Analysis of Interferon ELISA Data

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Abstract

Enzyme-linked immunosorbent assays (ELISA) are specific and highly sensitive procedures for identifying and quantifying analytes in samples.
This assay is based on the binding of a target molecule (analyte/antigen) to antibodies which recognize the compound. The presence of an antigen-antibody complex is detected using a secondary enzyme-conjugated antibody. Detection is obtained by addition of a substrate which yields a measurable product.
Enzyme-linked immunosorbent assays are routinely used in many areas of biological research.
The determination of the analyte concentration relies upon construction of a calibration curve. The standard curve is prepared by performing a dilution series of a known concentration of the analyte across a range of concentrations near the expected unknown concentration. The calibration curves are then used to calculate the concentration of an unknown sample.
For most analyses a plot of response versus concentration will create a linear relationship, at least within a certain range of concentrations, and can be analyzed with linear regression. However, for those calibration plots which are sigmoid that is, a curve having an "S" shape performing a linear fit leads to errors in estimating sample values. These 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.
In this study we compare the results of using linear fit and 4-parameter analysis on ELISA data and report our findings.

Methods

Standard curves were prepared using kits from two different ELISA products, Mouse Interferon Beta 42400 and Human Interferon Alpha 41100. The assays were performed following protocols for each product.
After completion of each assay, plates were read at OD 450 nm using Vmax Kinetic Plate Reader (Molecular Devices Corporation, CA, U.S.A). Triplicate measurements were performed for each data set.
Data was analyzed using SoftMax Pro software (Molecular Devices Corporation, CA, U.S.A).

Analysis

The Mean of the ODs @ 450 nm, % CV of the ODs @450 nm, and the Standard Deviation between the ODs@450 nm for each data point of the standard curve was calculated. The concentration was plotted on the X-axis.

Analysis

The Mean OD@ 450 nm was plotted on the Y-axis.
Standard curves were prepared using Mouse IFN Beta ELISA kit (product 42400)_ and Human Interferon Alpha ELISA kit (product 41100). For each data set, two standard curves were created. One standard curve was plotted using a 4 parameter fit algorithm and the second one generated using linear fit analysis. Mean OD@450 nm for all data points v/s the actual concentration in pg/ml corresponding to that data point were plotted.

Figure 1 A

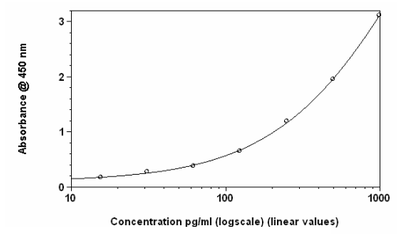

Figure 1 B

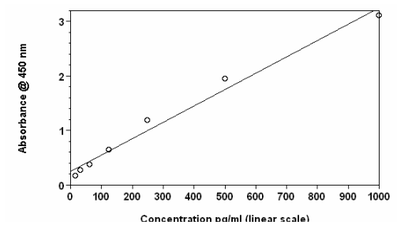

Figure 1 A and 1 B Standard curves for Mouse IFN Beta ELISA kit product 42400 with 4-Parameter fit [1. A] and Linear fit [1.B].

Figure 2 A

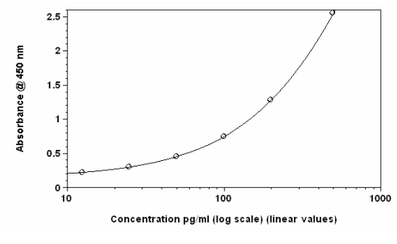

Figure 2B

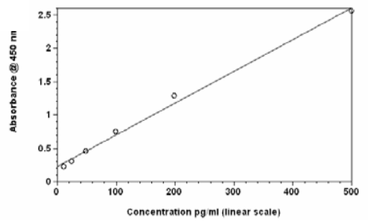

Figure 2 A and 2 B: High Range Sensitivity (HR) and Extended Range (ER) standard curves for Human Interferon Alpha ELISA product 41100, with 4-Parameter fit [2.A] and Linear fit [2.B].

Concentrations were interpolated from the fitted curves using the Mean OD values. The percent difference between actual concentrations and the backfit estimated concentrations obtained from a 4-parameter curve fit and linear fit equation are shown in Tables 1 and 2.

Actual Concentration (pg/ml)	Mean OD @ 450 nm	4-Parameter fit		Linear Fit	
		Backfit Concentration (pg/ml)	% Difference: Backfit and Actual Concentration	Backfit Concentration (pg/ml)	% Difference: Backfit and Actual Concentration
15.625	0.167	14.75	2.48	-27.05	278.84
31.25	0.268	35.86	14.75	6.59	78.91
62.5	0.374	58.72	6.05	41.90	32.96
125	0.645	120.42	3.66	132.16	5.73
250	1.186	258.74	3.50	312.26	24.90
500	1.943	494.51	1.10	564.52	12.90
1000	3.112	1001.53	0.15	954.01	4.60

Table 1: The backfit concentrations calculated with 4-parameter fit and linear fit for points on the standard curve for Mouse IFN Beta ELISA kit product 42400.

Actual Concentration (pg/ml)	Mean OD @450 nm	4-Parameter fit		Linear Fit	
		Backfit Concentration (pg/ml)	% Difference: Backfit and Actual Concentration	Backfit Concentration (pg/ml)	% Difference: Backfit and Actual Concentration
12.5	0.167	12.31	1.50	0.20	98.39
25	0.268	25.11	0.44	16.73	33.06
50	0.374	50.39	0.78	48.96	2.08
100	0.645	99.51	0.49	109.21	9.21
200	1.186	200.20	0.10	222.35	11.18
500	1.943	499.97	0.01	490.04	1.99

Table 2: The backfit concentrations calculated with 4-Parameter fit and Linear fit for points on the High Sensitivity standard curve for Human IFN Alpha ELISA product 41100..

Results

Comparing the backfitted interferon concentration values, 4 -Parameter fit versus Linear analysis, revealed disparities between the two methods. The largest discrepancies were noted at the lower concentrations. This trend was observed for both the Mouse IFN Beta and Human Alpha products. Overall the estimated interferon concentration values more closely matched the actual concentration when applying a 4-parameter fit. (Table 1 and Table 2).
Significant differences were seen for Mouse IFN beta at 15.125 pg/ml (~278%) and for Human IFN alpha interferon ~98 % at 12.5 pg/ml when using a linear fit equation.

Conclusions

Different methodologies are applied to mathematically fit data generated from ELISA experiments. The use of an inappropriate model to plot a standard curve can lead to inaccuracies in concentration determinations.

In this technical note we have demonstrated that there are calculated concentration differences obtained when using a linear fit and a 4-parameter curve fit. The backfit values derived from the 4-parameter fit standard curves varied less from the actual concentrations than did the backfit values from the linear fit

To force an assay to fit the best straight line when the response is nonlinear will certainly introduce inaccuracy into your results.

Thus, we recommend using 4-parameter fit algorithm for plotting standard curves prepared using ELISA kits manufactured by PBL Biomedical Laboratories.

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