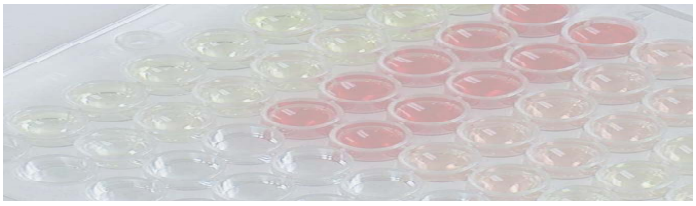
StatLIA® from Brendan



Weighting: The Good, The Bad and The Ugly

INFORMATIVE GRAPHS, METRICS ONLY POSSIBLE WITH GOOD (ACCURATE) WEIGHTING EQUATION

Weighting is a critical factor in the computations and analysis of an assay. A good weighting equation accurately models the variances in responses throughout the assay. Several powerful computations and informative graphs and metrics that analyze the assay and its performance are only possible with a good weighting determination. All of the graphs and metrics at right and below require accurate weighting:

- Weighted 5PL and 4PL
- Residual Error Plots
- Concentration Error Graphs
- Fit Probability for Curves
- Potency Assay Metrics
- Effective Outlier Detection
- Limits of Quantitation
- %CV Acceptability Metric

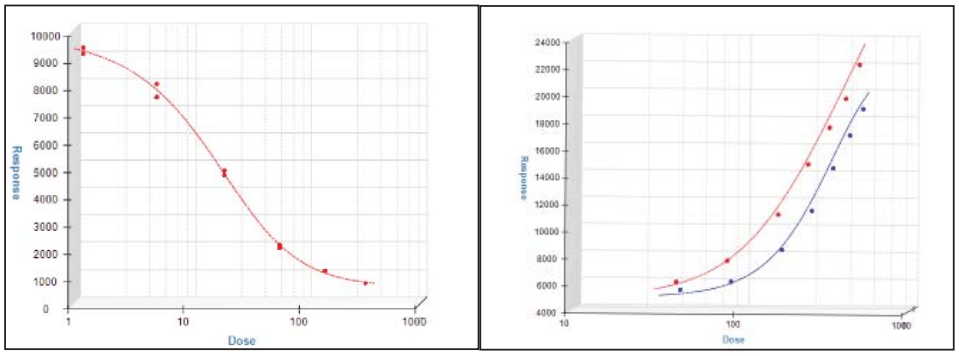
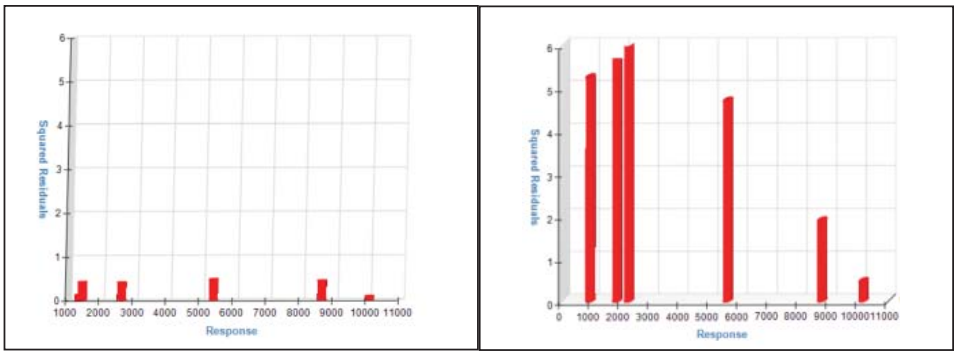


Figure 1

An accurate weighting equation used with a robust curve fitting model can increase the reportable range of an assay. Potency assays require a good weighting equation to accurately measure the parallelism (similarity) between the curves. Chi-square (χ2) metrics from the residual method used to measure parallelism are only possible with accurate weighting.

Figure 2

The Squared Residual plots show the residual error of each data point. The graph on the left shows all of the standard dilution points are good. The graph on the right shows the effect of a bad dilution point (dilution 3).



