

# Relative Calibration Issue Workaround

Update (July 2022): Aria Software v1.7 has provided a workaround for this issue. When the user has selected wells with multiple targets, a warning message will show to prevent further analysis. We recommend you upgrade your SW/FW version at your earliest convenience.

This document explains the recommended workaround for the defects that affect the Relative Quantity to calibrator results while using Aria software to analyze run files generated on both AriaMx (PN: G8830A) and AriaDx (PN: K8930AA) instruments.

## The issue:

It has been noted that when users are running Comparative Quantitation experiments, and analyzing targets collectively, where multiple assays with different Target names, each with their own calibrator, are set up on the same plate, there will be two potential outcomes on the analysis results as described below.

1. **When replicate numbers are assigned to the calibrator wells:** no Relative Quantity to Calibrator values are displayed for the Unknown wells, and the software displays the error “Selected wells contain multiple calibrator sets”.
2. **When no replicate numbers are assigned to the calibrator wells:** Incorrect Relative Quantity to Calibrator values are displayed for the Unknown wells.

## How to workaround this issue:

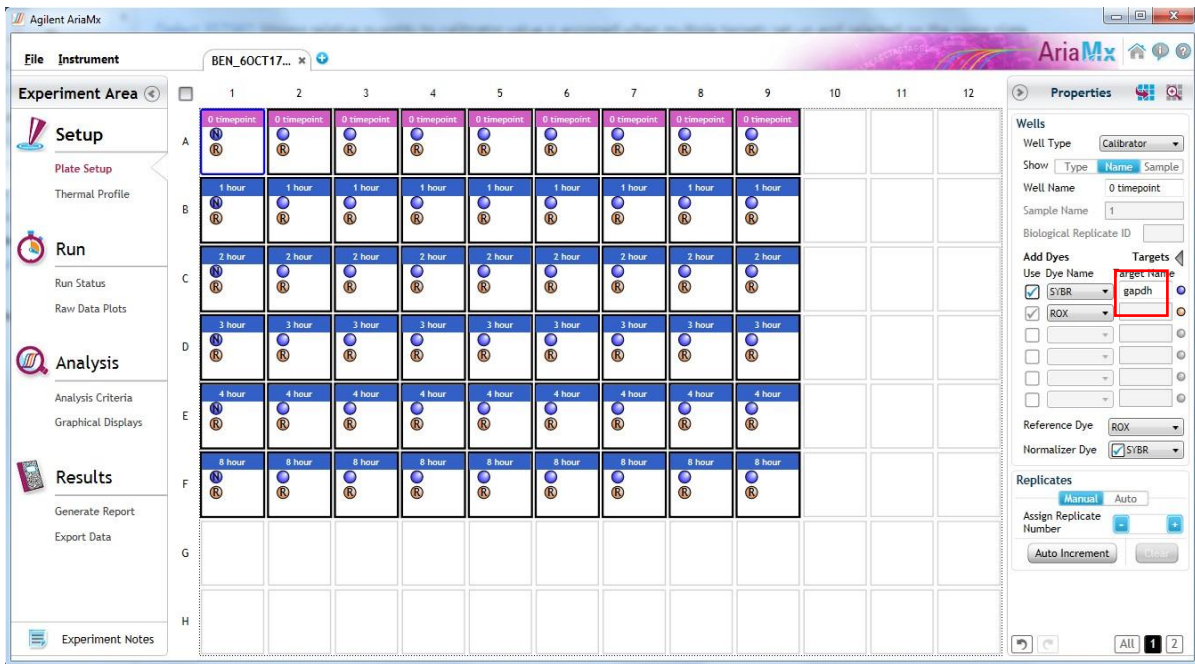
The defect can be avoided by making sure to only analyze one Gene of Interest target at a time. The following instructions will allow you to avoid these defects and allow for correct calculation of the Relative Quantity to Calibrator values.

The workaround is described here in terms of the example Comparative Experiment plate setup shown in the screenshot below. Please note that the workaround is identical on Aria software for run files from both AriaMx and AriaDx.

In this example, the plate is set up in the following way:

1. Primers for different target genes are being used in the wells in each column, so a different Target name assigned to the wells in each column in the Plate Setup.
2. The wells in column 1 contain primers to amplify the Normalizer target GAPDH.
3. Row A contains the Calibrator samples, and each successive row contains unknown samples taken at different timepoints.
4. The wells in each row are assigned the same Sample name. This associates the unknown samples in each row to the Normalizer in that same row as containing template taken from the same sample (in this case at the same time point).

In the screenshot below, N indicates the normalizer, and R indicates that ROX is assigned as the reference dye.

