Assays that have scared and scarred you!

4PL or 5PL or...both?

Can the same assay have different interpolation curve?

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Assay Type: [X] Binding [ ] Cell-Based [ ] Animal-Assay [ ] Other (Please fill in)

Tell us what happened:

The problem was data interpretation, curve data vs samples' data. New product needed a new assay ASAP. Production and development teams worked together to generate a new assay that could be used to test the product during in-process development efforts and to release and test the final DS and hopefully DP. As production studies were already ongoing, the team had a short window of time to generate data and bridge with the old assay. Thus historical DS where used to generate data. The team used DS stored at < -65C and heat treated samples. The assay was a quantitative assay, with a 7 points standard curve with known concentration and the test sample value (concentration) was determined by diluting within the curve. The team trended the data from the standard curves and % recoveries front he samples tested.
The development team used 4PL to interpolate the standard curve, the QC team used the 5PL.

The best fitting vs samples results

- To keep in mind:
  - The method was a microfluidic ELISA that had no a clear reading plateau. The samples and reagents were used in very small amounts, in the nl-scale and small errors in pipetting or Ab dilution, had an amplified effect on the assay. Training the analyst was key and essential to the successful run of the assay.
  - Not having a clear anchor points in the upper and lower asymptote can lead to different interpolation. This means that the algorithm used to interpolate the curve's points extract the upper and lower asymptotes.
- Solution:
  - The data from 10 independent curves generated were interpolated either using a 4PL or a 5PL equation, and the curves' 4 parameters and 5 parameters were plotted in 2 tables.
  - Looking at the data side by side it was noticeable that when using 5PL, even if the fitting of each individual curve was better, the variability of parameter "A" = upper asymptote, was > 25%CV
  - However the % recoveries of the samples that were tested at a reading close to the curve IC50, were not affected as much.
  - The development lab and the QC lab were able to move forward with the assay using 4PL and 5PL interpolation curves.