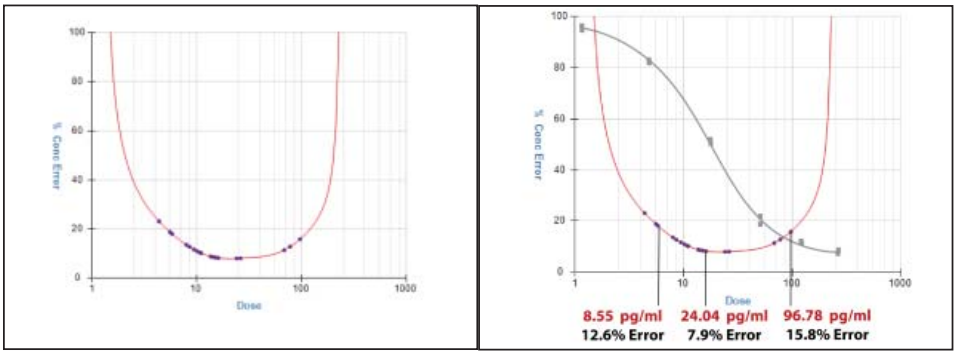


Figure 3

The Error Profile displays the % error at each concentration. The unknowns can be easily evaluated for reliability by their % error values. And the Limits of Quantitation can be determined by an acceptable % error value as a cutoff for the reportable range.[9]

ACCURATE WEIGHTING EQUATION CRITICAL FOR COMPUTATIONS AND ANALYSIS

As underscored in the literature, "Any sensible discussion of the design of an immunoassay, must take account of the variance per response."[1]

A good weighting equation that accurately models the variances of the responses significantly improves the sensitivity, reliability and accuracy of the computations and results in immunoassay and bioassay analysis.[1,2,3,4,5,6,7,8] (See Figure 4.) Conversely, a bad weighting equation (or no weighting) can adversely alter the results and metrics or make many of them meaningless.[2,3] Depending on the data, an accurate weighting equation used with an effective curve model can improve the sensitivity of an assay by an order of magnitude at the low end (unpublished study by manufacturer).

Good weighting equations model the non-uniform variances in responses at the different concentration levels in an assay. With the variances accurately modeled, the weighting equation normalizes the different variances observed in responses so that each data point contributes equally to the assay's computations. Without weighting, the curve fit is disproportionately biased toward the standard data points with the largest responses. (See Figures 5 and 6.) Weighting also ensures that those responses with the best precision are given more weight than responses with lower precision.[1,2,4,6]

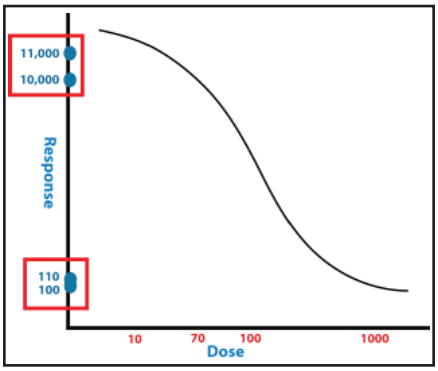
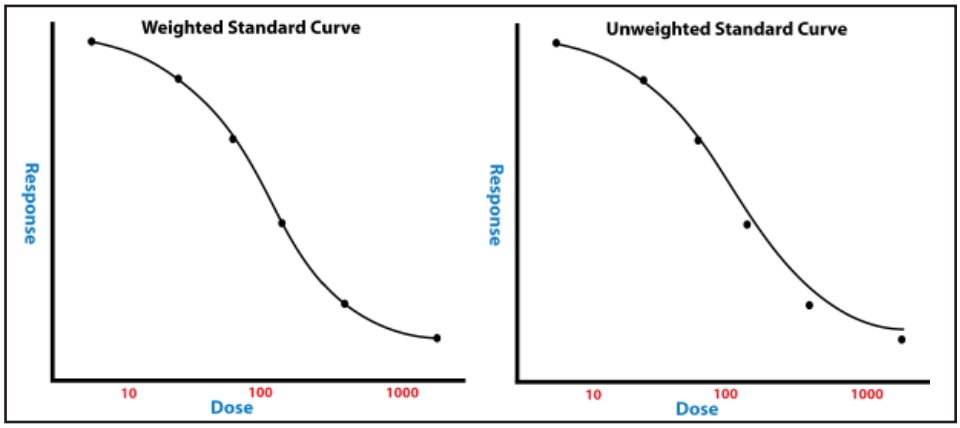


