STATLIA® MATRIX

See More. Know More. Do More.™

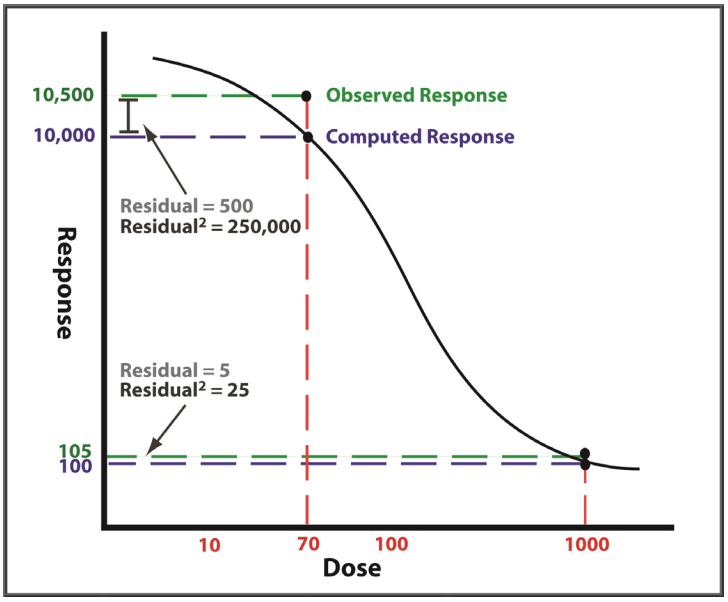
Tech Note

Logistic Curve Weighting

Weighting In Logistic Curve Regressions

FDA and European Pharmacopeia guidelines require appropriate weighting of responses with their estimated variances for all immunoassay and bioassay regression curves. This is because of the substantial effect weighting has on the sensitivity, reliability and accuracy of the results. Accurate weighting used with an appropriate curve fitting model can increase the reportable range of an assay. Other analytical computations that require accurate weighting include measuring the parallelism and relative potency between curves in potency tests, precision profiles of concentration error, limits of quantitation and detection, confidence limits around parameters, sample replicate precision, and identifying precision and residual outliers.

Heteroscedasticity of Variances Between Dilutions



Responses can differ by two or more orders of magnitude between end points, and their response variances (residuals^2) can differ by more than four orders of magnitude. In this illustration (not drawn to scale), both observed responses are 5% higher than the curve. But the squared error residual^2 of the high response point dwarfs that of the low response point. Without weighting each response with the inverse of its expected variance (residual^2), the curve will be fitted predominantly to the high responses with little influence from the low responses because that will yield the regression with the lowest sum of residuals^2 (RSSE). However, accurately weighting each response with its estimated variance ensures that all dilution points contribute equally to the final regression curve.

An accurate weighting regression of the response variance models the two sources of error, the *dilutional variance* and the *residual error*.

BRENDAN BIOANALYTICS

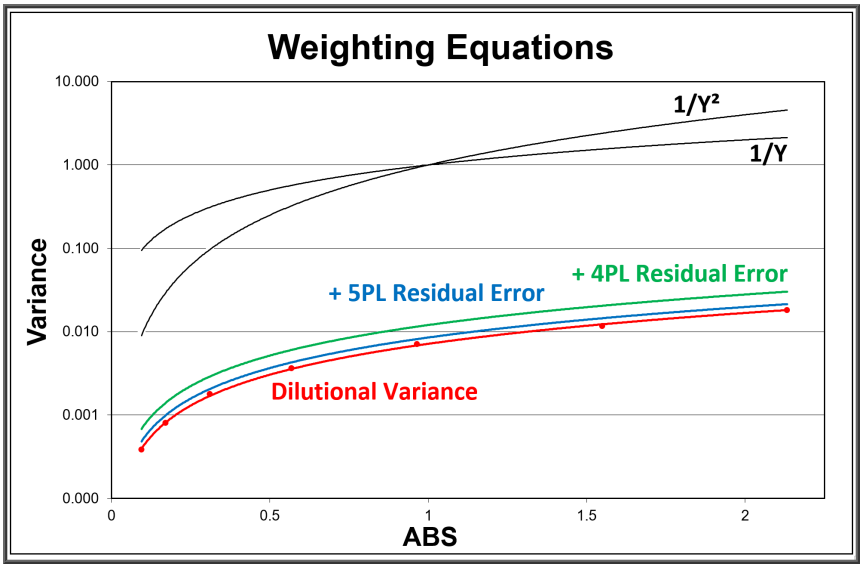
DILUTIONAL VARIANCE

Dilutional variance can be estimated from the replicate variances of each dilution. The dilutional variance contains the signal error from the detector, the systemic intra-assay error from the kinetic reactions, pipetting, reagents, incubation conditions, reactive phase separation, and random error. An initial dilutional variance estimate can be made from one assay, with more reliable variance estimates obtained from a pool of 6 or more historical assays using the error mean sum of squares from an analysis of variance (ANOVA) of each dilution. Pooled assays provide a larger sample size to determine a reliable estimate of the true variances. The responses and their variances are fitted to a power regression (log responses, log variances), which reduces the heteroscedasticity of the variances. This generates a variance regression in the form AY^B, where A is the error term, Y is the response, and B is the heteroscedasticity, or change, in the variances across dilutions. In our studies looking at over 25,000 assays from more than 200 immunoassay and potency tests, we have found that A can vary from 10^{-6} to 10^4 and B can vary from 0.5 to 3 between different test methods and different label types.

