Data Analysis for ELISA Assays

Immunoassays such as sandwich ELISAs are frequently used in research and diagnostics to detect and quantify specific protein targets in complex biological matrices. Accurate quantification requires assays which are validated for their intended purpose (see Technical Note #1). Additionally, correct data analysis and the application of appropriate curve fitting models are critical for reporting accurate results.

Running a quantitative ELISA assay usually results in plotting a 2-dimensional x-y-plot which describes the relationship of the concentration of the calibrator (dose), i.e. the protein of interest, with the response of the assay system, eg., optical density for colorimetric detection. Before samples can be evaluated, an appropriate curve fitting model has to be chosen and its goodness of fit assessed. This Technical Note discusses the most important curve fitting models for sandwich ELISA immunoassays and how the quality of fitting can be determined.

Curve Fitting: Linear vs. Non-Linear Regression Analysis

Linear regression is one of the most common models used in analytics. Immunoassays describe a biological system (antibody-antigen interaction) and as such **immunoassays usually do not follow a linear dose-response relationship**! ELISA assays often yield a sigmoidal curve (Figure 1) with only a limited linear concentration range. Thus, non-linear regression models such as 4- or 5-PL (-Parameter Logistic) regression should be applied which increase accuracy and the range of the calibration curve that can be used for quantification.

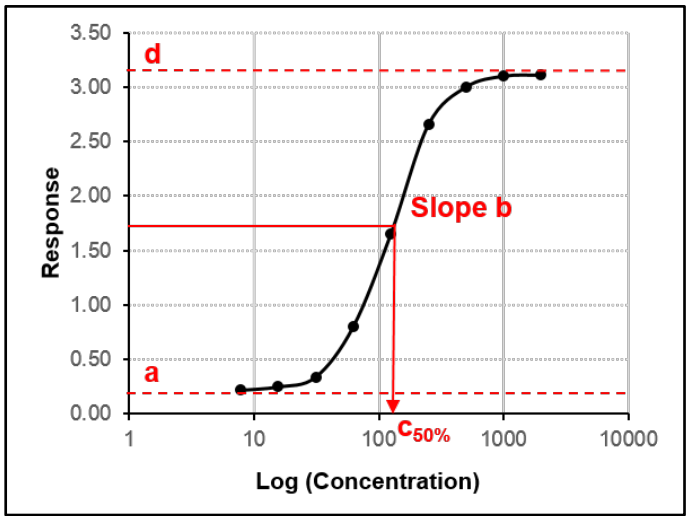

Figure 1: Typical sigmoidal immunoassay curve with parameters for non-linear regression analysis.

a = lower asymptote estimating the response at zero concentration
b = slope of curve
$c_{50\%}$ = concentration at mid-range (inflection point)
d = upper asymptote estimating the response at infinite concentration

For symmetric immunoassay curves (Figure 1), a 4-PL calibration model is the best choice and its equation can be expressed as:

$$\text{4-PL Regression: } y = d + \frac{a-d}{\left[1+\left(\frac{x}{c}\right)^{b}\right]}$$

For asymmetric calibration curves (Figure 2), a 5-PL regression analysis may give a better fit, because the regression equation takes into account the asymmetry with an additional parameter *g*:

$$\text{5-PL Regression: } y = d + \frac{a-d}{\left[1+\left(\frac{x}{c}\right)^{b}\right]^{g}}$$

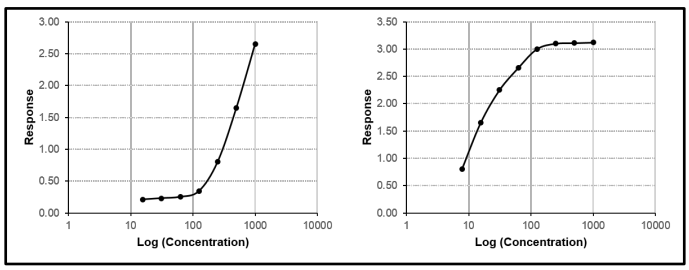

Figure 2: Examples of asymmetric immunoassay curves which may require 5-PL regression analysis.

How Well Does my Model Fit the Data?

The answer to this question is essential for generating high quality data. For linear regression analysis the regression coefficient R^2 is most commonly used to describe the goodness of fit. For non-linear regression models, the evaluation is slightly more complex and requires the investigation of residual variances over the calibration range. There are several practical ways to determine the goodness of fit without the need of sophisticated statistical software, two of them are presented here.

Residual Sum of Squares (RSS) Method

This method calculates the distance of the computed response (y-value) based on the chosen regression model from the measured response value at each concentration x. The Sum of Squares (SS) is calculated according to the following equation:

$$RSS = \sum(y_{observed} - y_{calculated})^2$$

A lower RSS value indicates a better fit.

Recovery of Calibration Standards

This method investigates the accuracy of the observed concentration calculated by the curve-fitting model for each calibrator concentration (expected value). The recovery for each concentration x is calculated according to this formula:

$$\text{Recovery} = \frac{c_{observed}}{c_{expected}} \text{ x } 100\%$$

The closer the recovery is to 100%, the better is the applied regression model. For accurate quantification, recovery values should be within 80 – 120%.

Weighting in Curve-Fitting Models

Unweighted 4-PL or 5-PL regression models assume equal response variance across all protein standards ('homoscedasticity'). However, immunoassays usually show unequal variances ('heteroscedasticity') across the calibration range. The variability in response and thus the measurement error usually increases with higher response values, and at lower protein concentrations small changes in response have a larger effect on accurate determination of protein concentration. Thus, weighting algorithms are often used to offset these effects which eventually leads to the optimization of the curve-fitting model.

One way of adjusting the weight is to use the reciprocal of the variance. By doing so, standards with high variance will have less weight on the calibration function, while standards with low variance will have more weight. Weighting in curve-fitting models requires statistical software tools and we refer to the respective instruction manuals for detailed information.

References

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