Figure 4

The large difference in variances between each set of replicates, from the high to low response ends, must be correctly modeled in the weighting equation for each test method. With a correct weighting equation, the differences in the magnitudes of responses are factored out of the computations. With no weighting or a bad weighting equation, these unequal variances will adversely alter the computations.

Figure 5

Without weighting, the 4PL curve fit is disproportionately biased toward the standard data points with the larger responses at the expense of the low response end. The same problem occurs with 5PL curve fits and linear regression fits.



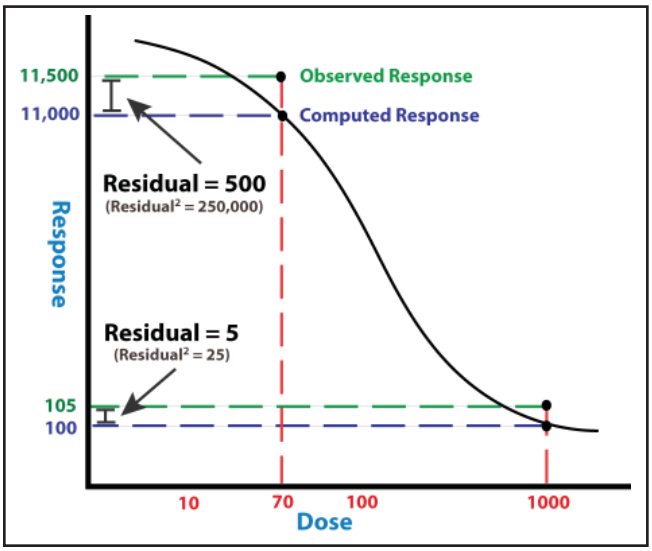


Figure 6

Least squares regression curve fitting models, such as the 5 parameter and 4 parameter logistic, compute the optimal curve that provides the lowest cumulative error determined by the distance between the observed data points and the curve. The total error is calculated by squaring the error at each data point and then adding the errors together. This is called the Sum of Squares Error (SSE). A 10 % error at each end of the curve, 5 and 500, yields significantly different values when squared, 25 and 250,000. As the curve fitting model searches for the best curve with the lowest SSE, the data points with the largest responses dominate the fitting because of the magnitude differences in their squared values. The low response end of the curve can have the most biological significance, yet have the worst fitting when weighting is not applied.[1,2,4,6]

NOT ALL WEIGHTING EQUATIONS ARE THE SAME

According to regression theory, the weights should be set equal to the inverse of the variance of the responses at each concentration. The equation is simple: 1/Variance. The question is: What is the variance for each test method? There are three approaches used in commercial software for weighting assay regressions. Only one of these approaches is considered acceptable in the literature.

