Curve Fitting and Data Analysis

Generally, we do not recommend the use of linear regression to fit ELISA data and particularly HCP assays. HCP ELISAs are rarely perfectly linear in dose response. Even though you may get an R squared value of .99, which looks to be good does not mean the assay is truly linear. To force an inherently non-linear method to fit the best straight line will lead to inaccuracies in reporting values on your samples. Those inaccuracies are most significant at the extremes of the standard curve, most often in the low end but sometimes in the high end as well. For this reason, we strongly urge our clients to use Point to Point, Cubic Spline or 4 Parameter as the curve fitting routines since these will yield the most accurate results.

It may be that curve fit routines other than the three we recommend will work satisfactorily for a given immunoassay method. If you decide to use another method for interpolation of sample values, it will be important for you to perform a careful analytical evaluation of your proposed algorithm. If after considering the points below, your method meets or exceeds the interpolation accuracy and precision of the other 3 methods then by all means use it. A word of caution, that just because your method works well in one immunoassay is no guarantee it will be optimal for another and therefore, each assay should be validated on a case by case basis. We recommend the 3 methods above because they are the most robust and the most accurate for immunoassay and have been shown to work for our assays. We specifically warn against the use of linear regression methods because most immunoassays are not linear nor is linearity a requirement for a good immunoassay. To force data from an assay to fit the best straight line when the inherent dose response is not a straight line is certainly a way to introduce inaccuracy into your results. Similarly, other more sophisticated regression methods can also introduce mathematical assumptions and enforce arbitrary and inappropriate rules that actually reduce the inherent accuracy and precision of the assay.

An easy way to determine the optimal curve fit routine is by “back fitting” the signals of your standards as unknowns. If the standards when “back fit’ as unknowns do not report back their nominal values, there may be artifacts introduced by inappropriate assumptions or restrictions in your curve fit algorithm. Finally, the most direct and objective way to assess the accuracy of an immunoassay is to assay controls with known levels of analyte across the important analytical range of the assay. Do not rely on arbitrary and indirect parameters such as R squared, slope, y-intercept, or asymptotes such as QC specifications. These parameters are often too insensitive to be useful in flagging a bad assay run.