upper limit = 5 mm Hg
- **Sub-Test − pH Data Stability:** This sub-test sends an error if the pH level of a well varies by more than 0.1 pH within a measurement period for each consecutive tick. If it is between 0.05 pH and 0.1 pH, a warning will be displayed.
  - Parameters: Lower limit = 0.05 pH; Upper limit = 0.1 pH

Data Drift

This sub-test is performed on all measurement periods. A data drift error can be displayed for one or more measurement periods.
- **Sub-Test − O_2 Data Drift:** This sub-test sends an error if the O_2 level during a measurement period is more than 20 mm Hg away from the first tick of this measurement period. If the tick difference is between 10 and 20 mm Hg, a warning will be displayed.
  - Parameters: Lower limit = 10 mm Hg; Upper limit = 20 mm Hg
- **Sub-Test − pH Data Drift:** This sub-test sends an error if the pH level during a measurement period is more than 0.4 pH away from the first tick of this measurement period.
  - Parameters: Lower limit = 0.2 pH; Upper limit = 0.4 pH

Measurement Jump

This sub-test is performed on all measurement periods. A measurement jump error can be displayed for 1 or more measurement periods.

- **Sub-Test – O_2 Measurement Jump:** This sub-test sends an error if the O_2 level of the first tick of 2 consecutive measurement period varies by more than 5 mm Hg. If the difference is between 2 and 5 mm Hg, a warning will be displayed.
  - Parameters: Lower limit = 2 mm Hg; Upper limit = 5 mm Hg
- **Sub-Test – pH Measurement Jump:** This sub-test sends an error if the pH level of the first tick of two consecutive measurement period varies by more than 0.1 pH.
  - Parameters: Lower limit = 0.05 pH; Upper limit = 0.1 pH

Cell Detection

This sub-test is performed on all measurement periods after an injection.

- **Sub-Test – OCR Cell Detection:** This sub-test sends an error if the OCR changes by more than 20 pmol/min. If the OCR changes by more than 10 pmol/min, a warning will be displayed.
  - Parameters: Lower limit = 10 pmol/min; Upper limit = 20 pmol/min
- **Sub-Test – ECAR Cell Detection:** This sub-test sends an error if the ECAR changes by more than 10 mpH/min. If the ECAR changes by more than 5 mpH/min a warning will be sent.
  - Parameters: Lower limit = 5 mpH/min; Upper limit = 10 mpH/min

Level

Assay result files are excluded from the level quality tests if:
- It is a hypoxia assay result file.
- The XF Pro or XF HS Mini set temperature is not 37 °C.
- The atmospheric pressure is not 760 mm Hg.
- There are no background wells assigned to the plate map.

Note: The level quality tests are not performed on background wells. The level quality tests use background-corrected data. The level quality tests do not use normalized rate data. If a well has a calibration error for a specific analyte (O_2 or pH), the level data quality tests will NOT be performed for the failed analyte.

Range Test

This sub-test sends an error if the first tick of a measurement period has level data outside the valid level data range.

- **Sub-Test – O_2 Range Test:** This sub-test sends an error if the O_2 level of a well is lower than 100 mm Hg or greater than 175 mm Hg for the first tick of a measurement period. For any measurement period, if O_2 levels drop below 40 mm Hg an error will be displayed in the well.
  - Parameters: Lower limit = 100 mm Hg; Upper limit = 175 mm Hg
- **Sub-Test – pH Range Test:** This sub-test sends a warning if the pH level (first tick of a measurement period only) of a well is lower than 6.5 pH or higher than 7.6 pH.
  - Parameters: First measurement period = 7.0 to 7.6 pH; All other measurement periods = 6.5 to 7.6 pH

Spike Test

This sub-test sends an error if the change in the level of two consecutive ticks of a well is unexpectedly different from the other wells in the same group.

- **Sub-Test – O_2 Spike Test:** This sub-test sends an error if the change in O_2 level of two consecutive ticks (within a measurement period) is unexpectedly different from the other wells in the same group. For each consecutive tick of a measurement period, we compare the level difference of a well to the average of the level differences of the other wells of the same group.
  - Parameters: Limit = 800%
- **Sub-Test – pH Spike Test:** This sub-test sends an error if the change in pH level of two consecutive ticks (within a measurement period) is unexpectedly different from the other wells in the same group. For each consecutive tick of a measurement period, we compare the level difference of a well to the average of the level differences of the other wells of the same group.
  - Parameters: Limit = 800%

Rate

Assay result files are excluded from the rate quality tests if:
- It is a hypoxia assay result file.
- The XF Pro or XF HS Mini set temperature is not 37 °C.
- The atmospheric pressure is not 760 mm Hg.
- There are no background wells assigned to the plate map.