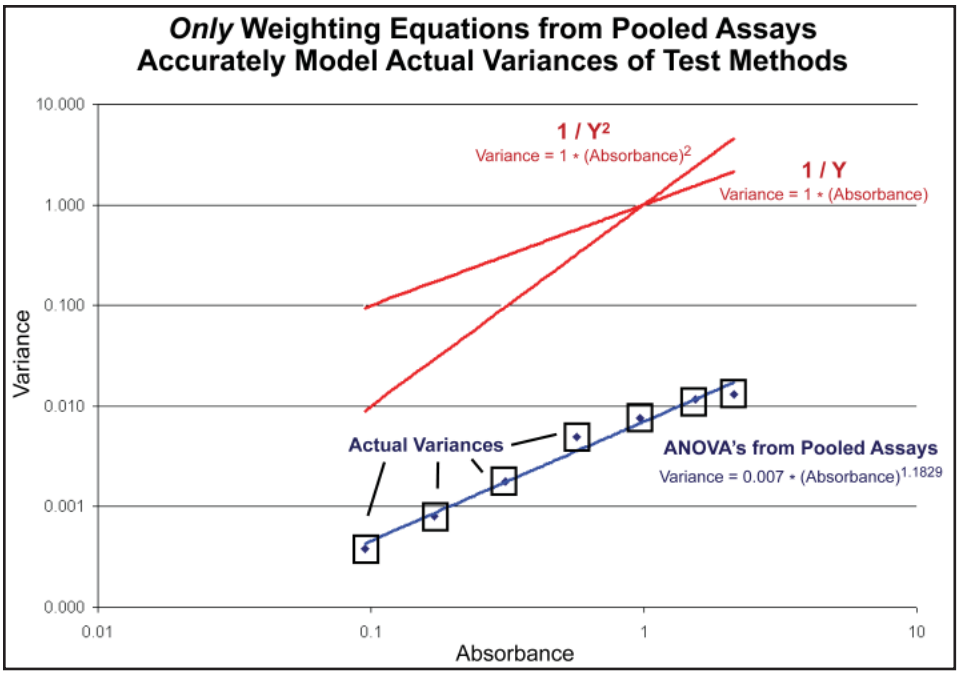


Figure 7

The weights are the inverse of the estimated variance of the responses at each concentration (1/Variance). The data points on the graph are the actual mean variances of the responses determined by an analysis of variance (ANOVA) of 30 pooled assays. The regression lines show the difference in accuracy between weighting expressions using the arbitrarily chosen values for the variances [1/Y or 1/Y^2] and Finney's power weighting equation [A (response)^B] computed from an ANOVA of pooled assays as described in the literature. [1,2,3,4,5,6,7,8]

THE GOOD. ***The weighting equation uses the variances determined by an analysis of variance (ANOVA) of the responses from pooled assays.***[2,3] Pooled assays are needed to provide enough degrees of freedom (sample size) for a reliable estimate of the true variances.[2] A separate weighting equation is needed for each test method to correctly model the unique variances generated by the different biological reactions. This is because the kinetics of the respective antibody (immunoassay) or reactive enzyme(s) (bioassay) vary widely, and these kinetics are a major contributor to the variance. That is why STATLIA automatically pools the assays for each test method to compute the actual variances from each test method. "*By pooling (variances) from all dose levels … and from many assays, we have drastically reduced the effect of sampling error and extracted information about the underlying nature of the error in the response variable.*"[2]

NOTE: ***STATLIA® uses Finney's power weighting equation*** [A (response)^B] ***with an Analysis of Variance (ANOVA) of pooled responses from historical assays stored in STATLIA's database.***[1,2,5] ***STATLIA computes a seperate weighting equation for each test method from these previously run historical (reference) assays.***

THE BAD. ***Weighting is determined from an arbitrary expression on a single assay.*** These arbitrary weighting expressions, such as 1/Y and 1/Y^2, are not determined from the actual variances of pooled assays in the test method. Consequently, they are very different than the actual variances they are supposed to estimate. They assume a uniform variance, when, in fact, immunoassay and bioassay variances are extremely non-uniform.[1,2,3,4,5,6,7,8,9] With inaccurate weighting, "the use of weights may actually seriously degrade the performance of the curve-fitting procedure."[2,3] Furthermore, the other powerful graphs and metrics found in STATLIA for analyzing an assay are not possible with these weighting schemes.

THE UGLY. ***No weighting equation is used to compute the assay.*** Without weighting, the least squares regression curve fitting models fit the high response data points with little contribution from the low response end, because the low responses are too small to materially influence the curve fitting. But with accurate weighting, all data points contribute equally to the curve fit. (See Figures 5 and 6.)

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