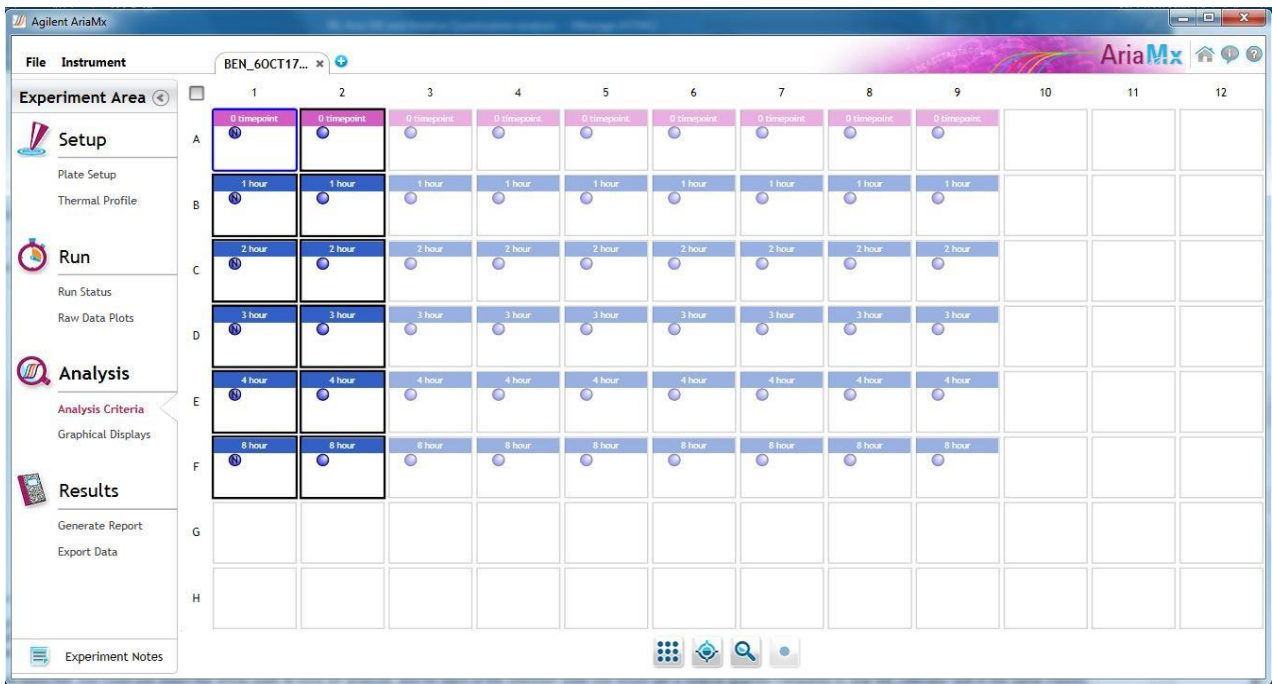


In the absence of the defect, analysis could be performed by just selecting the whole plate on the Analysis Criteria screen. For each of the unknown wells the Relative Quantity to Calibrator value would be calculated relative to the calibrator well in the same column, normalized to the unknown and calibrator wells from the same rows in column 1.