Note: The rate quality tests are not performed on background wells. The rate quality tests use background-corrected data. The range rate quality test does not use normalized rate data, the baseline rate quality test uses normalized rate data. If a well has a calibration error for a specific analyte (O_2 or pH), the rate data quality tests will NOT be performed for the failed analyte. If you normalize your result data, but a well does not have a normalization value (for any reason), then this well will be excluded from the rate data quality tests.

Range Test

This sub-test sends an error if baseline rate measurements are lower than the acceptable minimum rate threshold (determined by Agilent). Any rate measurements that occur before your first injection are called baseline rate measurements. The first baseline rate measurement is always excluded from the range quality test.

- **Sub-Test – OCR Range Test:** This sub-test sends an error if the OCR of a baseline rate measurement is below 13 pmol/min in a well.
  - Parameters: Lower limit = 13 pmol/min
- **Sub-Test – ECAR Range Test:** This sub-test sends an error if the ECAR of a baseline rate measurement is below 3 mpH/min in a well.
  - Parameters: Lower limit = 3 mpH/min

Baseline Test

This sub-test sends an error if the last baseline rate measurement (before your first injection) of a well is different from the last baseline rate of the other wells within the same group. A minimum of four valid wells in a group are required for this test (where "valid" = wells that pass calibration for both analytes).

- **Sub-Test – OCR Baseline Test:** This sub-test sends an error if the last OCR baseline measurement (last rate measurement before your first injection) of a well is different from the last OCR baseline measurement of the other wells within the same group. We measure the difference between the OCR of the test well and the average OCR of the other wells assigned to the group.
  - Parameters: Low limit = 25%; Upper limit: 40%
- **Sub-Test – ECAR Baseline Test:** This sub-test sends an error if the last ECAR baseline measurement (last rate measurement before your first injection) of a well is different from the last ECAR baseline measurement of the other wells within the same group. We measure the difference between the ECAR of the test well and the average ECAR of the other wells assigned to the group.
  - Parameters: Low limit = 35%; Upper limit: 50%

Injection
Assay result files are excluded from the injection quality tests if: - It is a hypoxia assay result file. - The XF Pro or XF HS Mini set temperature is not 37 °C. - The atmospheric pressure is not 760 mm Hg. - There are no background wells assigned to the plate map. **Note:** The injection quality tests are not performed on background wells. The injection quality tests use background-corrected data. The injection quality tests use normalized rate data that is background corrected (if normalization data is available). If a well has a calibration error for a specific analyte (O_2 or pH), the injection data quality tests will NOT be performed for the failed analyte. If there are no injections in your assay, the injection quality tests are not performed. If a group has less than four wells assigned, the injection quality tests are not performed. If you normalize your result data, but a well does not have a normalization value (for any reason), then this well will be excluded from the injection data quality tests.
Injection Failure
This sub-test sends an error if a well does not show a change in rates consistent with other wells in the group after an injection. - To identify an injection failure, the rate differences are calculated within each well (for each group) to first determine if the group has a response to an injection, then a threshold is calculated for each group. If the test well rate is below the calculated threshold for the group, then the well will be flagged as an injection failure. - This test is only performed on the rate measurement immediately after an injection. If there is no measurement period after an injection, this test will not be performed. This test is performed for both OCR and ECAR data.
Injection Problem
This sub-test sends a warning or an error if a well exhibits differences in the two rate measurements immediately preceding an injection, when compared to the same rate measurements in the other wells in the same group. - To identify if there is an injection problem, the z-score is calculated within each well. If rate data in a well is below the threshold (calculated within each group respectively), it is flagged and removed from subsequent calculations on the wells within that group to further identify any other wells with an injection problem warning or error. - This test is only performed on the two rate measurements immediately preceding an injection. If a well has an injection failure at an injection rate, then the injection problem test is not performed. This test is performed for both OCR and ECAR data.

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