RESIDUAL ERROR

Residual error is the imprecision of the individual dilution concentrations plus the lack of fit error of the curve model in each assay. The residual error of 4PL regressions is usually greater than of 5PL regressions because of the asymmetry of most assay dilution curves. Like the dilutional variance, the residual error is determined from pooled assays. This residual error is added to the dilutional variance error (A) for the final weighting regression of the response variances. In our studies, the residual error from 5PL curves has a median value from our 200 test methods of 6% of A, and the residual error from 4PL curves has a median value of 34% of A for the same test methods.

Accurate Weighting from Pooled Assays



The dilutional variance power regression (red) models the average variance of each standard dilution (red dots) from an ANOVA of pooled assays. The residual error from 5PL curves (blue) and 4PL curves (green) of the same pooled assays are added to the dilutional variance regression.

The weighting power regressions for the same pooled assays using a variance of Y (weight = 1/Y) and Y^2 (weight = $1/Y^2$) for each dilution response are plotted in black. These regressions shows how much empirical weighting models can differ from accurate weighting.

In regression statistics, an accurately weighted RSSE is a chi-square distributed value whose average from a pool of assay curves equals the degrees of freedom (number of points – number of parameters). The RSSE of a curve regression can be expressed as a chi-square probability (Fit Prob), simplifying evaluation.

As noted extensively in the literature, deriving the weighting from pooled assays is the only way to obtain accurate weights for immunoassay and bioassay dilution curves. In STATLIA MATRIX's Performance Analysis reports, the RSSEs from curves computed with the weighting regression from the pooled assays show that the average of the pooled RSSEs is within 5% of the number of degrees of freedom. For more information on weighting 5PL and 4PL curves, see Brendan Bioanalytics Tech Note: 5PL and 4PL Curve Fitting. To see how weighting is used for limits of quantitation (LOQ), see Brendan Bioanalytics Tech Note: LOQs, LODs.

REFERENCES

Belanger BA, Davidian M, Giltinan DM The Effect of Variance Function Estimation on Nonlinear Calibration Inference in Immunoassay Data. Biometrics: 52, 158-175, 1996.

Davidian M, Carroll R.J., Smith W. Variance Functions and the Minimum Detectable Concentration in Assays. Biometrika 1988, 75, 549–556.

DeSilva B, Smith W, Weiner R, Kelley M, Smolec J, Lee B, Khan M, Tacey R, Hill H, Celniker A. Recommendations for the Bioanalytical Method Validation of Ligand-binding Assays to Support Pharmacokinetic Assessments of Macromolecules. Pharmaceutical Research 2003, 20 (11), 1885-1900.

Dudley R.A., Edwards P, Ekins, R.P., Finney, D.J., McKenzie, I.G.M., Raab, G.M., Rodbard, D; Rodgers R.P.C. Guidelines for Immunoassay Data Processing. Clinical Chemistry 1985, 31 (8), 1264-1271.

Dunn JR, Wild D. Calibration Curve Fitting. The Immunoassay Handbook, Theory and Applications of Ligand Binding, ELISA and Related Techniques, 4th Edition, 323–336, 2013.

European Pharmacopeia 5.3. Statistical Analysis of Results of Biological Assays and Tests 2003, 4375-4406.

Finney D.J. Response Curves for Immunoassay. Clinical Chemistry 1983, 29 (10), 1762-1766.

Gottschalk PG, Dunn JR. Determining the Error of Dose Estimates and Minimum and Maximum Acceptable Concentrations from Assays with Nonlinear Dose-Response Curves. Computer Methods and Programs in Biomedicine, 204-215, 2005.

Gottschalk PG, Dunn JR. Measuring Parallelism, Linearity and Relative Potency in Immunoassay and Bioassay Data, Journal of Pharmaceutical Biostatistics 2005, 15 (3), 437–463.

Gottschalk PG, Dunn JR. The Five Parameter Logistic: A Characterization And Comparison With The Four Parameter Logistic. Analytical Biochemistry: 343, 54–65, 2005.

Raab, G.M. Estimation of a Variance Function, with Application to Immunoassay. Applied Statistics 1981, 3 (1), 32-40.

U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Veterinary Medicine (CVM). Bioanalytical Method Validation, Guidance for industry. Biopharmaceutics, May 2018.

Additional Information: *visit* www.brendan.com ■ *e-mail* sales@brendan.com ■ *call* 1-760-929-7500

Brendan Bioanalytics. 1947 Camino Vida Roble, Suite 215, Carlsbad, CA 92008 USA