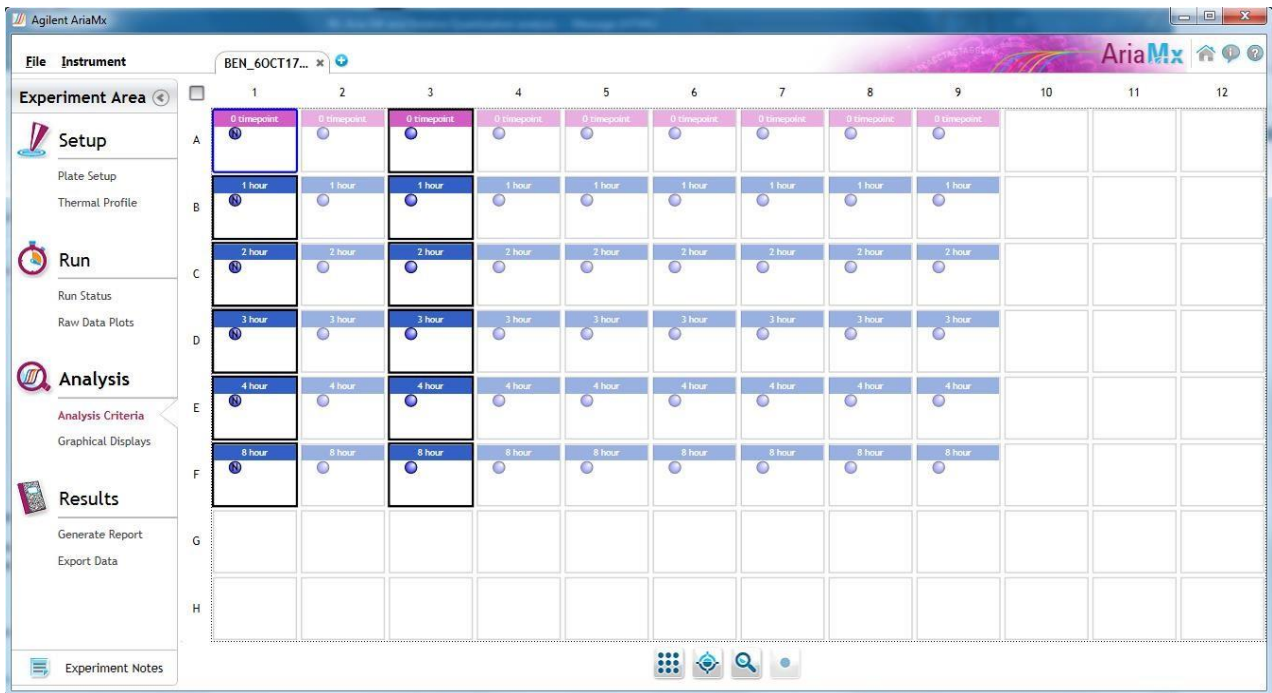
**Corrective Action by Customer:**

To ensure that the calculated relative quantities are correct:

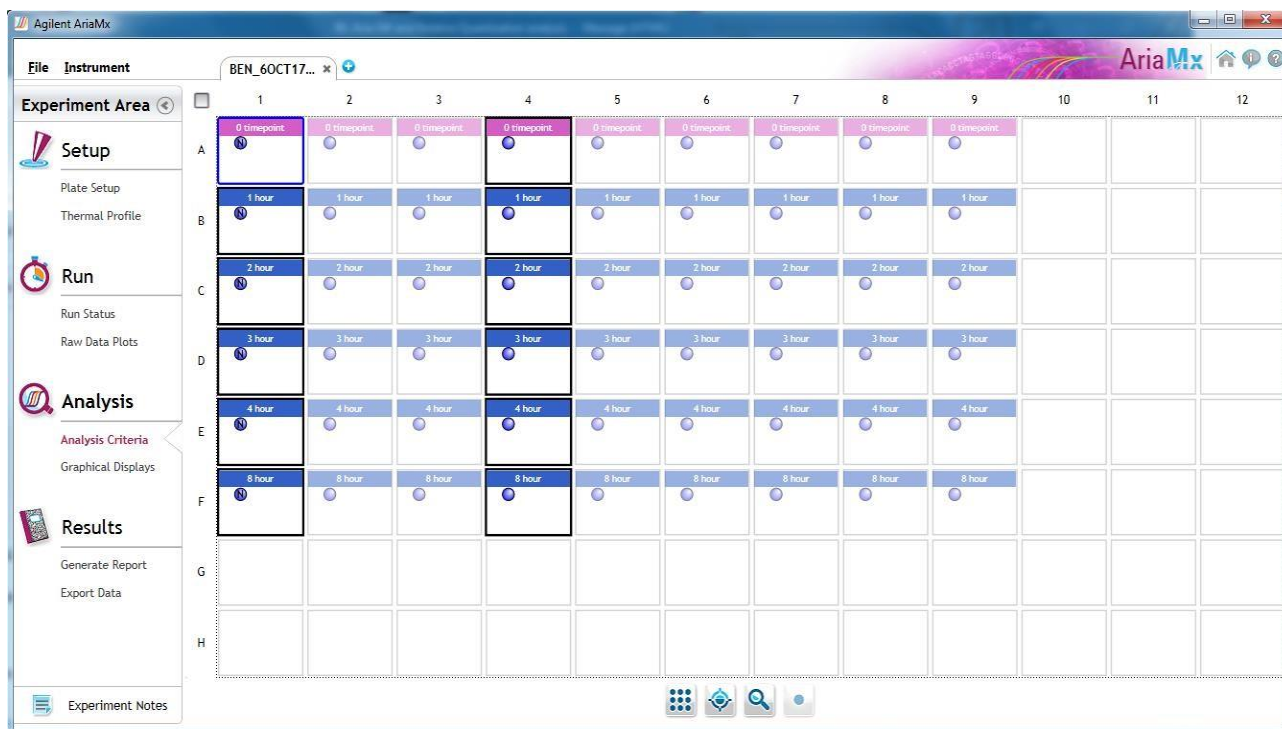
Step 1: Select one Gene of Interest target column and the normalizer column at a time on the Analysis Criteria screen (as shown below).



Step 2: Use the Graphical Displays screen and analyze the results for that specific GOI target. After analysis on graphical displays screen, use the Analysis Criteria Screen and select a different GOI column plus the normalizer column, then again Graphical Displays screen to analyze that GOI target.



Step 3: Repeat this for each GOI target, analyzing each separately.



**Contact information for questions:**

For additional questions or clarification please contact Agilent Technical Support at 1-800-227-9770.

*AriaDx: For In Vitro Diagnostic Use*

*AriaMx: For Research Use Only. Not for use in diagnostic procedures.*